

THE ENTOMOLOGICAL SOCIETY OF INDIA

New Delhi, 110012

ESI- BEST Ph.D. THESIS AWARD 2021

1. Name of the applicant: **Yana Venkanna**
2. Year of the award: **2021**
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S.No.	Degree	Institution	Year
1	BSc Ag	Acharya NG Ranga Agricultural University, A.P.	2001
2	MSc Ag., Entomology	Acharya NG Ranga Agricultural University, A.P.	2003
3	PhD in Entomology	ICAR-Indian Agricultural Research Institute, New Delhi.	2021

8. Date on which qualifying examination passed: **28-11-2019**
9. Transcript of the course work done, if any, as part of the Ph.D. degree programme. **(Enclosed)**
10. Was the thesis submitted in partial fulfillment of the requirement of the Degree? **Yes**
11. Date on which successfully completed thesis viva voce examination: **20-01-2021**
12. Date of the Degree/provisional certificate: **12-02-2021**
13. Have you received best thesis award earlier? **No**
14. Details of the thesis
 - (i) Title of the thesis: **Predatory behaviour of coccinellid beetle, *Cheilomenes sexmaculata* (Fab.) and its predation risk on prey**
 - (ii) Department/Division/Institution where research work was done: **Biological Control Laboratory, Division of Entomology, PG School, ICAR-Indian Agricultural Research Institute, New Delhi.**
 - (iii) Institution/University to which thesis was submitted as a part of the Ph.D. degree programme: **PG School, ICAR-Indian Agricultural Research Institute, New Delhi.**
 - (iv) Name and designation of the thesis supervisor:
Dr. Sachin Suresh Suroshe
Principal Scientist
Division of Entomology
ICAR-IARI, New Delhi.

(v) Research problem: Hypothesis, brief description, scientific, technological, socio-economic relevance and priority (limit to 1 page):

Coccinellid beetles are the most important predators which are being used against many soft bodied pests due to their voracious feeding potential (FP) and rapid numerical response (NR) in many agricultural and horticultural ecosystems. Introduction and augmentative releases of these beetles were proved for their efficacy to suppress certain crop pests. These traditional biological control methods mostly depend on feeding potential of predator on its prey. Recent advances in biological control studies have brought certain tri-trophic interactions related aspects such as non-consumptive effects of predator on its prey, indirect defense of plants through Herbivore Induced Plant Volatiles (HIPVs), induction of indirect defense through synthetic elicitors and development of genetically modified crops which change behavioral response of pest by release of olfactory cues (EBF-Wheat).

In order to have a robust and sustainable bio-control strategy, we must strive to decipher predator-prey relationship at each level, so that, the effect of biological control could be enhanced to maximum fold. Despite of its scope in biological and bio-rational method of pest control, a comprehensive study on predator-prey relationship and chemical mediated tri-trophic interactions on a particular crop-pest-predator is lacking.

Hence, the present investigations were conducted using a versatile native coccinellid species, *Cheilomenes sexmaculata* (Fab.) which is known to remains active throughout the year with a wide geographic distribution and vast host range in India. Cotton aphid, *Aphis gossypii* Glover has taken as prey being a key pest of many agri-horticultural crops with a wide host range of about 320 plant species covering 46 families. As it is known worldwide as a key pest of cotton, *Gossypium hirsutum* L., was taken as host plant.

Biological suppression of pest is a complex phenomenon which greatly determined by the foraging behaviour of its predator. A simplified depiction of prey population dynamics under predation of each stage of predator can provide information on effective stages of predator and their efficiency. To realize the concept like non-consumptive effect of predator on prey, a deep understanding of oxidative stress levels and its biological effects on prey must be worked out to explore and develop new tools for the insect pest suppression. Assessment of risk effect through bio-chemical oxidative stress markers can reveal possible mechanisms involved in non-consumptive effects.

Predator-prey interactions are strongly influenced by herbivore induced plant volatiles (HIPVs) released from the host plant which plays a crucial role in attraction of predators towards the habitat and pest's (prey) colonies. The chemicals involved in the tri-tropic interactions can alter the behavior, physiology and population buildup of prey and might be exploited to develop formulations to attract predatory beetles to a particular crop ecosystem for their further conservation.

Keeping this in view and the availability of scanty information, the present studies were conducted with the following objectives.

- a) To study the predatory behaviour of *C. sexmaculata* against cotton aphid, *A. gossypii*.
- b) To assess the predation risk induced by *C. sexmaculata* on the cotton aphid, *A. gossypii*.
- c) To study the orientation response of *C. sexmaculata* towards the prey and host plant extracts.

(vi) What standard methods and modern procedures were used in the experimental work? (limit to one page):

Biology of *C. sexmaculata* on *A. gossypii* was studied at controlled conditions of 25 ± 1 °C, $65 \pm 5\%$ RH and 14 hr of photo period in environmental chambers (Oliveira *et al.*, 2010). Cut leaf of cotton was used as substrate by keeping in a Petri plate. Agarose based Hoagland's media containing essential plant nutrients was used to keep the prolonged freshness of leaf (Nazarideljou *et al.* 2019). Biological and morphometrical observations were recorded on each stage of predator.

Feeding potential and life table studies were conducted in the Biological Control Laboratory, Division of Entomology, ICAR-IARI, Pusa at controlled conditions similar as followed for biology. Number of preys consumed was worked out by deduction of remaining aphid count and control mortality from the total number of aphids (Fand *et al.* 2010a). Feeding potential throughout the life of predator was analysed through 'CONSUME-MSChart' (Chi 2012a) to display age-specific consumption (kx), age-stage-specific consumption (Cxj) and the net consumption rate (C0). Data on duration, mortality and number of eggs laid was subjected to the construction of age-stage and two-sex life tables using TWOSEXMS-Chart (Chi 2012b).

A functional response study at various densities of aphid was conducted (Omkar, 2013). The polynomial logistic regression of the proportion of aphid killed in relation to the initial numbers was carried out for an insight about the type of functional response (Trexler and Travis, 1993; Juliano, 2001). As the consumed prey was not replaced during the foraging period, Roger's random attack equation (Rogers-II functional response equation) was used to fit the curve and estimate the functional response parameters (Royama, 1971; Rogers, 1972). Whereas, the numerical response determined by using a method given by Veeravel and Baskaran (1997).

Finite rate of increase (λ) of aphid per day was calculated from the constructed fecundity-life table (Fand *et al.* 2010b). The total response of predator was taken as a product of functional response and numerical response. All the parameters viz., finite rate of increase of prey (λ), functional response (FR), numerical response (NR) and total response (TR) of predator were assessed as a function of single factor i.e., initial prey density (N_0) by keeping the duration i.e., total time (Tt) as one day. The change in aphid population was projected graphically by simulating the respective equations for the different instars of grub, male and female of *C. sexmaculata*.

For non-consumptive effect studies, developed a simple method of aphid rearing on Mesta seedlings to get sufficient population, clear visibility for counting and to minimize the disturbance to the study organism (*A. gossypii*). A container was designed with a double cylinder system using Petri dishes and iron meshes for the treatment impose. Superoxide dismutase activity (SOD) was quantified following the method given by Lukasik *et al.* (2012). Catalase (CAT) activity (Aebi, 1984) and Malondialdehyde (MDA) content (Halliwell & Gutteridge, 1990) was assessed based on protein content determined using Bradford (1976) method.

Cotton leaves collected from different treatments were extracted for volatiles using two different solvents, n-Hexane and Dichloromethane (DCM). Extracts were analyzed by GC-MS for the identification of HIPVs (Magalhaes *et al.*, 2018). Orientation response of *C. sexmaculata* to the HIPVs was studied using Y-tube olfactometer (Oliveira and Pareja, 2014).

Data was analyzed through R software (R-Core Team 2020) using FRAIR package (Pritchard *et al.*, 2017) for functional response. Feeding potential was analyzed through 'CONSUME-MSChart' (Chi, 2012a). Data on duration, mortality and number of eggs laid was subjected to the construction of age-stage and two-sex life tables using TWOSEX-MS Chart (Chi, 2012b).

(vii) Briefly describe the significant results obtained? (Not more than 3 pages):

Host suitability and biology of *C. sexmaculata*: Cotton aphid *A. gossypii* was found to be the most suitable host for *C. sexmaculata* as evidenced by faster development (6.21 ± 0.03 days) and more longevity of female (67 ± 4.7 days) with high fecundity (756.5 eggs/ female). These positive biological attributes of *C. sexmaculata* on *A. gossypii* could contribute to its mass production for augmentative biological control.

Foraging behaviour of *C. sexmaculata*: For the grub stage, highest feeding potential (101.43 ± 3.04 aphids) was observed for the fourth instar which was higher than the gross feeding potential of all other instars. Among the adults, female recorded high feeding potential with a mean daily consumption of 43.13 ± 0.54 aphids. Life table studies showed intrinsic rate of increase (r) of 0.22 with the mean generation time of 27.29 days. The foraging behaviour studies revealed that, all the grub stages and adults displayed a type-II functional response. The IV instar grub has shown the lowest handling time with highest maximum predation (96.1aphids/day), followed by female (94.6), male (82.6) and other stages. However, female depicted a rapid extinction of aphid by keeping it below 25, as against fourth instar grub and male which affected extinction by keeping it below 30. So, the releases of fourth instar grub and female of *C. sexmaculata* could be advocated for the augmentative biological control of *A. gossypii*.

Oxidative stress in aphid under predation risk: We found that the oxidative stress is induced by olfactory cues, and even short duration exposure could raise antioxidant enzyme levels in the cotton aphid. Aphid's antioxidant mechanism uses up regulation of superoxide dismutase (SOD) and catalase (CAT) but not sufficiently to offset the oxidative damage that led to increased Malondialdehyde (MDA) content. SOD activity was significantly higher for active predation (9.75 ± 0.60 units/mg protein) and risk of predation (9.30 ± 0.40 units/mg protein) over controls (5.39 ± 0.13 units/mg protein). Catalase (CAT) showed highest activity in the aphids exposed to active predation (78.12 ± 5.26 H₂O₂ decomposed in $\mu\text{M}/\text{min.}/\text{mg}$ protein) followed by risk of predation (62.62 ± 5.22) and no-predation (26.48 ± 5.99) with significant differences between each other. A similar trend was also observed for Malondialdehyde (MDA) content as recorded 3.41 ± 0.30 , 2.73 ± 0.39 and 1.40 ± 0.04 nM/mg protein in aphids exposed to active predation, risk of predation and no-predation, respectively. It is a clear indication of oxidative stress induction through the olfactory cues released by the predator as expressed stress enzyme levels with the same magnitude under active and risk of predation without significant differences between them.

Non-consumptive effects (NCEs) of *C. sexmaculata* on *A. gossypii*: NCEs did not influence the longevity, mortality and developmental period of aphids as there is no significant difference between the treatments and control. NCEs did have significant effects on the reproduction of aphids with reduced reproductive period and reduction in the number of nymphs produced per female during the total life span. A significant decrease in reproduction was recorded in aphids under active predation (32.35 ± 4.65 nymphs/female) as well as risk of predation (30.00 ± 4.40 nymphs/female) compared to the no-predation treatment (41.62 ± 5.60 nymphs/female). Both the active predation and risk of predation treatments resulted in a similar trend in lowering aphid reproduction, albeit with no significant difference between them. Despite the fact that there was no influence of NCEs on mortality and developmental period of nymphs produced by the parthenogenetic adults of *A. gossypii* which were exposed to predators, exposure clearly paved the way for the production of winged morphs. There was more than three-fold increase in the per cent of winged morphs produced by the aphids under predation-risk compared to the control. However,

there was no significant difference observed for winged morphs between the active-predation and risk-predation treatments (Fig. 1).

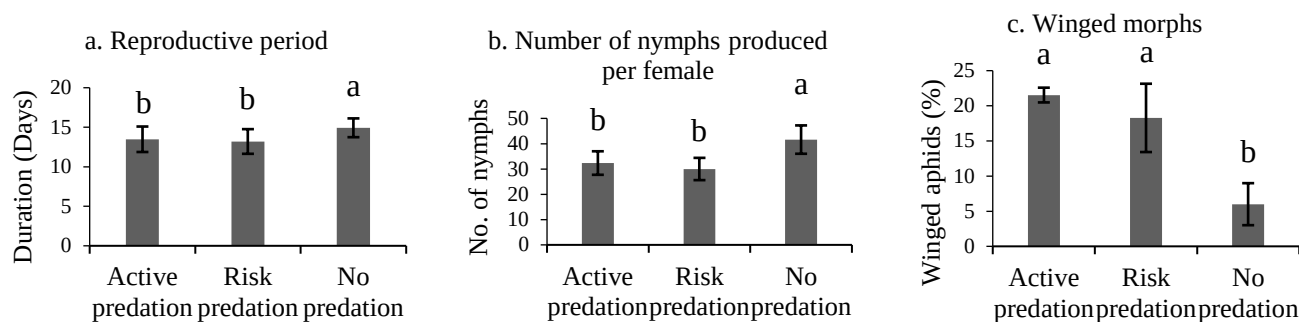


Fig. 1: Biological parameters differentially expressed by cotton aphid, *A. gossypii* under predation-risk of *C. sexmaculata*

A simple rearing technology of *A. gossypii*: Seeds of mesta, *H. cannabinus* grew well on absorbent cotton with germination per cent more than 90. In just three days after sowing, the seedlings with fully opened cotyledonary leaves were ready to host the aphids. Each Petri plate (10 cm Ø) could accommodate a stand of 25 seedlings. These seedlings supported the population buildup of *A. gossypii* up to 33 days after sowing. Nymphal population increased geometrically from first day to about 12 days after releasing, then increased exponentially up to 21-24 days and thereafter a faster decline was observed. The highest value of 568.9 ± 24.8 nymphs per seedling was recorded at 24 °C and lowest at 27 °C (312 ± 11 /plant). Wingless adult population was obtained from sixth day after releasing at 24 and 27 °C whereas, it was observed from 12th day after releasing at 18 and 21 °C. The adult population was noticed highest at 24 °C (30 ± 1.8) followed by 21 (27.3 ± 0.9), 27 (22.2 ± 3.4) and 18 °C (19.2 ± 3.0) at 21, 24, 15 and 24 days after release, respectively. The cumulative total for winged adults was higher at 27 °C, which significantly differed with all other temperatures.

Orientation response of *C. sexmaculata* to the aphid induced HIPVs of cotton: Female and male beetles of *C. sexmaculata* have not shown any significant attraction to the un-infested leaf (UL) of neither DCM nor n-Hexane extractant at any of the tested concentration. Among the other extracts, infested leaf with aphid (WA) extracted with DCM as well as n-Hexane have shown significant attraction for both female and male beetles at all the concentrations. In the remaining extracts, aphid only (AO) and infested leaf without aphid (WOA) have shown significant attraction at one or other concentrations. Among all the extracts which have shown significant attraction over control, DCM-WA 5% was attracted the highest number of female beetles ($83.8 \pm 3.24\%$) followed by Hexane-WA 5% ($72.5 \pm 2.50\%$) and others. Similarly, male beetles were also mostly attracted to DCM-WA 5% ($72.5 \pm 3.66\%$) and Hexane-WA 5% ($70.0 \pm 4.23\%$). Male beetle has shown significant variation to the concentration gradient of WA by displaying 1, 5 and 10% on par with each other. Whereas, female has shown significant variation to the concentration gradient of both WA and aphid only (AO) extracts with higher number of groups with significant difference. Whereas, the extracts drawn from MeSA sprayed plants have not shown any significant attraction of beetles.

GC-MS analysis of different extracts: Volatile profile of extracts extracted through DCM exhibited an increase in number as well as abundance of certain compounds on infestation of aphid. It displayed 27 compounds by un-infested leaf (UL), increased to 38 for infested leaf with

aphid (WA) and 34 for infested leaf without aphid (WOA). Whereas, aphid only (AO) extracts could record only 22 compounds. UL and WOA have shown similarity in compounds and their abundance. Whereas, aphid infested leaf (WA) depicted a different pattern with the highest quantity of 3-Carene ($20.15 \pm 1.56\%$) followed by (E)- β -Caryophyllene ($13.06 \pm 1.51\%$), 1,4,7-Cycloundecatriene-1,5,9,9-tetramethyl-,Z,Z,Z- ($8.98 \pm 0.38\%$), α -Bulnesene ($4.89 \pm 0.96\%$) and 3-Hexenal ($4.88 \pm 0.54\%$). Similar trend of increase in number of compounds was also recorded from the extracts drawn through the other solvent n-Hexane. n-Hexane extracts of WA resulted additional compounds viz., Heptane ($17.36 \pm 5.09\%$), Pentadecane ($10.39 \pm 5.21\%$), 3-Hexenal ($7.93 \pm 1.79\%$), 2-Hexenal ($7.09 \pm 0.89\%$) and α -Linolenic acid (2.79 ± 0.99).

Comparative analysis of extracts revealed certain compounds as aphid induced HIPVs as they found in the extract of infested leaf but not in un-infested one. The DCM extract showed 14 additional compounds as HIPVs in which 3-Carene was found as predominant. Similarly, n-Hexane extract displayed 11 additional compounds as HIPVs in which Pentadecane was the highest. With the obtained results and analysis of volatile profile of different extracts, some of the compounds were identified as major aphid induced HIPVs namely, 3-Carene, Heptane, Pentadecane, 3-Hexenal, α -Copaene and α -Linolenic acid (Fig. 2). A blend of aphid induced HIPVs consisting of six major and eleven minor compounds was assumed to be responsible for the higher attraction of ladybird beetle, *C. sexmaculata* (Fig.3).

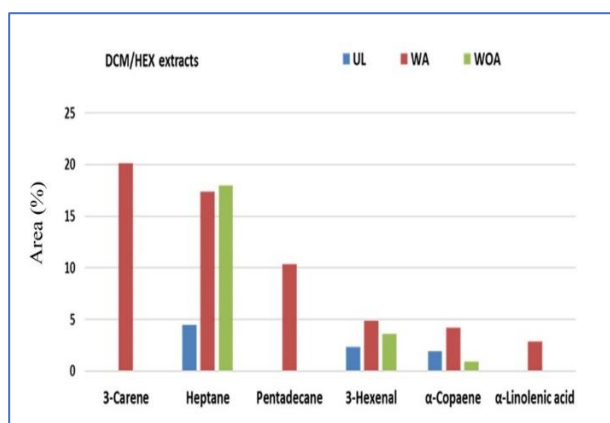


Fig. 2. Major aphid induced HIPVs identified through comparative analysis of different extracts for volatiles with their relative abundances [UL-Un infested leaf, WA-Infested leaf with aphid, WOA-Infested leaf without aphid]

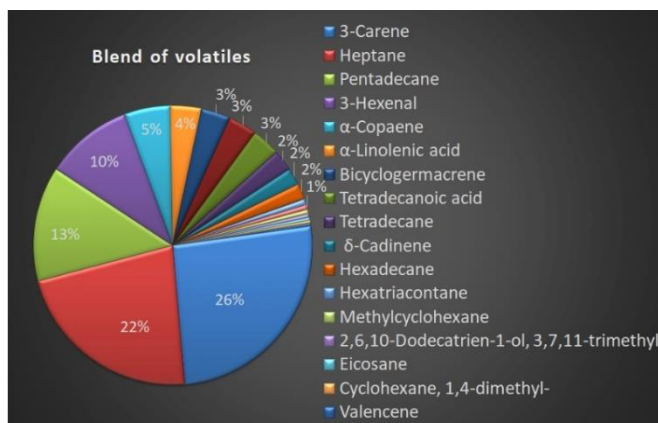


Fig. 3. Proposed blend of volatile compounds assumed to be responsible for higher attraction of *C. sexmaculata* adults

Volatile profile of the extracts collected from MeSA sprayed plants revealed that the emission profile at 2 and 4 h after spraying is very closure to that of un-infested plant as it has shown similarity in the number of compounds and their abundance. Whereas, the number of compounds increased from 20 to 29 at 10 h after spraying in which 13 compounds are additionally released after MeSA spraying. Among the additional compounds elicited by the MeSA, 3-Carene, Tetra decanoic acid, 3-Hexenal and Pentadecane were found common with that of aphid infested HIPVs. However, the MeSA elicited volatiles have not shown any significant attraction of *C. sexmaculata* beetles.

(viii) In what way these results have made an original contribution to science and have impact:

These positive biological attributes of *C. sexmaculata* on *A. gossypii* could contribute to its mass production for augmentative biological control.

Fourth instar grub, female and male of *C. sexmaculata* are found to be the effective stages against *A. gossypii* as it exhibited rapid reduction of prey with high attack rate and low handling time. Female beetles, in addition to their immediate functional response also contribute to the prolonged numerical response. So, the use of female and fourth instar grub could be advocated for the release towards the augmentative biological control of *A. gossypii*.

In the present investigations, we found that the NCEs resulting from predation-risk by *C. sexmaculata* on cotton aphid, *A. gossypii* was likely transmitted through olfactory cues, and even short exposure could raise the antioxidant enzyme levels in the aphids. The adult aphids exhibited antioxidant mechanisms through the up regulation of SOD and CAT but not sufficient to avoid oxidative damage that led to increased MDA content. As a result, the cost of production of SOD and CAT might have been compensated for with reduced reproduction in cotton aphid by adopting a 'sit and wait' strategy, and also by triggering winged morphs production for resorting to a 'fight or flight' strategy. Synthetic olfactory cues which mimic predator presence may act as biorational insecticide by reducing aphid population similar to non-consumptive effect found in this study.

We have developed a simple rearing technique of cotton aphid, *A. gossypii* on Mesta seedlings raised in a Petri plate. It is an easy and simple method as it does away with maintenance of host plants in pots. It is very effective as we can quickly produce nymphs, wingless and winged adults in a larger number in short time. With this technique, continuous rearing is possible so that, lab studies and mass production of predators and parasitoids can be carried out easily in laboratory.

Based on the knowledge gained and comprehensive observations from the results obtained, we identified a possible blend of volatiles to be used as an effective attractant to enhance the foraging behavior of aphidophagous coccinellid beetles in the cotton crop. Proposed blend of volatile consists 17 compounds which include 3-carene (20.15%), Heptane (17.36%), Pentadecane (10.39%), 3-Hexenal (7.93%) and α -copaene (4.18%) as major compounds. It facilitates development of an attractant of coccinellid beetles using one or few of these identified compounds.

- (ix) **Publications (in journals/along with NAAS rating as on 1.1.2021, Seminar proceedings, University/Institute Newsletter, Lecture delivered, Book chapter etc.(publications only from the thesis to be included, only those publications in published/accepted/proof stage will be considered, no marks will be given for other publications not related with thesis works):**

S.No.	Publication	Title	Authors	Publisher with volume	NAAS rating
1	Research Article	Biology of <i>Cheilomenes sexmaculata</i> (F.) on cotton aphid <i>Aphis gossypii</i> Glover	Venkanna, Y, Sachin S Suroshe*, Bhagyasree S N and Sweetee Kumari	Indian Journal of Entomology, 82(1): 75-79 (2020)	5.08
2	Research Article	Feeding potential and foraging behaviour of <i>Cheilomenes sexmaculata</i> (F.) on the cotton aphid, <i>Aphis gossypii</i> glover	Y. Venkanna, Sachin S. Suroshe, Subhash Chander & Sweetee Kumari	International Journal of Tropical Insect Science, 28: 1-12 (2021)	6.54
3	Research Article	Non-consumptive effects of the zigzag ladybird beetle, <i>Cheilomenes sexmaculata</i> (Fab.) on its prey, the cotton aphid, <i>Aphis gossypii</i> Glover	Y. Venkanna, Sachin S. Suroshe & Anil Dahuja	Biocontrol Science and Technology, DOI: 10.1080/09583157.2021.1937521	7.22
4	Research Article	A simple rearing technique for the cotton aphid, <i>Aphis gossypii</i> Glover	Y. Venkanna & Sachin S. Suroshe	International Journal of Tropical Insect Science (Under final review)	6.54
5	Research Article	Orientation response of <i>Cheilomenes sexmaculata</i> L. to the cotton HIPVs	Y. Venkanna, Sachin S. Suroshe & Suresh Nebapure	Communicated	-
6	Newsletter	A technique for continuous rearing of cotton aphid (<i>Aphis gossypii</i>)	Y. Venkanna & Sachin S. Suroshe	IARI NEWS, 35 (4): 4 (2019)	-

- (x) Soft copy in PDF format needs to be submitted for the above including whole thesis (Enclosed)

15. Whether any patents have been taken/applied for, if yes, attach evidence: No
16. Professional recognition, awards, fellowships received (In a separate sheet give full particulars such as the agency/ organization which gave the award, purpose, the nature of the award, etc.): No
17. A concise statement (about 150 words) highlighting the most significant aspects of the research work done that you would like to see in your citation of award, if chosen.

Dr. Y. Venkanna, Subject Matter Specialist (Entomology), Krishi Vigyan Kendra, Ramagirikhilla, Peddapally District, Telangana, has been awarded **ESI- BEST Ph.D. THESIS AWARD 2021** for Outstanding Doctoral Thesis Research in Agricultural Entomology for his doctoral thesis on the topic "**Predatory behaviour of coccinellid beetle, *Cheilomenes sexmaculata* (Fab.) and its predation risk on prey**" pursued from ICAR-Indian Agricultural Research Institute, New Delhi. Dr. Venkanna studied foraging behaviour of native coccinellid predator, *C. sexmaculata* on cotton aphid, *Aphis gossypii* with a comprehensive investigation including biology, life table, feeding potential, functional response, numerical response with which worked out total response equation and drawn prey population dynamics under predation of each stage of predator. Also studied predation risk effect through enzyme analysis of SOD, CAT and MDA content and found increased oxidative stress in aphids under predation risk of *C. sexmaculata* which ultimately effected reproductive performance of aphid and also induced the winged morphs production as a non-consumptive of predator. Identified certain volatiles as aphid induced HIPVs released by cotton and proved their attractiveness of *C. sexmaculata* beetles. Proposed a blend of volatile consisting 17 compounds includes 3-carene (20.15%), Heptane (17.36%), Pentadecane (10.39%), 3-Hexenal (7.93%) and α -copaene (4.18%) as major compounds.

18. Certificate by the student that all the details given are true



Signature

19. Certificate by the supervisor/guide that the information submitted has been verified and true

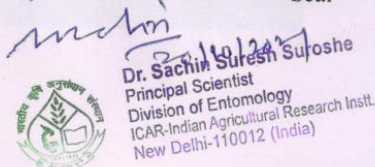

Dr. Sachin Suresh Suroshe
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Signature

20. Forwarding note by the Head of the Department/professor

I have forwarded students application twice to Professor/Head of entomology; student has also sent mail to Prof./Head and also spoke for necessary action at Prof./Head end. But Prof/Head is not interested in forwarding application of student for Best thesis award instituted by ESI.

Name and Designation

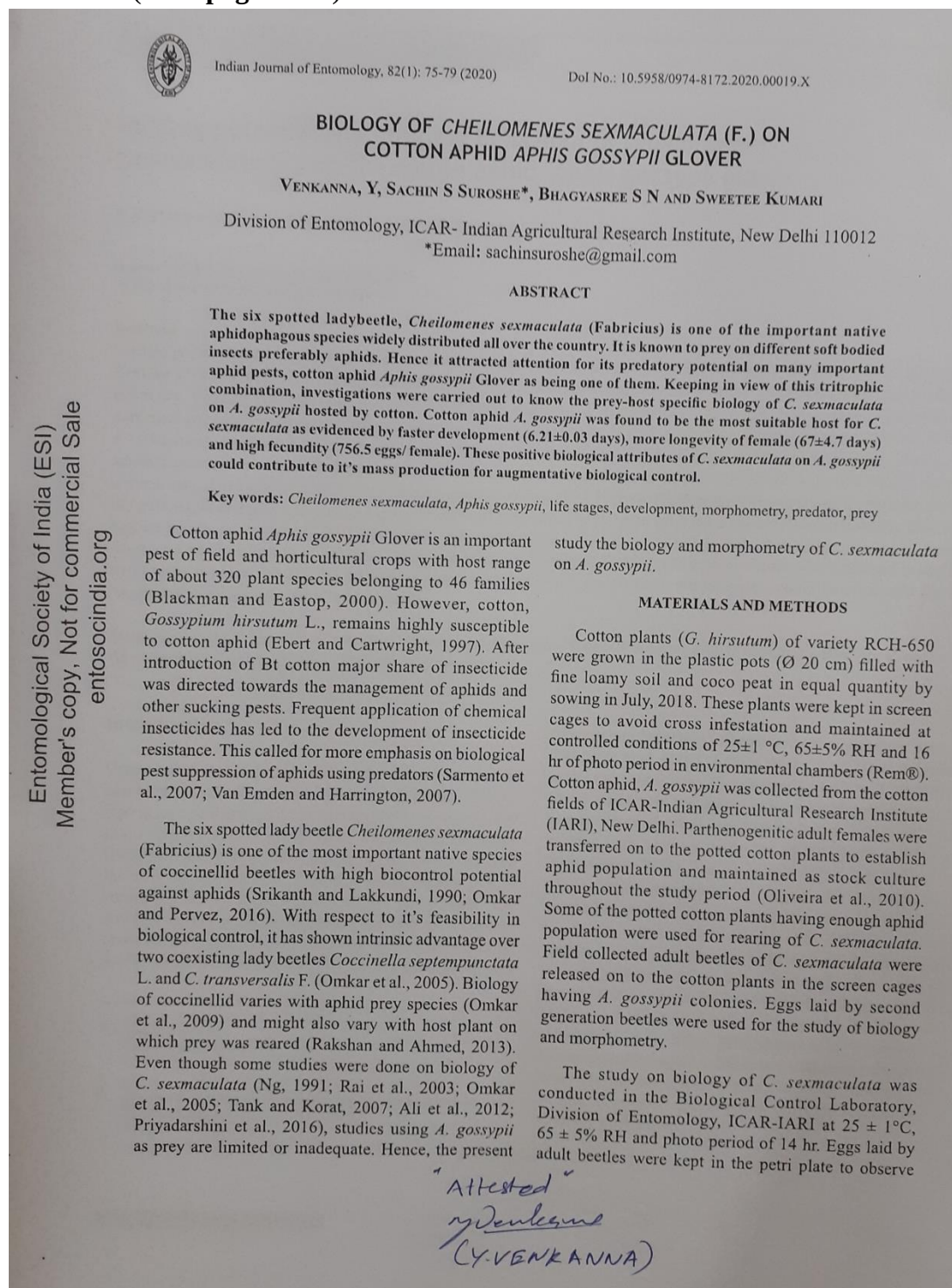
Seal


Dr. Sachin Suresh Suroshe
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Annexures

List of self-attested scanned documents to be enclosed:

1. Publications (First page alone)





Feeding potential and foraging behaviour of *Cheilomenes sexmaculata* (F.) on the cotton aphid, *Aphis gossypii* glover

Y. Venkanna¹ · Sachin S. Suroshe¹ · Subhash Chander¹ · Sweetee Kumari¹

Received: 9 May 2020 / Accepted: 26 December 2020
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Abstract

Feeding potential and foraging behaviour are two important factors which determine the predator's efficiency of managing prey. Predator-prey system of *Cheilomenes sexmaculata* (Fabricius) and *Aphis gossypii* Glover was evaluated to find out the predation efficiency. Feeding potential was observed for different stages of *C. sexmaculatus* on *A. gossypii* whereas, functional and numerical responses were ascertained as a function of prey/host density. For the grub stage, highest feeding potential (101.43 ± 3.04 aphids) was observed for the fourth instar which was higher than the gross feeding potential of all other instars. Among the adults, female recorded high feeding potential with a mean daily consumption of 43.13 ± 0.54 aphids. Life table studies showed intrinsic rate of increase (r) of 0.22 with the mean generation time of 27.29 days. The foraging behaviour studies revealed that all the grub stages and adults displayed a type-II functional response. The IV instar grub has shown the lowest handling time with highest maximum predation (96.1aphids/day), followed by female (94.6), male (82.6) and other stages. However, female depicted a rapid extinction of aphid by keeping it below 25, as against fourth instar grub and male which affected extinction by keeping it below 30. So, the releases of fourth instar grub and female of *C. sexmaculatus* could be advocated for the augmentative biological control of *A. gossypii*.

Keywords Type-II functional response · Numerical response · Roger's random attack equation · Biological control · Age-stage-specific consumption · Predator-prey relationship

Introduction

Cotton aphid, *Aphis gossypii* Glover has been a key pest of agri-horticultural crops due its economic importance. This polyphagous aphid species is known to attack more than 300 plant species across 46 families all around the globe (Blackman and Eastop 2000) among which cotton has been the most susceptible host. Cotton aphid is the key pest of cotton and known to be a vector of leaf curl disease. The developments of pesticide resistance and associated concern about the environmental hazards caused by frequent pesticidal applications have warranted efforts on biological pest

suppression, especially of aphids by the predatory beetles (Samento et al. 2007). Among different predators prevailed in the crop-ecosystem, the coccinellid beetles (Coleoptera: Coccinellidae) are considered as the most important group as they are known for their voracious feeding on aphids and other sucking pests both by grubs and adults (Dixon and Dixon 2000; Omkar 2016). The six spotted zigzag ladybird beetle, *Cheilomenes sexmaculatus* (F.) is one such aphidophagous coccinellid predator known to suppress the aphid population to a greater extent in nature (Reddy et al. 2001). It is a native species of India, remains active throughout the year with a wide geographic distribution and host range. It was also observed for its voracious feeding on aphids with high searching ability and could be handle with ease in the laboratory (Venkatesan et al. 2006). With respect to its feasibility as a biological control agent, it has shown intrinsic advantage over other two coexisting species viz., *Coccinella septempunctata* L. and *Coccinella transversalis* F. (Omkar et al. 2005; Rakshith et al. 2018).

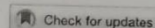
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(Y. VENKANNA)



RESEARCH ARTICLE



Non-consumptive effects of the zigzag ladybird beetle, *Cheilomenes sexmaculata* (Fab.) on its prey, the cotton aphid, *Aphis gossypii* Glover

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ABSTRACT

Non-consumptive effects (NCEs) of predators include non-lethal changes in prey altering their behaviour and physiology with increased oxidative stress under predation-risk. Although prey species may exhibit a complex of antioxidant mechanisms, these may be inadequate to result in a reduction of adverse biological consequences in the prey. Reduced prey fitness may result and could contribute to enhancing biological control. Cotton aphid, *Aphis gossypii* is an important polyphagous pest predated by a variety of coccinellids and very much preferred by zigzag ladybird beetle, *Cheilomenes sexmaculata* (F.). Possible NCEs of *C. sexmaculata* on cotton aphid were studied to understand the underlying anti-oxidative mechanisms. We found that the non-consumptive effect is induced by olfactory cues, and even short-duration exposure could raise antioxidant enzyme levels in the cotton aphid. Aphids antioxidant mechanism uses upregulation of superoxide dismutase (SOD) and catalase (CAT) but not sufficiently to offset the oxidative damage that led to increased Malondialdehyde (MDA) content. Affected aphid biology with reduced reproductive performance as recorded 30.0 ± 4.40 nymphs/female under predation-risk compared to no-predation (41.6 ± 5.60). Further, the induction of winged morphs increased with active predation ($21.53 \pm 1.05\%$) and increased risk of predation ($18.28 \pm 4.86\%$) compared to aphids not exposed to predators ($6.00 \pm 2.99\%$). Antioxidant production costs were compensated by the exposed aphids directly through reduced reproduction and also triggered winged morphs in progeny. Non-consumptive effect might directly contribute to declines in aphid populations through reduced reproduction and may also stimulate dispersal of winged morphs.

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Introduction

Aphis gossypii Glover (Aphididae: Homoptera) is one of the most important sucking insects damaging agriculture and horticultural crops. It is a highly polyphagous pest infesting more than 320 plant species of 46 families, and a major pest of cucurbits and

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plasma isolate identified it as a member of the peanut witches' broom phytoplasma group (*Candidatus* Phytoplasma aurantifolia).

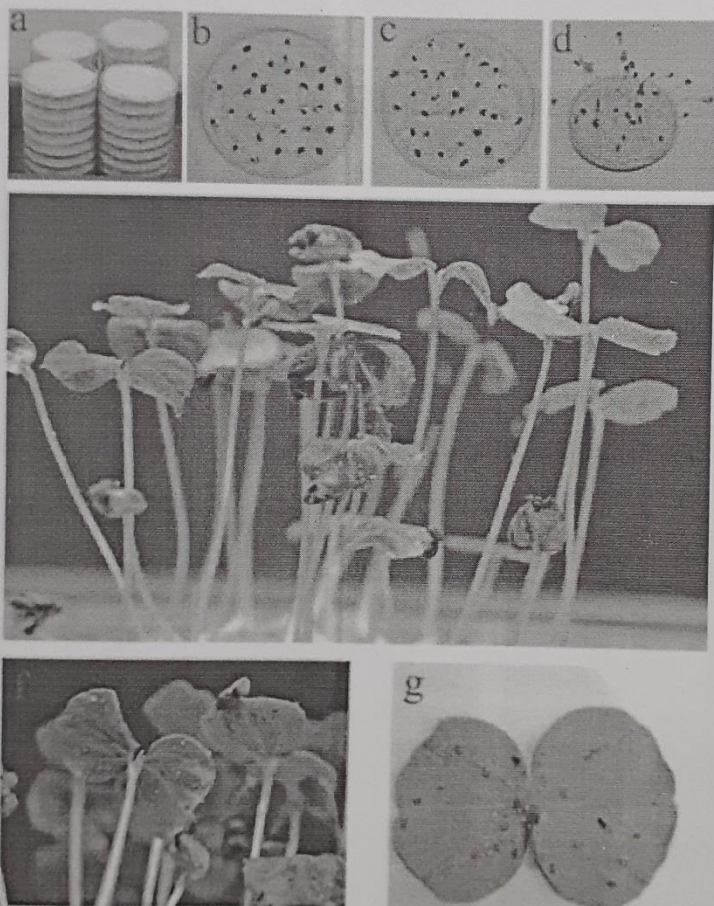
Also, on the basis of computer-simulated RFLP analysis of amplified 16S rRNA gene, assigned the peanut strain to subgroup D. This is the first report on the association of 'Ca. P.

aurantifolia' (16SrII-D subgroup phytoplasma) with peanut in India.

A Technique for Continuous Rearing of Cotton Aphid (*Aphis gossypii*)

For rearing of cotton aphid, *Aphis gossypii* potted cotton plant method has been extensively used which is a cumbersome process requires more time and space. The other methods such as

rearing on excised cotton leaves and using artificial diet are also not suitable for rearing in high number for longer periods. A simple rearing technique of *A. gossypii* on a natural host system of *Hibiscus cannabinus* L. was developed in the Division of Entomology, ICAR-IARI, New Delhi. Mesta (*H. cannabinus*) was selected for rearing of *A. gossypii* based on screening of different host plants. In this period, seedlings of *H. cannabinus* were grown on absorbent cotton in a Petri plate (10 cm ø) as substrate. This technique was found to be effective for continuous rearing throughout the year. Among different temperatures, 24°C was found most suitable to produce nymphs and wingless adults in high number. However, for the production of winged adults, rearing at 27°C was found to be best. This technique made simple and easy to practice as it avoids growing and maintenance of host plants in pots throughout the year. It is effective as it facilitates quick start of rearing and produce nymphs, wingless adults and winged adults in high numbers and feasible for continuous rearing on *H. cannabinus* seedlings for 30 days without transfer. This technique is simple and suitable for continuous rearing in lab studies and mass production of predators and parasitoids would be possible for laboratory.

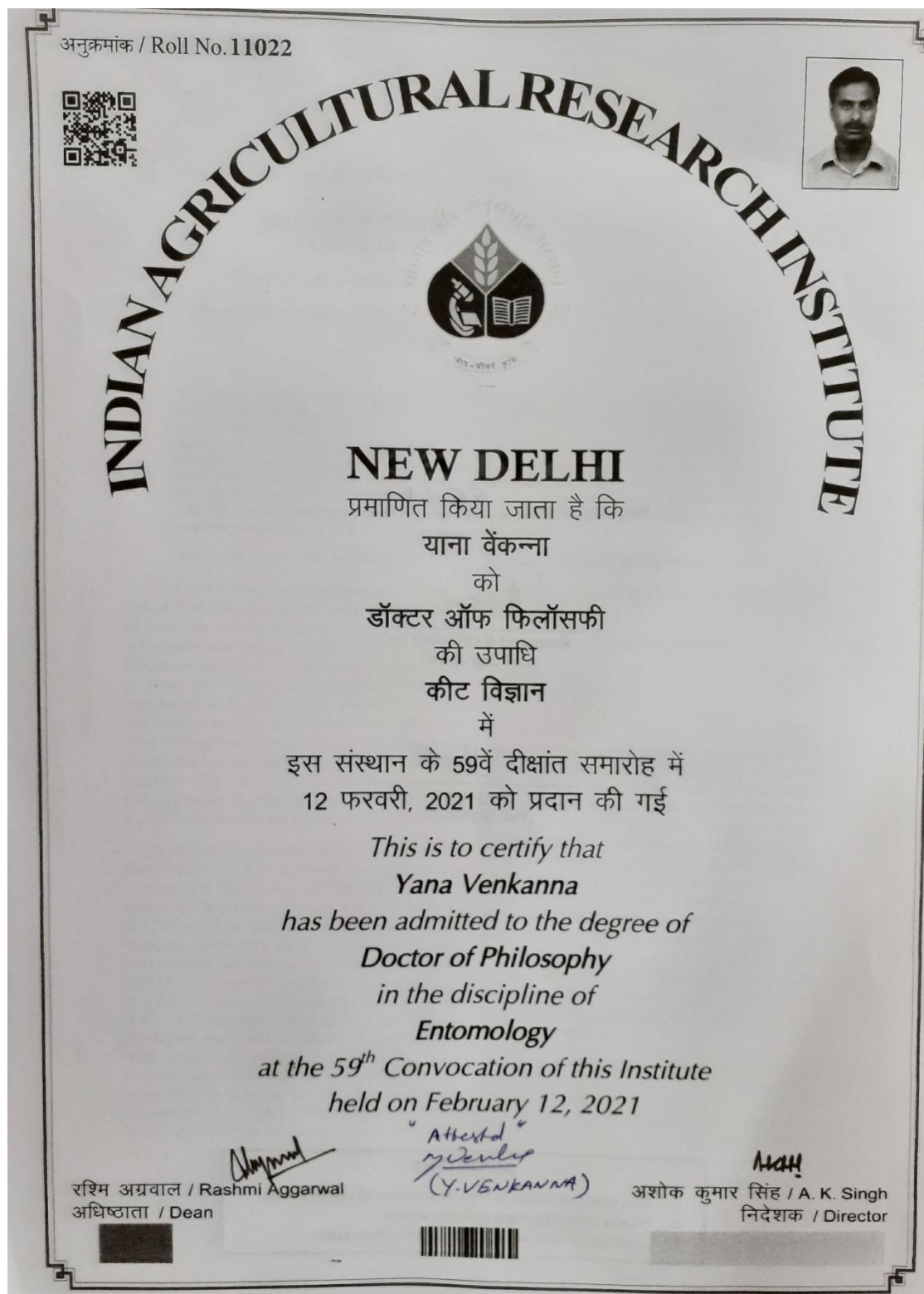


Seed kept for germination: (a-d) 0, 1, 2 and 3 days after keeping for germination. (e) Aphid establishment at 18 days after release, (f) Aphid on both sides of leaf, (g) Different stages of aphid on underside of leaf.

Procedure of new rearing method of Aphis

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2. Photo copy of PhD degree



3. Age proof



4. Best passport size photograph



5. Thesis (soft copy) (Enclosed pdf file)

Predatory behaviour of coccinellid beetle, *Cheilomenes sexmaculata* (Fab.) and its predation risk on prey

BY

VENKANNA. Y

A Thesis Submitted to the Faculty of Post-Graduate School,
ICAR-Indian Agricultural Research Institute, New Delhi
in partial fulfillment of the requirements
for the award of the degree of

DOCTOR OF PHILOSOPHY

IN

ENTOMOLOGY

2020

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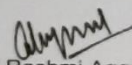
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6. Transcript of the course work done, if any, as part of the Ph.D. degree programme.
(Enclosed)

	POST GRADUATE SCHOOL INDIAN AGRICULTURAL RESEARCH INSTITUTE NEW DELHI - 110012 (Deemed University under Sec. 3 of UGC Act, 1956)	
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9. Title of Thesis	:	PREDATORY BEHAVIOUR OF COCCINELLID BEETLE, <i>Cheilomenes sexmaculata</i> (FEB.) AND ITS PREDATION RISK ON PREY
10. Date of Submission of Thesis	:	12 November 2020
11. Date of Passing the Thesis Viva-voce Examination	:	20 January 2021
12. Details of Course Credit	:	Reverse
13. Overall Grade Point Average	:	7.49/10.00 SEVEN POINT FOUR-NINE OUT OF TEN
		
New Delhi Friday, February 12, 2021	 रश्मि अग्रवाल / Rashmi Aggarwal अधिष्ठाता / Dean	

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Details of Course Work and Overall Grade Point Average

TRIME- STER	COURSE CODE	TITLE	CREDIT	GRADE
I				
	ENT503-2017	PRINCIPLES OF INSECT PEST MANAGEMENT	3	8.14
	ENT606	INSECT BIOCHEMISTRY	3	7.43
	MBB501	PRINCIPLES OF BIOTECHNOLOGY	4	6.95
	PGS501	LIBRARY AND INFORMATION SERVICES	1	9.80
	PGS502	TECHNICAL WRITING AND COMMUNICATION SKILLS	2	8.80
II				
	BIO606	INTERMEDIARY METABOLISM	3	6.00
	ENT607-2017	ADVANCES IN INSECT PHYSIOLOGY	3	6.90
	ENT691	SEMINAR	1	8.44
	MBB505-2017	MOLECULAR GENETICS OF EUKARYOTES	3	6.20
	PGS503	INTELLECTUAL PROPERTY AND ITS MANAGEMENT IN AGRICULTURE	1	7.45
	PGS505	AGRICULTURAL RESEARCH, RESEARCH ETHICS AND RURAL DEVELOPMENT PROGRAMES	1	8.25
III				
	ENT603-2018	ADVANCES IN INSECT PEST MANAGEMENT	3	7.60
	ENT605-2017	ADVANCES IN INSECT TOXICOLOGY	4	8.25
	ENT608-2017	RECENT TRENDS IN ENTOMOLOGY	3	8.35
	ENT691	SEMINAR	1	8.85
	MBB506	BIOSAFETY, IPR AND BIOETHICS	2	7.40
	PGS-506	HISTORY OF AGRICULTURE		Satisfactory
IV				
	BIO501	BASIC BIOCHEMISTRY	5	6.75
	BIO691	SEMINAR	1	8.72
VII				
	QVVE	QUALIFYING EXAMINATION	2	7.14

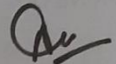
Total Credits: 46**Remarks:****O.G.P.A.:** 7.49/10.00

SEVEN POINT FOUR-NINE OUT OF TEN

Note:

OGPA	Description of Performance
(1) 9.0 to 10.0	(90 to 100%) 1st Class with distinction
8.0 to 8.99	(80 to 89%) 1st Class
7.0 to 7.99	(70 to 79%) 2nd Class
6.0 to 6.99	(60 to 69%) Pass
Below 6.00	(Less than 60%) Fail
Incomplete	I
(4)	The date of Final Viva-voce Examination may be treated as the date of completion of the degree programme for all purposes.

- (2) O.G.P.A. = $\frac{\text{Total points earned}}{\text{Divided by Total credits taken}}$
- (3) One hour lecture or three hours practical per week for 12 weeks carries one credit hour.

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BIOLOGY OF *CHEILOMENES SEXMACULATA* (F.) ON COTTON APHID *APHIS GOSSYPYII* GLOVER

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ABSTRACT

The six spotted ladybeetle, *Cheilomenes sexmaculata* (Fabricius) is one of the important native aphidophagous species widely distributed all over the country. It is known to prey on different soft bodied insects preferably aphids. Hence it attracted attention for its predatory potential on many important aphid pests, cotton aphid *Aphis gossypii* Glover as being one of them. Keeping in view of this tritrophic combination, investigations were carried out to know the prey-host specific biology of *C. sexmaculata* on *A. gossypii* hosted by cotton. Cotton aphid *A. gossypii* was found to be the most suitable host for *C. sexmaculata* as evidenced by faster development (6.21 ± 0.03 days), more longevity of female (67 ± 4.7 days) and high fecundity (756.5 eggs/ female). These positive biological attributes of *C. sexmaculata* on *A. gossypii* could contribute to its mass production for augmentative biological control.

Key words: *Cheilomenes sexmaculata*, *Aphis gossypii*, life stages, development, morphometry, predator, prey

Cotton aphid *Aphis gossypii* Glover is an important pest of field and horticultural crops with host range of about 320 plant species belonging to 46 families (Blackman and Eastop, 2000). However, cotton, *Gossypium hirsutum* L., remains highly susceptible to cotton aphid (Ebert and Cartwright, 1997). After introduction of Bt cotton major share of insecticide was directed towards the management of aphids and other sucking pests. Frequent application of chemical insecticides has led to the development of insecticide resistance. This called for more emphasis on biological pest suppression of aphids using predators (Sarmiento et al., 2007; Van Emden and Harrington, 2007).

The six spotted lady beetle *Cheilomenes sexmaculata* (Fabricius) is one of the most important native species of coccinellid beetles with high biocontrol potential against aphids (Srikanth and Lakkundi, 1990; Omkar and Pervez, 2016). With respect to its feasibility in biological control, it has shown intrinsic advantage over two coexisting lady beetles *Coccinella septempunctata* L. and *C. transversalis* F. (Omkar et al., 2005). Biology of coccinellid varies with aphid prey species (Omkar et al., 2009) and might also vary with host plant on which prey was reared (Rakshan and Ahmed, 2013). Even though some studies were done on biology of *C. sexmaculata* (Ng, 1991; Rai et al., 2003; Omkar et al., 2005; Tank and Korat, 2007; Ali et al., 2012; Priyadarshini et al., 2016), studies using *A. gossypii* as prey are limited or inadequate. Hence, the present

study the biology and morphometry of *C. sexmaculata* on *A. gossypii*.

MATERIALS AND METHODS

Cotton plants (*G. hirsutum*) of variety RCH-650 were grown in the plastic pots ($\varnothing$ 20 cm) filled with fine loamy soil and coco peat in equal quantity by sowing in July, 2018. These plants were kept in screen cages to avoid cross infestation and maintained at controlled conditions of 25 ± 1 °C, $65 \pm 5\%$ RH and 16 hr of photo period in environmental chambers (Rem®). Cotton aphid, *A. gossypii* was collected from the cotton fields of ICAR-Indian Agricultural Research Institute (IARI), New Delhi. Parthenogenetic adult females were transferred on to the potted cotton plants to establish aphid population and maintained as stock culture throughout the study period (Oliveira et al., 2010). Some of the potted cotton plants having enough aphid population were used for rearing of *C. sexmaculata*. Field collected adult beetles of *C. sexmaculata* were released on to the cotton plants in the screen cages having *A. gossypii* colonies. Eggs laid by second generation beetles were used for the study of biology and morphometry.

The study on biology of *C. sexmaculata* was conducted in the Biological Control Laboratory, Division of Entomology, ICAR-IARI at 25 ± 1 °C, $65 \pm 5\%$ RH and photo period of 14 hr. Eggs laid by adult beetles were kept in the petri plate to observe

incubation period and hatching percent. Growth and development were observed by providing aphid on cotton leaf substrate kept in a petri plate (100 mm Ø). Freshly collected cotton leaf was spread upside down in the petri plate, mixed population of second and third instar nymphs of aphid were gently transferred on to the leaf and allowed to settle. Newly hatched grub of *C. sexmaculata* was transferred on to the leaf (Dixon and Agarwala, 1999). Agarose based Hoagland's media containing essential plant nutrients was used to keep the prolonged freshness of leaf. In each petri plate 5 ml of Hoagland's media (1.5 %) was poured at one side by keeping in slant position and kept for solidifying. Cut end of petiole of cotton leaf was inserted in to Hoagland's media before transferring the aphids. Total 60 plates were maintained as replicates. Aphids were replaced every day with fresh ones where as cotton leaf for every three days. The grubs were observed for every 3 hr for moulting, pre-pupation, pupation, adult emergence and mortality. For Ist instar grub 50, IInd instar 100, IIIrd and IVth instar 150 aphids were provided as prey/ day.

The same procedure was followed for adult beetles. One day old adult beetles were transferred individually in to a petri plate containing 150 aphids. After pre-mating period, pairing was done and allowed for 24 hr, and then the pair was separated and maintained individually till their mortality. Different biological parameters viz., fecundity, incubation period, hatching per cent, duration of each stage, sex ratio, oviposition period, longevity and mortality were recorded. During each stage, observed under stereo-binocular microscope for colour pattern. To ascertain proper growth, morphometrical measurements were recorded for each stage using Nikon microscope (SMZ 800N) having camera (USB 500) with software, "VIMAGE 2016".

RESULTS AND DISCUSSION

The details of biology and morphometrics are given in Table 1. Eggs were laid mostly on cotton leaf sometimes on the surface of petri plate in clusters of 6 to 24 eggs. Freshly laid eggs were cigar shaped, yellow in colour with smooth chorion and without any reticulations. With advancement of age, eggs changed to pale yellow then turned greyish and became completely black just before hatching. Length of eggs varied from 0.88 to 1.18 mm with a mean of 1.06 ± 0.06 mm and the breadth ranged from 0.34 to 0.45 mm with a mean of 0.38 ± 0.02 mm. Incubation period showed consistency and ranged from 3 to 3.6 days with a mean

of 3.2 ± 0.1 days. In a cluster, almost all eggs hatched simultaneously without any lagging period, particularly in small clusters of 6 to 12 eggs. Hatching varied from 69.2 to 100% with a mean of 89.9 ± 4.7 .

The grubs of *C. sexmaculata* completed development through four instars with three moults then went under pupation with considerable pre-pupation period. Freshly hatched grubs were dark grey with shining dark head capsule and legs. Length and breadth of grub ranged from 1.33 to 1.64 mm and 0.38 to 0.47 mm, respectively. Mean length and breadth was 1.49 ± 0.05 and 0.42 ± 0.01 mm, respectively. Mean width of head capsule of Ist instar grub was 0.23 ± 0.01 mm. Second instar grubs were greyish black in colour with black head capsule and legs, and white coloured patches on first and fourth abdominal segments. Length and breadth of IInd instar grub ranged from 3.94 to 4.42 mm and 0.58 to 0.75 mm, respectively. Mean length and breadth was 4.13 ± 0.09 and 0.68 ± 0.03 mm, respectively. Mean width of head capsule was 0.45 ± 0.03 mm.

Third instar grub was glistening black in colour with development of white spots on mid-dorsal line of meso thorax, meta thorax and first abdominal segments. Length and breadth of IIIrd instar grub ranged from 5.26 to 6.13 mm and 0.76 to 0.88 mm, respectively. Mean length and breadth was 5.74 ± 0.15 and 0.83 ± 0.02 mm, respectively. Mean width of head capsule was 0.55 ± 0.02 mm. Fourth instar grubs were yellowish black in colour with black colour legs. Length and breadth of IVth instar grub was ranged from 6.94 to 7.63 mm and 1.28 to 1.43 mm, respectively. Mean length and breadth was 7.37 ± 0.12 and 1.36 ± 0.02 mm, respectively. Mean width of head capsule was 0.68 ± 0.02 mm. The duration of Ist, IInd, IIIrd and IVth instar grub ranged from 1.625 to 1.875, 1 to 1.375, 1.125 to 1.625 and 1.75 to 2.25 days, respectively with a mean duration of 1.729 ± 0.018 , 1.161 ± 0.022 , 1.323 ± 0.072 and 2 ± 0.027 days, respectively. The total grub period ranged from 6 to 6.375 days with mean developmental period of 6.214 ± 0.03 days. Pre-pupation period was 1.318 ± 0.05 days.

The results of present study signify the suitability of *A. gossypii* for *C. sexmaculata* as shown faster development of grub in just 6.21 days. The faster development might be attributed to the quality of prey possessing essential nutrients required for growth and development of predator. Even though many studies had reported *A. craccivora* as the most suitable prey for ladybird beetles, viz. *C. septempunctata* (Hodek,

Table 1. Biology and morphometrics of *C. sexmaculata* on *A. gossypii*

S. No.	Particulars	Period (Days)			Measurements			Number observed
		Minimum	Maximum	Mean±SE	Length (mm)±SE	Breadth (mm)±SE	Head width (mm)±SE	
1	Egg	3.0	3.6	3.2±0.1	1.06±0.06	0.38±0.02	-	60
2	First instar	1.625	1.875	1.729±0.018	1.49±0.05	0.42±0.01	0.23±0.01	60
3	Second instar	1.000	1.375	1.161±0.022	4.13±0.09	0.68±0.03	0.45±0.03	56
4	Third instar	1.125	1.625	1.323±0.022	5.74±0.15	0.83±0.02	0.55±0.02	55
5	Fourth instar	1.750	2.250	2.000±0.027	7.37±0.12	1.36±0.02	0.65±0.02	54
6	Total grub period	6.000	6.375	6.214±0.030	-	-	-	54
7	Pre-pupa	1.000	1.875	1.318±0.050	-	-	-	54
8	Pupa	3.250	3.875	3.585±0.043	3.83±0.13	2.32±0.1	-	54
9	Adult-Male	19.0	86.0	65.1±7.3	4.23±0.09	3.75±0.09	-	24
10	Adult-Female	34.0	87.0	67.0±4.7	5.35±0.25	4.06±0.15	-	26
11	Pre-oviposition	5.0	8.0	6.0±0.4	-	-	-	26
12	Oviposition	20.0	49.0	42.0±3.3	-	-	-	26
13	Post-oviposition	9.0	36.0	19.0±3.1	-	-	-	26
14	Total life cycle of male	97.25	30.12	75.56±8.76	-	-	-	24
15	Total life cycle of female	98.0	45.0	78.06±5.96	-	-	-	26
16	Survival of grub (%)	-	-	90.0	-	-	-	60
17	Survival of pupa (%)	-	-	92.6	-	-	-	54
18	Adult emergence (%)	-	-	83.33	-	-	-	60
19	Sex ratio (Male:Female)	-	-	0.92:1	-	-	-	54
20	Fecundity (No. of eggs/female)	241	1377	756.5±128.9	-	-	-	26
21	Hatching %	69.2	100.0	94.9±4.7	-	-	-	100 clusters

1960), *Propylea dissecta* Mulsant (Omkar and Mishra, 2005) and *C. sexmaculata* (Omkar and Bind, 2004), the present combination of predator-prey is not known. Suitability of prey depends on lady bird species and also varies with predator-prey combination (Omkar et al., 2009). Interestingly, Sugiura and Takada (1998) reported *A. craccivora* as the most suitable prey for *C. sexmaculata* compared to *A. gossypii* but the host species used was a pumpkin *Cucurbita moschata* Duchesne.

It was evidenced that the prey suitability and developmental period also influenced by host species on which the prey was reared as earlier studied by Rattanapun (2012) who reported significant variation in developmental period of *C. sexmaculata* on *A. gossypii* when reared on two different host species. Similarly Rakshan and Ahmed (2013) found morphological and developmental variations in *C. sexmaculata* on *A. craccivora* reared on different hosts. Faster development of *C. sexmaculata* on *A. gossypii* found in present study was strongly supported by Omkar et

al. (2009) who found fastest development of lady beetle *Anegleis cardoni* (Weise) on *A. gossypii* followed by *A. craccivora* and *Lipaphis erysimi* Kalt and reported *A. gossypii* as most suitable prey.

Freshly formed pupae were whitish yellow then turned to pale orange yellow. Pupa had symmetrically arranged black spots on each segment. Mean length and breadth of pupae was 3.83 ± 0.13 mm and 2.32 ± 0.1 mm., respectively. Pupation period ranged from 3.25 to 3.875 days with mean of 3.585 ± 0.043 days. Newly emerged adults were yellowish, which later turned to shining yellow with black spots. Later adult spotted zigzag markings on elytra. Length and breadth of male ranged from 3.98 to 4.47 and 3.56 to 4.02 mm with a mean of 4.23 ± 0.09 and 3.75 ± 0.09 mm, respectively. Whereas, in case of female, it ranged from 4.46 to 5.84 and 3.57 to 4.46 mm, with a mean of 5.35 ± 0.25 and 4.06 ± 0.15 mm, respectively. After adult emergence, three days of pre-mating period was observed whereas, pairing was noticed from fourth day onwards. Total longevity of male and female ranged from 19 to 86 and

34 to 87 days, respectively. However, mean duration of longevity was noticed as 65.1 ± 7.3 and 67 ± 4.7 days. Despite of shorter duration of grub stage, longevity of adults was found on higher side. Adult longevity was higher when compared with earlier studies as reported from 30 to 40 days (Tank and Korat, 2007; Priyadarshini et al., 2016; Sanghani et al., 2017). However, Singh et al. (2008) reported higher longevity (92.25 days) which is in accordance with present study. Male has shown longer duration compared to female as reported earlier (Tank and Korat, 2007; Priyadarshini et al., 2016). In a total life span of 67 days of female, 6 ± 0.4 days were devoted for pre-oviposition, 42 ± 3.3 days for oviposition and 19 ± 3.1 days for post oviposition.

During the mean oviposition period of 42 days, fecundity has been found to increase for first 30 days and there after decreased gradually by exhibiting a triangular fecundity function (Dixon and Agarwala, 2002). The highest per day fecundity for individual was 72 whereas, the mean fecundity was found to be 18.01 ± 0.88 eggs/day/female. The total fecundity varied between 241 and 1377 with pooled mean of 756.5 ± 128.96 eggs/female. Higher longevity and oviposition period were contributed to yield higher fecundity (756.5). High fecundity and oviposition period of *C. sexmaculata* on *A. craccivora* was also reported by Omkar et al. (2005). Present results are in closer agreement with Rajamohan and Jayaraj (1973) who reported fecundity of 717.7 for *C. sexmaculata* when reared on *A. craccivora*. Total life cycle was completed with a mean duration of 78.06 ± 5.96 days in female and was found higher than male (75.56 ± 8.76). Survival percentage of egg, larva and pupa were 94.9, 90.0 and 92.59, respectively. Morphometrical observations recorded in the present study were in line with the earlier reports (Rai et al., 2003; Tank and Korat, 2007; Rakshan and Ahmed, 2013) without much deviation indicates proper growth and development of *C. sexmaculata* on *A. gossypii*.

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Feeding potential and foraging behaviour of *Cheilomenes sexmaculata* (F.) on the cotton aphid, *Aphis gossypii* glover

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Abstract

Feeding potential and foraging behaviour are two important factors which determine the predator's efficiency of managing prey. Predator-prey system of *Cheilomenes sexmaculata* (Fabricius) and *Aphis gossypii* Glover was evaluated to find out the predation efficiency. Feeding potential was observed for different stages of *C. sexmaculatus* on *A. gossypii* whereas, functional and numerical responses were ascertained as a function of prey/host density. For the grub stage, highest feeding potential (101.43 ± 3.04 aphids) was observed for the fourth instar which was higher than the gross feeding potential of all other instars. Among the adults, female recorded high feeding potential with a mean daily consumption of 43.13 ± 0.54 aphids. Life table studies showed intrinsic rate of increase (r) of 0.22 with the mean generation time of 27.29 days. The foraging behaviour studies revealed that all the grub stages and adults displayed a type-II functional response. The IV instar grub has shown the lowest handling time with highest maximum predation (96.1aphids/day), followed by female (94.6), male (82.6) and other stages. However, female depicted a rapid extinction of aphid by keeping it below 25, as against fourth instar grub and male which affected extinction by keeping it below 30. So, the releases of fourth instar grub and female of *C. sexmaculatus* could be advocated for the augmentative biological control of *A. gossypii*.

Keywords Type-II functional response · Numerical response · Roger's random attack equation · Biological control · Age-stage-specific consumption · Predator-prey relationship

Introduction

Cotton aphid, *Aphis gossypii* Glover has been a key pest of agri-horticultural crops due its economic importance. This polyphagous aphid species is known to attack more than 300 plant species across 46 families all around the globe (Blackman and Eastop 2000) among which cotton has been the most susceptible host. Cotton aphid is the key pest of cotton and known to be a vector of leaf curl disease. The developments of pesticide resistance and associated concern about the environmental hazards caused by frequent pesticidal applications have warranted efforts on biological pest

suppression, especially of aphids by the predatory beetles (Sarmiento et al. 2007). Among different predators prevailed in the crop-ecosystem, the coccinellid beetles (Coleoptera: Coccinellidae) are considered as the most important group as they are known for their voracious feeding on aphids and other sucking pests both by grubs and adults (Dixon and Dixon 2000; Omkar 2016). The six spotted zigzag ladybird beetle, *Cheilomenes sexmaculatus* (F.) is one such aphidophagous coccinellid predator known to suppress the aphid population to a greater extent in nature (Reddy et al. 2001). It is a native species of India, remains active throughout the year with a wide geographic distribution and host range. It was also observed for its voracious feeding on aphids with high searching ability and could be handle with ease in the laboratory (Venkatesan et al. 2006). With respect to its feasibility as a biological control agent, it has shown intrinsic advantage over other two coexisting species viz., *Coccinella septempunctata* L. and *Coccinella transversalis* F. (Omkar et al. 2005; Rakshith et al. 2018).

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Even though the aphid is the most preferred prey over a variety of other soft bodied insects, the interactive biological parameters may vary with the combination of ladybird beetle and the aphid species. Among the various interactive parameters, feeding potential and the foraging behavior are two important factors to be considered as essential to determine predator's efficacy against its prey.

Feeding potential is the number of preys consumed per unit time when the availability of prey is abundant and continuous. It shows the rate and trend of prey consumption with age-stage specificity of a predator. Hence, it is considered as an important criterion for the selection of a predator as it influences the uptake rate of prey and also the reproduction level of predator. Foraging behavior elaborates the way predator reacts to the changing prey density leading to two kinds of responses namely, functional and the numerical response (Price 1975). Functional response describes the rate of predation of a predator at different prey densities whereas; the numerical response is the progressive change of predator population in relation to increasing prey density. Bio-ecology experts have propounded three types of functional response viz., Type-I, II and III, which represents increasing linear relationship, a decelerating curve and a sigmoid curve, respectively (Holling 1966; Trexler et al. 1988).

Coefficient of attack (a') and handling time (T_h) are important parameters of functional response. Coefficient of attack (a') estimates the steepness of increase in predation which controls the consumption rate at low prey densities whereas; handling time (T_h) speaks of the time require for handling each prey for the estimation of satiation threshold. Thus, the functional response (FR) indicates the rate of prey predation by an individual predator at varying densities of prey. The total prey consumption is the result of multiplicative effect of predator population which is described by the numerical response. Numerical response (NR) is the prey density dependent reproductive performance of a predator which is influenced by the rate of prey consumption and efficiency of conversion of ingested food (ECI). The total response (TR) of the predator is the product of functional response (FR) and numerical response (NR). In the predator-prey interaction, initial prey density is driving factor for predator and prey dynamics. As the prey tends to increase at finite rate of increase (λ), the predator feed the prey at the rate of total response, and the difference would be the remaining prey population for its further build-up.

Although, there are many studies on foraging behavior of *C. sexmaculata* on different insect pests, a detailed study involving feeding potential, functional response, numerical response of *C. sexmaculata* and its ultimate influences on population dynamics of *A. gossypii* was lacking. Hence, the present investigations have been carried out to address the same for predator-prey model of *C. sexmaculata* (predator) and *A. gossypii* (prey). We also attempted the impact of total

response of *C. sexmaculata* on population dynamics of *A. gossypii*.

Materials and methods

Host plant

Cotton plants (*G. hirsutum*) of variety RCH-650 were grown in the environmental chambers at 25 ± 1 °C, $60 \pm 5\%$ RH and a photophase of 14 h. Seeds were grown in the plastic pots filled with an equal quantity of fine loamy soil and coco peat. These plants were maintained throughout the study period to rear cotton aphid and ladybird beetle. Screen cages were used to avoid cross infestation.

Rearing of *A. gossypii*

Cotton aphid, *A. gossypii* was collected from the infested cotton fields of ICAR-IARI, Pusa, New Delhi. Parthenogenetic females were transferred on to the potted cotton plants grown in the laboratory. In about 30 days, aphid population was established. This population was maintained as a stock culture throughout the study period.

Rearing of *C. sexmaculata*

Adult beetles of *C. sexmaculata* were collected from different crops grown in the fields of ICAR-IARI, Pusa. The collected adult beetles were released in to screen cages having cotton plants with aphid. Eggs of the beetle were collected periodically from these plants and kept in a Petri plate for incubation. After incubation, newly hatched grubs were reared individually in the Petri plates provided with *A. gossypii* nymphs on cotton leaf substrate. Aphids were replaced daily along with cotton leaf to provide fresh food to the growing grub and maintained till the pupation. The pupae were kept separately and emerged adults were transferred on to aphid infested cotton plants grown in the screen cages. This continuous rearing of grubs in Petri plates and adults on cotton plants in screen cages was followed throughout the study period for stock culture (Omkar et al. 2005; Fand et al. 2010a; Venkanna et al. 2020).

Feeding potential and life table studies

These studies were conducted in the Biological Control Laboratory, Division of Entomology, ICAR-IARI, Pusa at controlled conditions of 25 ± 1 °C, $60 \pm 5\%$ RH and a photo period of 14 h. In the Petri plate (10 cm) about 5 ml of agarose-based Hoagland's media containing essential plant nutrients was poured at one side by keeping Petri plate in slant position for solidifying. Freshly collected cotton leaf was

spread upside down in the Petri plate by inserting cut end of petiole in to Hoagland's media to maintain prolonged freshness of leaf. Mixed population containing second and third instar of aphid was gently transferred with a camel hair brush on to the cotton leaf and allowed to settle. Newly hatched grubs of *C. sexmaculata* were collected from the stock culture and released individually on to aphids. Total of 30 plates were maintained as replicates, each containing a single predator and 160 aphids, whereas two Petri plates were kept without

predator as a control to observe natural mortality of prey. After every 24 h, number of remaining aphids was noted and replaced with fresh aphids to maintain density of 160. Grubs were observed daily for moulting, pre-pupation, pupation, adult emergence and mortality. Number of preys consumed was worked out by deduction of remaining aphid count and control mortality from the total number of aphids (Fand et al. 2010b).

Prey consumed = No.of prey provided–(No.of prey remained + No.of control mortality)

The same procedure was followed for male and female beetles. One day old adult beetles were transferred individually in to a Petri plate containing 160 aphids and daily count of remaining aphids was noted. Feeding potential throughout the life of predator was analysed through 'CONSUME-MSChart' (Chi 2012a) to display age-specific consumption (k_x), age-stage-specific consumption (C_{xj}) and the net consumption rate (C_0). Data on duration, mortality and number of eggs laid was subjected to the construction of age-stage and two-sex life tables (Chi and Liu 1985; Chi 1988) using TWOSEX-MSChart (Chi 2012b).

Functional response studies

Various densities of aphid viz., 5, 10, 20, 40, 80, 120 and 160 were provided on cotton leaf substrate in a Petri plate. Aphids were gently transferred from stock culture on to the cotton leaf substrate and allowed for three hours to settle. Then, the leaf substrates with unevenly distributed aphids were discarded to ensure proper density of aphids. Mixed population containing second and third instar of aphid was used and adults were discarded to avoid offspring production during the experiment. A single randomly selected individual of grub (I to IV instar), male and female was released in to Petri plate having particular density of prey. First instar grub was released on to the prey immediately after hatching, whereas second instar was pre-starved for 3 h, third instar for 6 h, fourth instar for 12 h and adult for 24 h to normalise their appetite. For adults, fifteen days old mated adults were used to represent an active reproductive adult. After releasing the particular stage of the predator, it was allowed to feed for 24 h. Thereafter, the predatory stage was removed and remaining aphids were counted. Ten replicates were maintained for each density of prey and one was kept without predator as a control to observe natural mortality of prey. Prey consumption was worked out by taking an account of aphids mortality in control (Omkar 2013). The polynomial logistic regression of the proportion of aphid killed in relation to the initial numbers was carried out for an

insight about the type of functional response (Trexler and Travis 1993; Juliano 2001).

$$\frac{N_e}{N_0} = \frac{\exp(P_0 + P_1 N_0 + P_2 N_0^2)}{1 + \exp(P_0 + P_1 N_0 + P_2 N_0^2)}$$

Where, N_e is the number of preys eaten, N_0 is initial prey density, and P_0 , P_1 and P_2 are the intercept, linear and quadratic coefficients (maximum likelihood estimates), respectively. A significant negative value of P_1 on polynomial logistic regression indicates the Type-II functional response. Otherwise a positive value of P_1 with a significant negative value of P_2 confirms the Type-III functional response (Juliano 2001). As the consumed prey was not replaced during the foraging period, Roger's random attack equation (Rogers-II functional response equation) of type-II functional response was used to fit the curve and estimate the functional response parameters (Royama 1971; Rogers 1972).

Roger's random attack equation:

$$N_e = N_0 \left[1 - \exp \left(a' (T_h N_e - T_t) \right) \right]$$

Where,

N_e is the number of preys eaten

N_0 is initial prey density

a' is coefficient of attack

T_h and T_t is handling time and total time, respectively.

Numerical response studies

Numerical response was determined by using the method given by Veeravel and Baskaran (1997). Fifteen days old active egg laying adult female beetles were starved for 24 h prior to the experimentation. These pre-starved female beetles were released individually on to different densities of aphid viz., 5, 10, 20, 40, 80, 120 and 160 settled on cotton leaf substrate in a Petri plate. Each Petri plate carried a particular density of prey and a single female beetle. Five replicates for each prey

density were kept and one plate without beetle as a control to record natural mortality of prey. After 24 h, the beetles were removed and the data on number of aphid predated and eggs laid by beetle was noted. Fecundity curve was drawn with number of eggs laid as a function of initial prey density then the resulted regression equation was used to work out the numerical response equation. The numerical response (NR) as a net reproductive turnout was worked out with the obtained fecundity curve equation by taking in to consideration the sex ratio and fraction of oviposition period to the total life period of female. The contribution of ingested food towards egg biomass at different prey densities was worked out using the equation given below. It is also referred to as efficiency of conversion of ingested food (ECI).

$$\text{ECI} = \frac{\text{Number of eggs laid}}{\text{Number of prey consumed}} \times 100$$

Rate of natural increase of prey, *A. gossypii*

Fecundity life table of cotton aphid, *A. gossypii* was constructed to know the natural increase. Newly formed adult females were transferred individually on to a cotton leaf kept in a Petri plate. Daily observations on mortality and fecundity were recorded for a total of 30 individual females until their death. Finite rate of increase (λ) per day was calculated from the constructed table (Southwood 1966; Jervis et al. 2005; Fand et al. 2010c).

Total response of *C. sexmaculata* and population change in *A. gossypii*

The total response of predator was taken as a product of functional response and numerical response. All the parameters viz., finite rate of increase of prey (λ), functional response (FR), numerical response (NR) and total response (TR) of predator were assessed as a function of single factor i.e., initial prey density (N_0) by keeping the duration i.e. total time (T_1) as one day. The change in aphid population was projected graphically by simulating the respective equations for the different instars of grub, male and female of *C. sexmaculata*.

Data analysis

Polynomial logistic regression was used to have insight on the type of functional response. Rogers-II equation was performed to know the parameters like a' and T_h . R software (R-Core Team 2013) was used for the statistical analysis pertaining to the foraging behavior. Logistic regression analysis was done using 'glm' function and got confirmed with the

'fair_test' of FRAIR package (Pritchard et al. 2017). The same package was used for estimation of functional response parameters and pair-wise comparison of different stages of predator for the parameters.

Results

Feeding potential and life table of *C. sexmaculata* on *A. gossypii*

The grub of *C. sexmaculata* developed faster with a mean duration of 6.66 days and displayed increasing consumption with the advancement of age and stage. Grub stages showed significant difference for predation rate with each other by displaying increase in consumption with advancement of stage and attaining maximum consumption (101.43 ± 3.04) at fourth instar grub. Adult beetles exhibited high consumption rate over grub because of long duration of adult life with significant high consumption by female compared to male. Mean daily consumption analysed for all the stages of grub and adults showed significant superiority of female with the consumption of 43.13 ± 0.54 aphids/day followed by fourth instar grub and male. The total consumption of the cohort of size 33 was 96,842.5 aphids with net predation rate of 2934.62 aphids/predator (Table 1).

When sex and stage differentiation was taken in to consideration and analysed using CONSUME-MSChart, the age-specific consumption rate (k_x) and age-specific net consumption rate (q_x) and cumulative net consumption rate (C_y) of *C. sexmaculata* on *A. gossypii* has been depicted in Fig. 1. The maximum value for k_x and q_x was obtained at the age of 33 days with net predation rate (C_o) of 2934.62aphids/predator. Under life table studies, age-specific survival rate (l_x) ranged from 0.03 to 091. The age-specific fecundity (m_x), age-specific maternity ($l_x m_x$) and age-stage-specific fecundity (f_x) of *C. sexmaculata* preyed on *A. gossypii* was maximum at 27 days age with the values of 14.63, 13.3 and 27.44, respectively (Fig. 2). Life table studies showed intrinsic rate of increase (r) of 0.22, net reproductive rate of 383.6, mean fecundity of 790.19 and the mean generation time of 27.29 days.

Functional response

Polynomial logistic regression analysis of proportion of prey consumed (N_c/N_0) to the initial prey density (N_0) is resulted in significant negative linear parameters ($P_1 < 0$) and confirmed the type-II response for all the predatory stages (Table 2). The line fitted with Roger's model exhibited a characteristic polynomial decelerating type-II curve for all predatory stages. The curves displayed the increase in predation with the increase in prey density until reaching the satiation for all the stages of

Table 1 Predation rate of *C. sexmaculata* on *A. gossypii*

Predator stage		Predation rate	
		No. of aphids consumed per individual	No. of aphids consumed per day
Grub	First instar	16.24±1.26a	9.74±0.74a
	Second instar	20.3±1.89b	18.61±1.31b
	Third instar	37.13±2.10c	26.04±2.33c
	Fourth instar	101.43±3.04d	36.66±2.02d
Pre-adult	1st to 4th instar	173.69±3.71	—
Adult	Male	2856.36±3.74a	36.62±1.01d
	Female	3216.16±4.00b	43.13±0.54e
Total lifespan	Male	3036.93±109.37	—
	Female	3389.84±70.26	—
Total consumption of the cohort (N=33)		96,842.5	—
Net predation rate (C_o)		2934.62	—
Tukey's HSD		$P<0.01$	$P<0.001$

Predation rate (Mean ± SE) analysed with least square means and separated using Tukey's HSD test. Different letters next to the results denotes significant differences

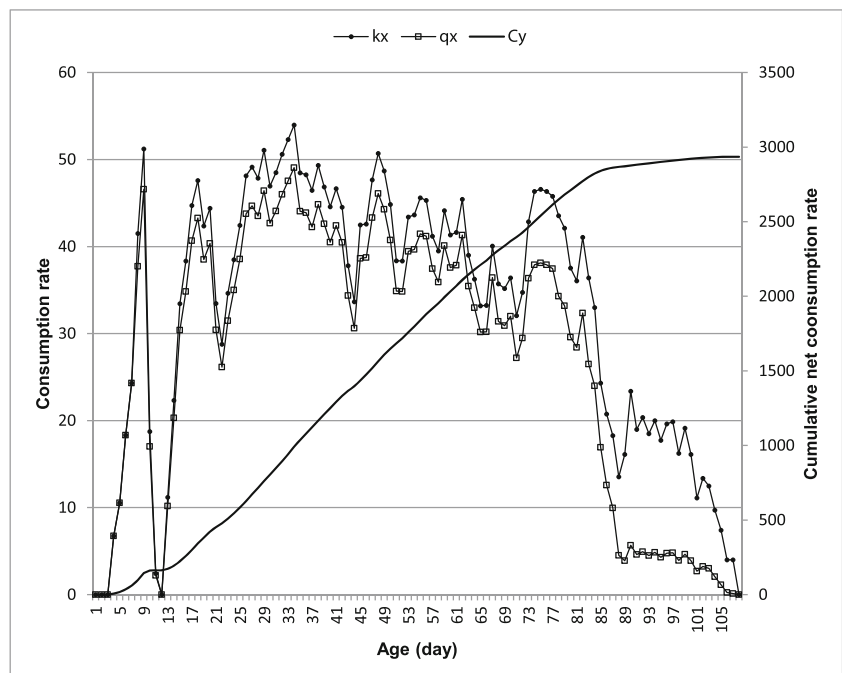
predator (Fig. 3). Polynomial logistic regression curves also confirmed the type-II response by showing declining in proportion of predation with the increasing prey density (Fig. 4). Female beetle has shown the highest coefficient of attack (a') followed by male, fourth and other instar grubs. However, the fourth instar recorded lowest handling time (0.01 day) followed by female, male and other instar grubs (Table 3). Pair-wise comparison of different stages of predator for the functional response parameters viz., coefficient of attack (a') and handling time (T_h) revealed the significant difference among each

other except for fourth instar grub which is non-significant with male for attack rate (a'), and female for handling time (T_h) (Table 4).

Numerical response

Egg laying by female has started at prey density of 20 with a mean fecundity of 8.4 ± 1.2 and increased with the prey density to reach the maximum of 21.8 ± 2.2 eggs at a prey density of 160. Fecundity has shown a strong correlation ($R^2 = 0.822$)

Fig. 1 Age-specific consumption rate (k_x), age-specific net consumption rate (q_x) and cumulative net consumption rate (C_y) of *C. sexmaculata* on *A. gossypii*



with the prey density by exhibiting logarithmic curvilinear regression line (Fig. 5). ECI was highest ($47.6 \pm 8.1\%$) at prey density of 20 which then decreased ($33.9 \pm 3.1\%$) with an increase in prey density by displaying a linear regression relationship (Fig. 6). Oviposition period (32 days), total period of female (67 days) and female to adult ratio (0.53) obtained in the present study was used to draw numerical response equation. Fecundity regression curve resulted in the following equation.

$$\text{No. of eggs laid/female} = 7.17 \log_e N_0 - 13.42$$

$$\text{Numerical response (NR)} = (7.17 \log_e N_0 - 13.42) 32/67 \times 0.53$$

Where, N_0 is the initial prey density.

Natural increase of prey, *A. gossypii*

In the absence of predator, the fecundity life table of parthenogenetic aphid population is resulted in the finite rate of increase (λ) of 2.16 per day.

Total response of predator and population change in prey

The total response of predator (TR) was attained by multiplying numerical response equation with the respective functional response equations for different stages. Increase in prey population at a finite rate of increase ($\lambda = 2.16$) was taken as a prey recruitment factor while depletion at a rate of total response (TR) of predator. The difference of total response to the natural increase of prey was considered as a net population to be resulted for the next day.

Table 2 Maximum likelihood estimates from polynomial logistic regression analysis of the proportion of prey eaten (N_e/N_0) by different stages of *C. sexmaculata* against initial number of aphids offered (N_0)

Growth stage	Parameter	Estimate	S.E.	Z value	P value
First instar	Intercept (P_0)	-1.40	0.11	-12.29	< 2e-16 ***
	Linear (P_1)	-0.01	0.00	-7.59	3.1e-14 ***
Second instar	Intercept (P_0)	-0.30	0.086	-3.51	4.5e-4 ***
	Linear (P_1)	-0.01	0.00	-11.50	< 2e-16 ***
Third instar	Intercept (P_0)	0.76	0.09	8.81	< 2e-16 ***
	Linear (P_1)	-0.01	0.00	-14.93	< 2e-16 ***
Fourth instar	Intercept (P_0)	2.08	0.11	19.44	< 2e-16 ***
	Linear (P_1)	-0.02	0.00	-17.30	< 2e-16 ***
Male	Intercept (P_0)	1.68	0.10	16.74	< 2e-16 ***
	Linear (P_1)	-0.01	0.00	-15.95	< 2e-16 ***
Female	Intercept (P_0)	2.85	0.13	22.73	< 2e-16 ***
	Linear (P_1)	-0.02	0.00	-20.18	< 2e-16 ***

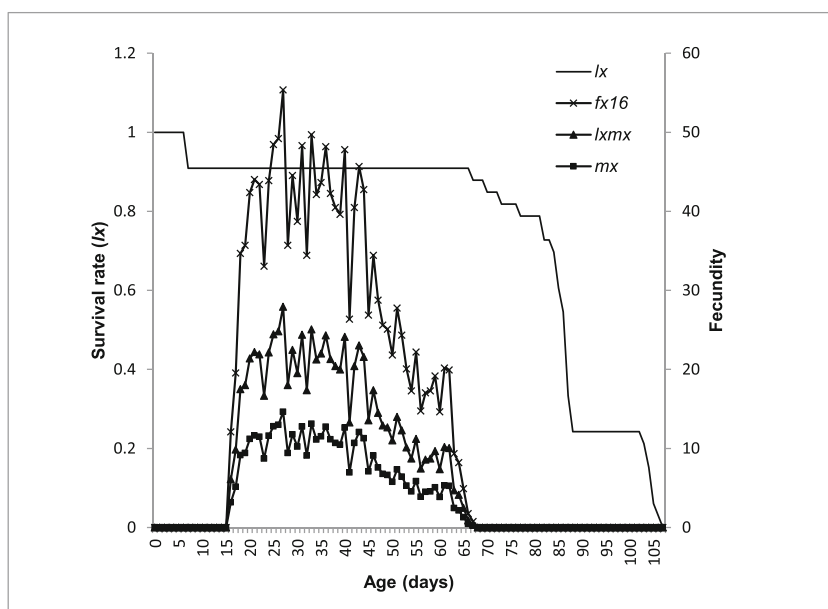
* Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$

Significant negative value of linear parameter (P_1) confirmed the type-II functional response for all the stages of *C. sexmaculata* on *A. gossypii*

Prey recruitment = $N_0\lambda$; Depletion of prey = TR; Net population = $N_0\lambda - \text{TR}$; Time = 1 day.

Population change of prey was projected by simulating respective equations. Initial prey density (N_0) was taken on x-axis and the expected net population for the next day on y-axis. The simulated curves for different stages of predator exhibited prey elimination by female, male and fourth instar grub of predator, while other instars could not. Among all the stages, female was the most effective as it showed rapid prey elimination by keeping it below 25 while male and fourth instar grub kept it below 30 (Fig. 7).

Fig. 2 Age-specific survival rate (l_x), age-specific fecundity (m_x), age-specific maternity ($l_x m_x$) and age-stage-specific fecundity (f_x) of *C. sexmaculata* on *A. gossypii*



Discussion

Predation efficiency of *C. sexmaculata* on *A. gossypii* increased with the age of the grub which might have supported faster development. Even though the consumption was comparatively low for early instars, the faster development

indicates quality and quantity of nutrition attained by the grub. Similar findings were reported for feeding potential of *C. sexmaculata* grub with respect to mean daily consumption on *A. gossypii* (Saha 1987; Sanghani et al. 2017) and *Aphis nerii* Boyer de Fonsc (Patro 2004). However, some other studies showed little deviation for total consumption which might

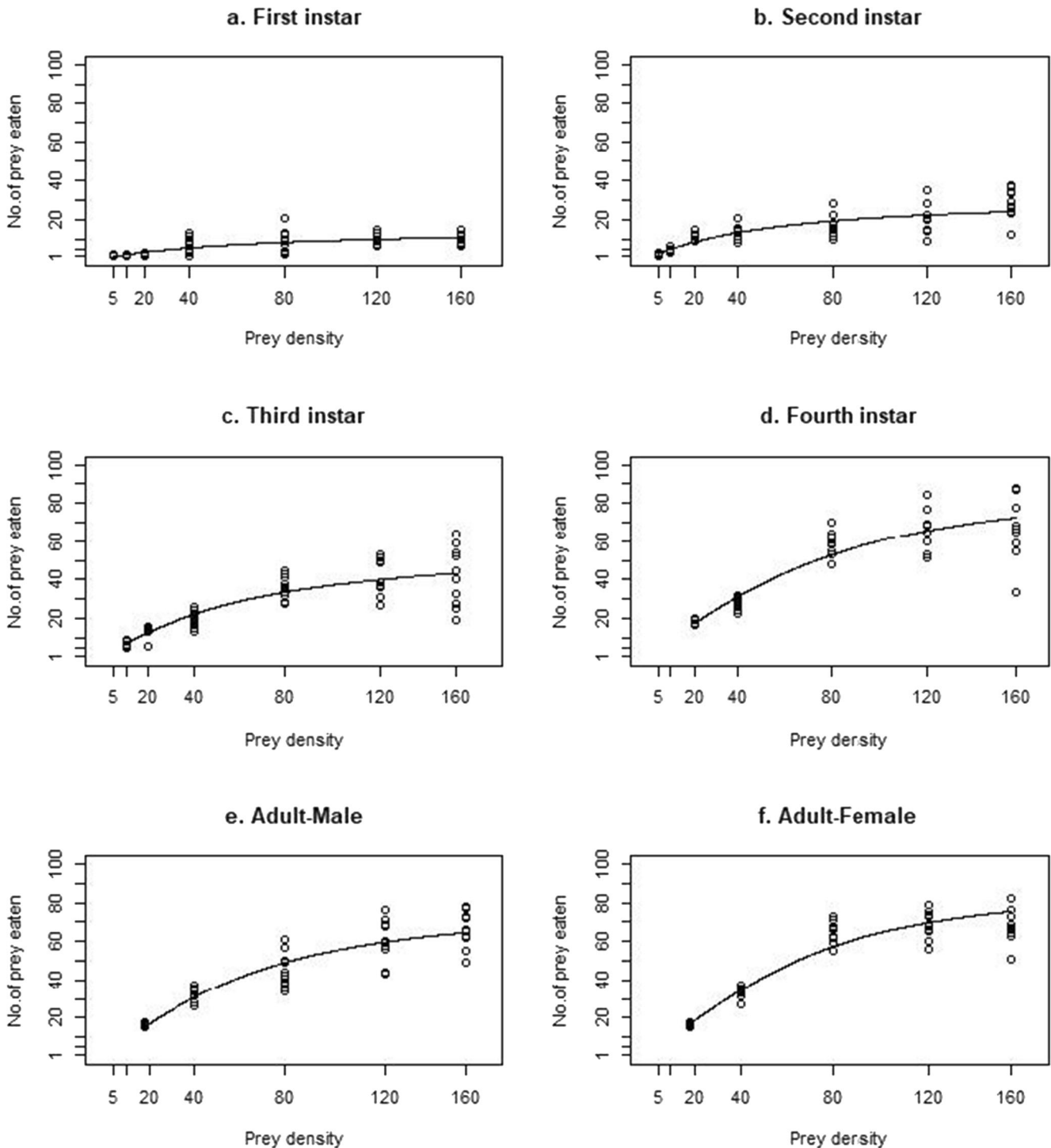


Fig. 3 Functional response of *C. sexmaculata* to different densities of *A. gossypii* when subjected to the Rogers-II model. Fitted lines exhibited decelerating curves which indicates type-II functional response for all the

four instars of grub (a, b, c, d), male (e) and female (f). Here, slope of curve indicates coefficient of attack (a') whereas, height of peak indicates lower handling time

be due to variations in the developmental period of grub (Sanghani et al. 2017; Varma et al. 1993).

Despite of shorter duration of grub, adult has shown high longevity with substantially longer oviposition period. Adult has exhibited high feeding potential throughout the life except for last few days in both the sexes. High feeding potential of adult of *C. sexmaculata* on *A. gossypii* has already been reported earlier for a mean daily consumption of 34.24 (Sanghani et al. 2017) and 35.25 (Kumar et al. 2016) aphids. However, the total consumption of adult has shown variation (Sanghani et al. 2017) which might be due to the differences in longevity. Hence, per day consumption must be more

appropriate parameter rather than total consumption to express feeding potential of adult when the developmental period differs. Range of age-specific survival rate (l_x) and irregular peaks in age-specific fecundity (m_x) was found in conformity with earlier works on coccinellids (Yu et al. 2005; Boopathi et al. 2020).

All the stages of *C. sexmaculata* displayed type-II functional response which is considered more effective than type-III. Type-II functional response of all instars of grub and adults might be a result of their innate capacity of searching and consumption capability even at low prey density. It shows the predatory efficiency of *C. sexmaculata* and indicates

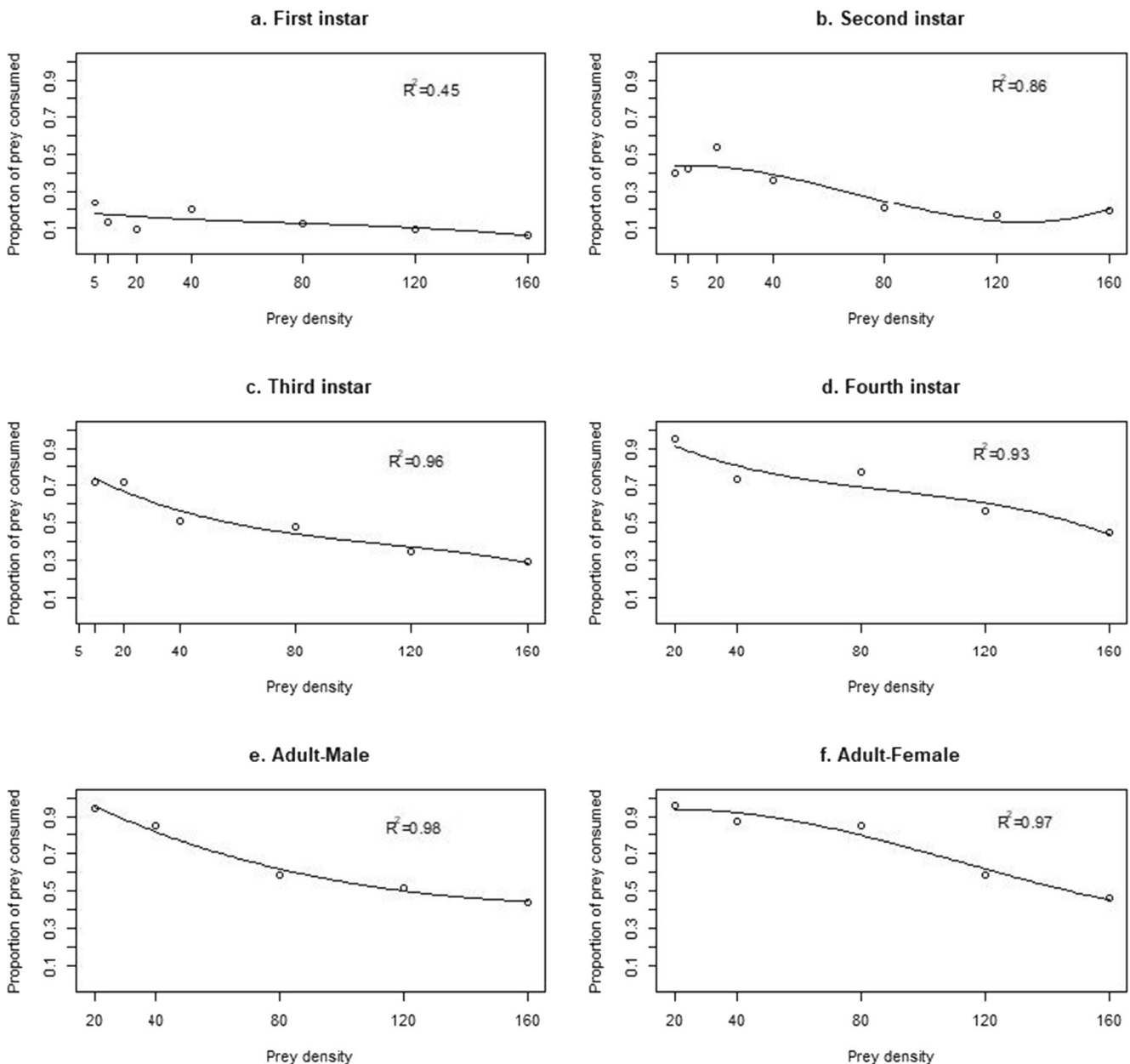


Fig. 4 Polynomial logistic regression of proportion of prey consumed to the initial prey density. Gradual reduction in proportion of prey consumption with increasing prey density confirms type-II functional response

Table 3 Estimates of coefficient of attack (a') and handling time (T_h) for different stages of *C. sexmaculata* on *A. gossypii* ($T_t = 1$ day) when subjected to Rogers-II functional response equation

Predator stage	Coefficient of attack (a')	95% CI		Handling time (T_h)	95% CI		Maximum predation per day (T_t/T_h)
		Lower	Upper		Lower	Upper	
First instar	0.23	0.18	0.32	0.06	0.05	0.08	15.6
Second instar	0.75	0.60	0.94	0.03	0.03	0.04	31.3
Third instar	1.41	1.13	1.75	0.02	0.01	0.02	57.3
Fourth instar	2.60	2.14	3.23	0.01	0.01	0.01	96.1
Male	2.54	2.08	3.14	0.01	0.01	0.01	82.6
Female	3.45	2.85	4.22	0.01	0.01	0.01	94.6

A. gossypii as a preferential prey, and the same was reflected with faster development of grub and higher longevity of adults. Earlier studies on different species have established the predominance of type-II functional response of ladybird beetles against the different aphid species (Omkar 2011; Gupta et al. 2012). Few species of *Cheilomenes* viz., *C. propingua* Mulsant, *C. lunata* F. and *C. sulphurea* Olivier predation behaviour against *A. gossypii* also showed Type-II response (Mrosso et al. 2013). Even though, type-II functional response has been observed in many ladybird beetles against various preys; the studies on functional response of immature stages is limited. In few cases, type-III response was noticed, specifically in fourth instar grub and female of *Nephus includens* Boheman against *A. gossypii* (Bayoumy

2011) and in *Harmonia axyridis* Pallas against pine aphid, *Cinara* sp. (Hu et al. 1989).

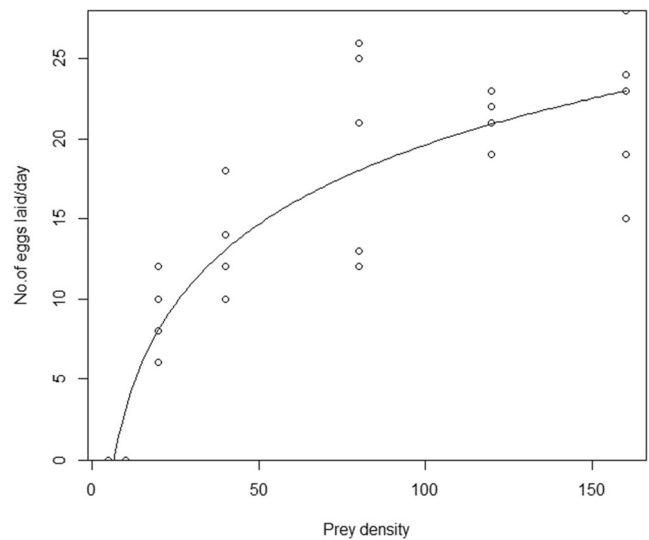
Coocinellid predator, *Stethorus gilvifrons* Mulsant has exhibited a modified type-II response for a mite, *Tetranychus urticae* Koch. A decelerating curve (type-II) at low to medium mite density and an accelerating curve (type-III) at high density are seen (Osman and Bayoumy 2011). We too observed a similar waving of polynomial regression curve in case of second instar grub as other stages have showed gradual decline (Fig. 4). Even though, it appeared to be a shift between type-II and type-III. However, mostly confirmed a type-II response as a best fit (Bayoumy 2011; Osman and Bayoumy 2011; Kumar et al. 2014).

An increase in coefficient of attack rate with the advancement of stage of predator signifies the order for their aggressiveness. Higher values for coefficient of attack rate recorded for predators revealed their superior predatory efficiency as reported earlier for adults of *C. sexmaculatus* on *A. craccivora* and *M. persicae* (Pervez and Omkar 2005),

Table 4 Pair-wise differences between functional response parameters of different stages estimated through functional response coefficient test using delta parameter

Stages of comparison	$D_{a'}$ (SE)	D_{T_h} (SE)
1st instar-2nd instar	-0.51 (0.08)***	0.032 (0.01)***
1st instar-3rd instar	-1.18 (0.11)***	0.05 (0.01)***
1st instar-4th instar	-2.37 (0.17)***	0.05 (0.01)***
1st instar-Male	-2.31 (0.19)***	0.05 (0.01)***
1st instar-Female	-3.22 (0.21)***	0.05 (0.01)***
2nd instar-3rd instar	-0.67 (0.13)***	0.01 (0.00)***
2nd instar-4th instar	-1.86 (0.18)***	0.02 (0.00)***
2nd instar-Male	-1.79 (0.20)***	0.02 (0.00)***
2nd instar-Female	-2.71 (0.22)***	0.02 (0.00)***
3rd instar-4th instar	-1.19 (0.19)***	0.01 (0.00)***
3rd instar-Male	-1.13 (0.21)***	0.01 (0.00)***
3rd instar-Female	-2.04 (0.23)***	0.01 (0.00)***
4th instar-Male	0.06 (0.24)NS	-0.00 (0.00)*
4th instar-Female	-0.85 (0.26)**	-0.00 (0.00)NS
Male-Female	-0.91 (0.28)***	0.00 (0.00)*

* Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$, NS-Non significant. $D_{a'}$ and D_{T_h} are estimates of the differences between stages for the parameters a' and T_h , respectively

**Fig. 5** Fecundity of *C. sexmaculata* in response to the initial prey density of *A. gossypii* exhibited logarithmic regression to the prey density with an equation, $y = 7.17 \log_e(X) - 13.42$, $R^2 = 0.82$, $t = 11.38$, $P < 0.001$ (5.19e-12) with 28 d.f

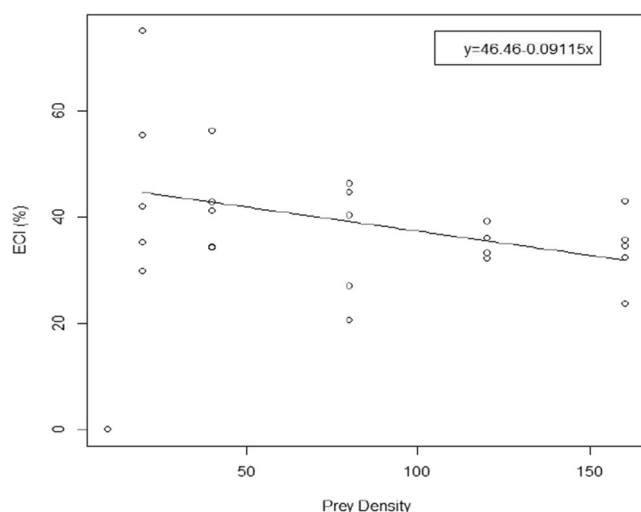


Fig. 6 Efficiency of conversion of ingested food (ECI) of *C. sexmaculata* on *A. gossypii* exhibiting a negative linear regression with prey density ($y = 46.46 - 0.09x$, $R^2 = 0.18$, $t = -2.24$, $P < 0.05$ (0.03), with 23 d.f)

for adults of *C. propingua*, *C. lunata* and *C. sulphurea* on *A. gossypii* (Mrosso et al. 2013) and same work on other aphidophagous ladybird beetles (Shah and Khan 2013).

Lower handling time for fourth instar grub and female is in line with the earlier studies conducted on three different *Cheilomenes* sp., (Mrosso et al. 2013; Pervez and Omkar

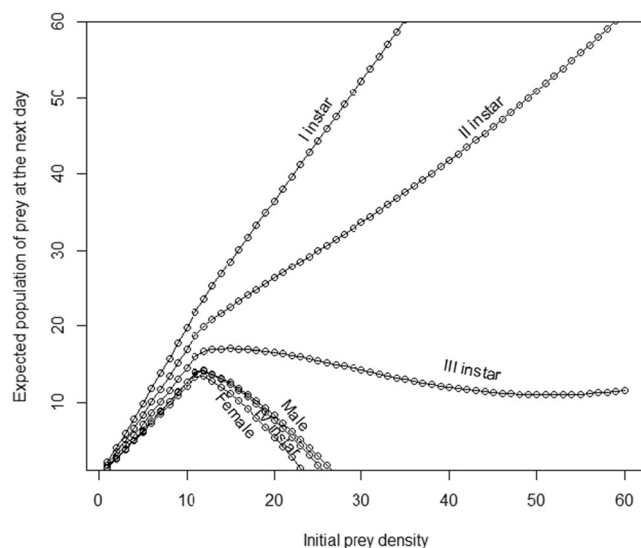


Fig. 7 Population change of *A. gossypii* according to the total response of different stages of predator, *C. sexmaculata*. A continuous vector taken on X-axis as initial prey density (N_0), simulated for expected net population to be remained for the next day as a result of difference between increase of prey at finite rate of increase ($N_0\lambda$) and removal of prey at a rate of total response ($FR \times NR$). Numerical response values ≤ 1 were considered as 1. Respective total response equations for different instars of grub, male and female were used for simulation of curves for respective stages. Curves for first and second instar showed continuous increase of aphid whereas, fourth instar grub, male and female has brought down the aphid population to zero by keeping it below 30

2005). The similar results were also reported for *Adalia tetraspilota* Hope, *Hippodamia variegata* Goeze and *Coccinella undecimpunctata* L. on *A. gossypii* (Al-Deghairi et al. 2014). In spite of having moderate attack rate, the fourth instar grub has shown highest theoretical maximum predation as a result of lower handling time which indicates its consistency for predation. The differences among various stages for the parametric values for functional response could be due to differences in predator size, its physical strength, voracity, satiation time, digestion ability, walking speed and learned behaviour for predation (Pervez and Omkar 2005; Mills 1982). Even though attack rate is a key factor, many workers have explained the efficiency of a predator using handling time (Veeravel and Baskaran 1997).

The present study tried to explain the effectiveness of a predator by taking in to an account of functional response equation as a whole which includes both the parameters i.e., attack rate and handling time. In addition to this the other parameters viz., numerical response of predator and the natural increase of prey too were addressed. By simulating the obtained equations for total response of predator and natural increase of prey, the change in aphid population in response to predation by different stages was depicted (Fig. 5). It clearly exhibited the prey elimination by female, male and fourth instar grub of predator. Among the three stages, female was the most effective as it resulted in rapid elimination of prey by keeping it below 25, while male and fourth instar grub could eliminate prey by keeping it below 30. In the field, predator-prey interaction could be influenced by other physical and biological factors such as weather, alternate prey, migration, intra-guild predation etc.; the present study is the first little step to understand predator-prey interaction between *C. sexmaculata* and *A. gossypii*.

Conclusion

Fourth instar grub, female and male of *C. sexmaculata* are found to be the effective stages against *A. gossypii* as its exhibited rapid reduction of prey with high attack rate and low handling time. Female beetles, in addition to their immediate functional response also contribute to the prolonged numerical response. So, the use of female and fourth instar grub could be advocated for the release towards the augmentative biological control of *A. gossypii*.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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IARI News



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RESEARCH

New Yellow Mutant Variety of Chrysanthemum

Irradiation with gamma rays (10, 15 and 20 Gy) of 14 cultivars viz. Star Yellow, Star White, Pusa Anmol (yellow), Pusa Kesari, Vjay Kiran, Himanshu, Pusa Sona, Sadbhavana, White Prolific, Ajay, Datymed, Mother Teresa and Liliput were done with 30 uniform rooted cutting for each treatment during August 2017.



Himanshu

A yellow coloured mutant of white coloured spray type cultivar Himanshu have been identified and developed after irradiation of 15 Gy. This identified plant attains similar attributes of their parent except having yellow colour and best suited for pot cultivation.

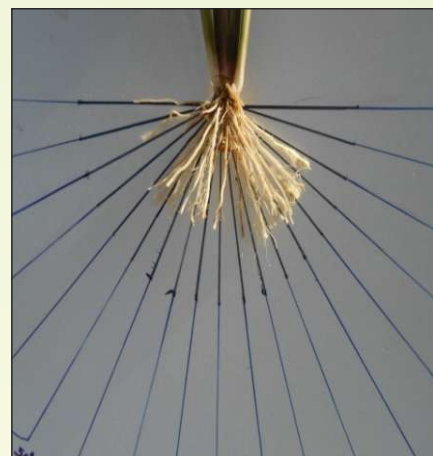
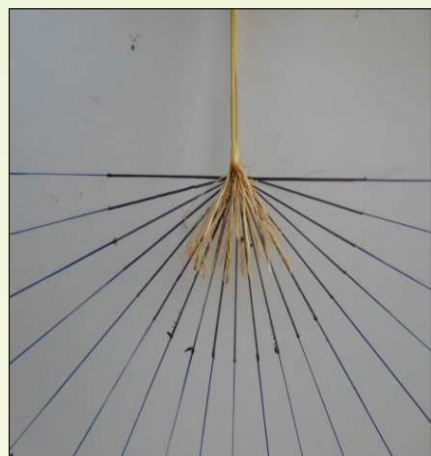
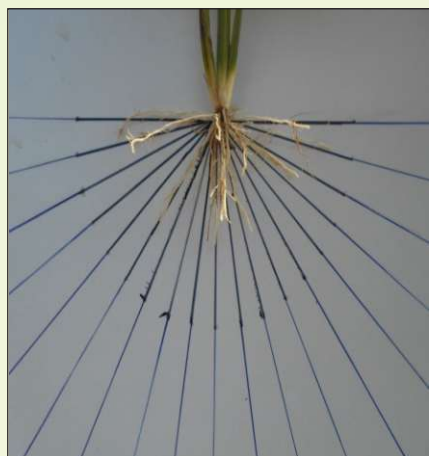
Identification of Donor for Dual-Nutrient Efficiency in Wheat

Nitrogen (N) and phosphorus (P) are the crucial inputs for



Himanshu Yellow Mutant

sustainable crop production. Seventy bread wheat genotypes were phenotyped in field for tolerance to low stresses for four years of individual nutrient treatments. Field evaluation was followed by physiological and biochemical evaluation of selected genotypes (HD 2781, Ajantha, HD 2824 and C 306) with precise N and P concentration in hydroponics for dual-nutrient stress tolerance. Traits such as root morphology, enzymes of nitrate assimilation, acid phosphatase activity in root exudate, leaf area and biomass were evaluated to identify the contrasting genotypes. Based on these responses, HD 2781 (tolerant/efficient) and C 306 (sensitive/inefficient) were selected to understand the molecular basis of dual-nutrient stress tolerance. Expression analysis of candidate genes involved in transport of N (NRT1.1, NRT1.2, NRT2.1) and P (PT2.1, PT1.2, PHT1.4, PT8) in roots, as well as genes involved in N assimilation (Nia2, GS1, AlaAT, GDH), P remobilization (PAP, SQD2, NPC4, DGDG1, MGDG1) and transcriptional



Method to measure root angle of cereals

regulators (PHO1:H2, PHO2, PHR1, IPS1.1, NLA1, SPX1) showed that HD 2781 is genetically efficient and responsive genotype, and can be used as 'donor' for developing varieties for N and P use efficiency.

An Easy, Robust and Cheaper Method to Measure Root Angle of Cereals

Cereals are the most important component of the human diet. Abiotic stresses such as drought possess serious hindrance to increase in productivity of cereals. Deep rooting is a major component trait that contributes to dehydration avoidance. Root angle is surrogate trait for deep rooting. There is wide variability among genotypes for root angle. This variability is not utilized to a great extent due to the phenotyping bottlenecks. Available methods are expensive, complicated and time-consuming. Therefore, a reliable and high throughput method was developed to screen a large number of genotypes. To measure root angle, plant shoot was cut at 5-10 cm above the ground level along with cut roots

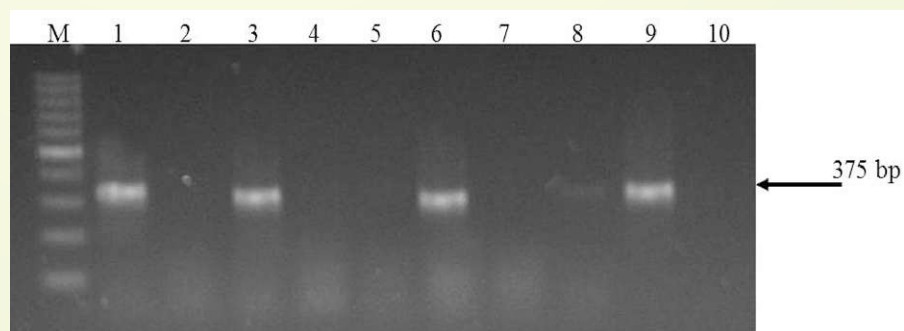
at 2-3 cm distance from the base of the stem. Entangled lateral roots were removed. The shoot along with the roots is held perpendicular and root angle was measured for one root. Same procedure was adopted for all the roots.

Viroids Infecting Grapevine Genotypes in India

A study was conducted at IARI on detection and characterization of 5 viroids in 22 commercial grapevine genotypes grown in Germplasm Block of Indian Agricultural Research Institute during May-November, 2017. Symptoms of red leaf or leaf roll including yellow speckle

characteristic of grapevine yellow speckle disease was observed in genotype Sharad Seedless.

Four viroids viz., Grapevine yellow speckle viroid-1 (GYSVd-1), Grapevine yellow speckle viroid-2 (GYSVd-2), Australian grapevine viroid (AGVd) and Hop Stunt viroid (HSVd) were detected either individually or in mixed infection in the tested samples by using specific primers through reverse-transcriptase polymerase chain reaction. GYSVd-2 was reported for the first time from India. No viroids could be detected in genotypes viz., Tempronillo,



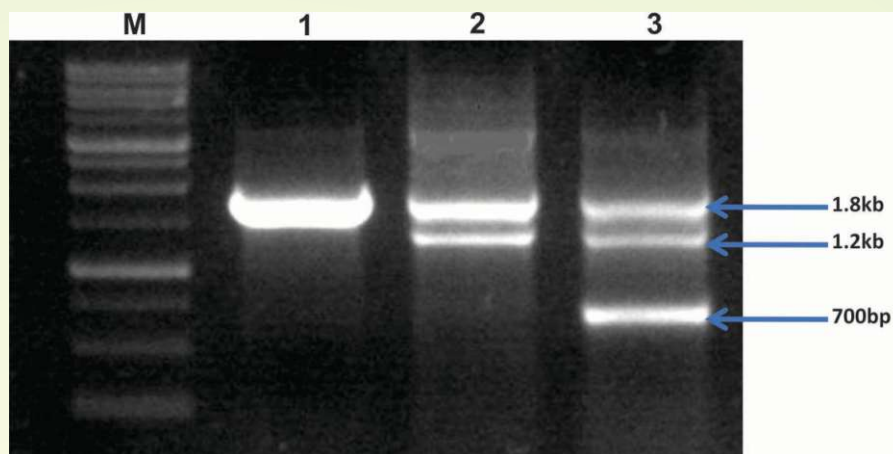
Reverse-transcriptase polymerase chain reaction for viroid detection Lanes: M:Marker 100bpDNA ladder ; 1: Thompson Seedless; 2:Chardonnay; 3: Flame Seedless; 4: Julesky Muscat, 5: Beauty Seedless; 6: Pusa Navrang; 7: Black Muscat; 8: Syrah ; 9:Black Prince; 10: NRC grape genotype

Syrah accessions, Black Muscat, Bharat early*Syrah, Bharat early and beauty seedless and were found free from these viroids. The phylogenetic analysis of all the four viroids indicated that they might have been introduced in India from other Asian, European or South American countries through the introduction of infected propagative material.

Development of a Multiplex PCR Assay for Detection and Identification of Chickpea Phytoplasma Utilizing Multilocus Gene Specific Primers

A multiplex PCR assay was developed by optimizing reaction components and cycles for the detection of phytoplasma associated with the chickpea phyllody disease. Three sets of primers corresponding to 16S rRNA universal primer pair P1/P7, nested primer pair R16F2n/R16R2 and *imp* gene specific primers (IMPIIF2/IMPIIR1) were used together.

Different concentrations of the PCR components such as primers, template DNA and PCR annealing temperature were examined and optimized for better amplification of phytoplasma DNA in a multiplex PCR assay. Expected fragments of 1.8kb (P1/P7), 1.2kb (R16F2n/R2) and 717bp (IMPIIF2/IMPIIR1) were amplified. The developed multiplex PCR assay provided a sensitive, rapid and cheap assay in identifying the phytoplasma



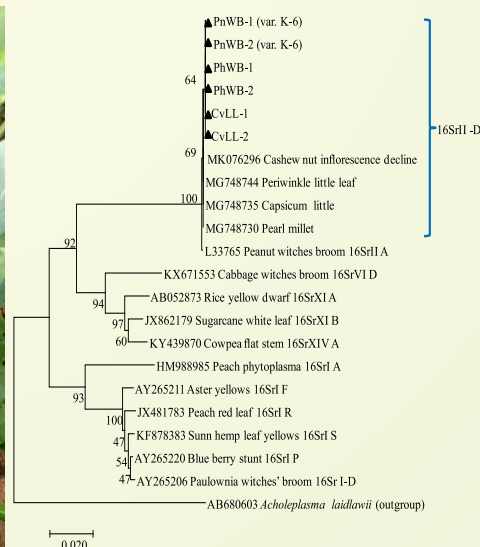
Multiplex PCR assay results for phytoplasma DNA amplification, M: marker 1 kb DNA ladder, 1: P1/P7 primer, 2: P1/P7 and R16F2n/R16R2 primers, 3: P1/P7, R16F2n/R16R2 and IMPIIF2/IMPIIR1 primers

associated with the chickpea phyllody disease. The phytoplasma strain was efficiently identified as 16SrII-D subgroup based on 16S rRNA and *imp* genes both by PCR and the new developed multiplex PCR assay approach.

Report of a New Phytoplasma Disease Associated with Little Leaf and Shoot Proliferation in Peanut

'*Candidatus* Phytoplasma aurantifolia' was identified on

peanut variety K-6, at Kadiri and Gooty regions of Andhra Pradesh causing little leaf and shoot proliferation during the monsoon season of 2018-19. The phytoplasma association was confirmed by amplifying ~1.25 kb, 840 bp, 1094 bp and 465 bp DNA products of 16S rRNA, *secA*, *tuf* and *SAP11* genes from symptomatic plants using phytoplasma specific primers of these genes. The sequences comparison of peanut phyto-



Little leaf and witches' broom disease in peanut and phylogenetic tree constructed by the neighbor-joining method of 16S rRNA gene of the peanut phytoplasma strains

plasma isolate identified it as a member of the peanut witches' broom phytoplasma group (*Candidatus* Phytoplasma aurantifolia).

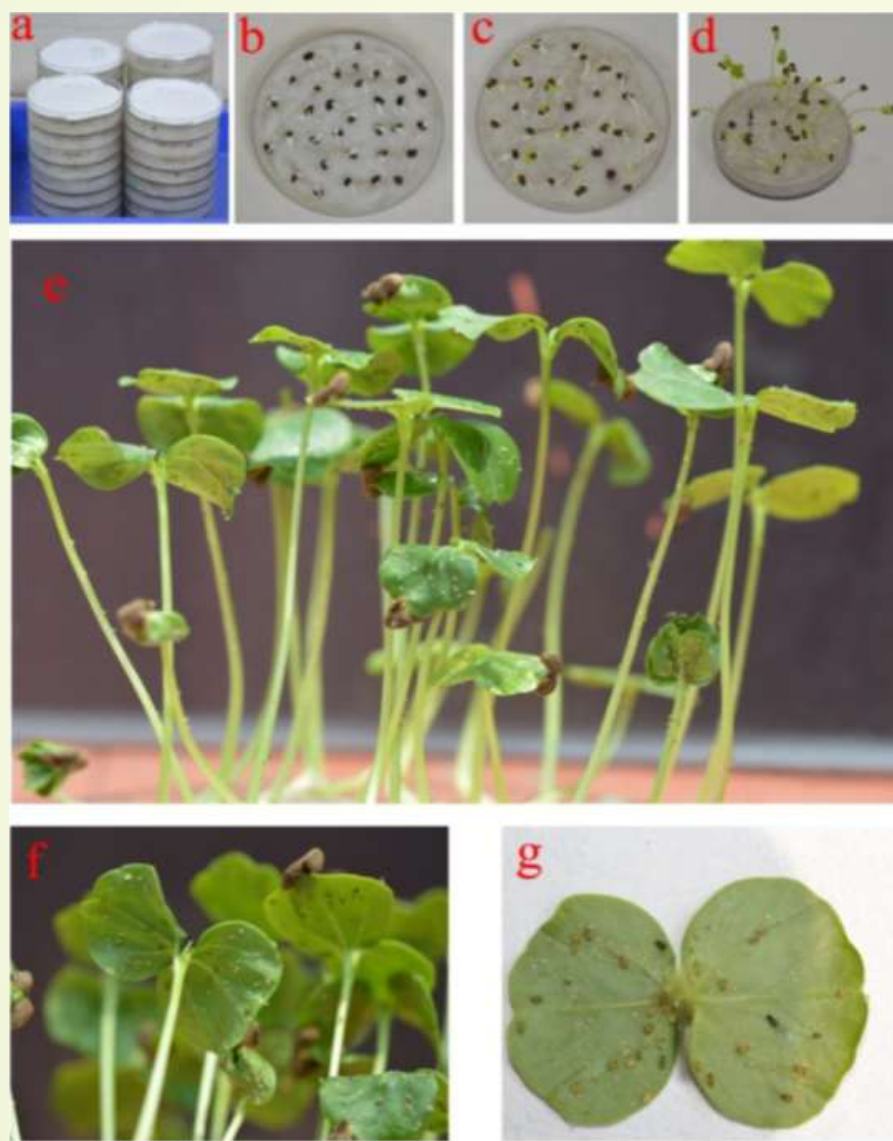
Also, on the basis of computer-simulated RFLP analysis of amplified 16S rRNA gene, assigned the peanut strain to subgroup D. This is the first report on the association of 'Ca. P.

aurantifolia' (16SrII-D subgroup phytoplasma) with peanut in India.

A Technique for Continuous Rearing of Cotton Aphid (*Aphis gossypii*)

For rearing of cotton aphid, *Aphis gossypii* potted cotton plant method has been extensively used which is a cumbersome process requires more time and space. The other methods such as

rearing on excised cotton leaves and using artificial diet are also not suitable for rearing in high number for longer periods. A simple rearing technique of *A. gossypii* on a natural host system of *Hibiscus cannabinus* L. was developed in the Division of Entomology, ICAR-IARI, New Delhi. Mesta (*H. cannabinus*) was selected for rearing of *A. gossypii* based on screening of different host plants. In this period, seedlings of *H. cannabinus* were grown on absorbent cotton in a Petri plate (10 cm ø) as substrate. This technique was found to be effective for continuous rearing throughout the year. Among different temperatures, 24°C was found most suitable to produce nymphs and wingless adults in high number. However, for the production of winged adults, rearing at 27°C was found to be best. This technique made simple and easy to practice as it avoids growing and maintenance of host plants in pots throughout the year. It is effective as it facilitate quick start of rearing and produce nymphs, wingless adults and winged adults in high numbers and feasible for continuous rearing on *H. cannabinus* seedlings for 30 days without transfer. This technique is simple and suitable for continuous rearing in lab studies and mass production of predators and parasitoids would be possible for laboratory.



Seed kept for germination: (a-d) 0, 1, 2 and 3 days after keeping for germination. (e) Aphid establishment at 18 days after release, (f) Aphid on both sides of leaf, (g) Different stages of aphid on underside of leaf.

Procedure of new rearing method of Aphid



Agriculture Education Day Celebrated at IARI

EDUCATION

Agriculture Education Day Celebrated at IARI on December 3, 2019

ICAR-IARI celebrated December 3, 2019 as "Agricultural Education Day", which is the birthday of the first Union Minister of Agriculture of India and the first President of independent India, *Bharat Ratna* Dr. Rajendra Prasad. In this programme, several events of painting, essay and debate competition on the theme "Mechanization in context of Indian Agriculture" were organized in Dr. B.P. Pal Auditorium, IARI. In after-noon session, Dr. Rashmi Aggarwal, Dean & Joint Director (Education), welcomed the speaker of the Day, Prof. R.B. Singh, Former Director IARI, Chairman ASRB, Chancellor CAU and President NAAS.

Prof. R.B. Singh delivered Agricultural Education Day Lecture on the topic "Transforming

Agricultural Education to Build New India". During his lecture, he emphasized the need of education in agriculture and its implications in the transformation of existing agriculture system. He emphasized that education will help in utilizing the information related to weather, new crop varieties, resource conserving cultivation practices as well as farm machinery and its implementation for increasing farmer's returns. He said that only agriculture can help to make India

free from hunger and poverty. He suggested that our goal should be to alleviate hunger and poverty by 2030 and for this goal we have to shift from Land grant system to World grant system. Prof. R.B. Singh felicitated the participants. Dr A.K. Singh, Director (ICAR-IARI) & DDG (Agriculture Extension) briefed the audience about the event and also introduced the speaker. At the end of the programme Dr. K.M. Manjaiah proposed formal vote of thanks.

Orientation Programme for M.Sc. students of the Afghanistan National Agricultural Science and Technology University (ANASTU)

An 'Orientation Programme' was organized on October 7, 2019 for the new batch of M.Sc. students of ANASTU, Kandahar, Afghanistan in the discipline of Horticulture, Plant Protection, and Animal Husbandry in the Virology Auditorium, Division of Plant Pathology, IARI.



Research Advisory Committee (RAC) Meeting of the Institute

Research Advisory Committee (RAC) Meeting

The Research Advisory Committee (RAC) Meeting of the Institute was held from December 12-13, 2019 to under the Chairpersonship of Dr. V. L. Chopra, former Director General, ICAR & Secretary, DARE and former Member Planning Commission. The respective School Coordinators presented the Action Taken Report & the research achievements. The RAC Members Dr. K.V. Peter, Dr. A.K. Sikka, Dr. J.P. Khurana, Dr. B.S. Parmar, Dr. J.P. Tandon, Dr. R.S. Deshpande and Dr. D.K. Yadav attended the meeting.

EXTENSION

Field Day on Pigeon Pea

Two field days on pigeon pea under CFLD were organized on October 15 & 30, 2019 in Tajnagar and Bhorakalan village of Gurugram district, respectively. In these field days, 57 & 65 farmers and farm women were participated, respectively. During this programme the SMS of KVK provided the technologies of how to control pod borer insect and post harvest technology in pigeon pea pulses crop under Cluster Front Line Demonstration Programme and given the production technology like soil sampling, seed treatment, use of balance fertilizer, irrigation scheduling, weed control and plant protection measures etc. for *rabi* crops.

Soil health & Water Sampling Campaign

Four days campaign on soil sampling was organized to aware

the farmers about the soil health in Langra, Teekli, Bhorakalan, Khanpur of Gurugram district and a total of 109 farmers were benefitted. Farmers were demonstrated how to collect the soil sample and importance of soil testing. They were also suggested to apply the fertilizer and manures based on the soil test to reduce the cost of cultivation and maximize the yield.

Mahila Kisan Diwas

Krishi Vigyan Kendra, Shikohpur, Gurugram organized a Mahila Kisan Diwas on October 15, 2019 at village Chandu. On this occasion Head, Subject Matter Specialists and staff of KVK were given their valuable suggestions on role of farm women in agriculture as they are backbone of Indian Agriculture, and they are doing so hard work in the fields, crop management and livestock production management. In this programme 37 farm women and farmers were participated.

World Food Day

World Food Day was organized in KVK, Shikohpur, Gurugram on October 16, 2019 in which 61 farmers, farm women and students were participated. They were informed about the agricultural technologies like improved seed variety, use of fertilizer and insecticides on soil test based, drip irrigation to make it more profitable. They were also exposed about various

entrepreneurial skills like Dairy, Mushroom, Bee keeping, Vermicompost and Food processing that can make them earn money without migrating to cities.

Krishi Shiksha Diwas

The Krishi Vigyan Kendra, Shikohpur, Gurugram organized Krishi Shiksha Diwas on December 03, 2019. In this programme, 68 students of class 9th & 10th standard of Govt. Senior Secondary School, Shikohpur were participated. The students visited different demonstration units like polyhouse, crop, vegetable, dairy, mushroom, vermicompost, IT and soil testing laboratory and availed knowledge about these agriculture related enterprises.

Kisan Diwas

Krishi Vigyan Kendra, Shikohpur, Gurugram organized a Kisan Diwas on the birth anniversary of the 5th Prime Minister of India, Shri Chaudhary Charan Singh, on December 23, 2019 at village Bar Gujjar, Distt. Gurugram. In this training programme 80 farmers were participated. Several subject matter specialists had given their valuable suggestions about farming like INM, IPM and protected cultivation. Both SMS and farmers shared their experiences of farming.

Participation in Exhibitions

The Institute participated in following exhibitions:

- ❖ Vibrant India 2019' held at Delhi Haat, Pitam Pura, New Delhi from 18-19 October, 2019.
- ❖ India International Trade Fare, 2019 at Pragati Maidan from 14-27 November, 2019.

CAPACITY BUILDING

Trainings

- ❖ Institute's KVK at Shikohpur has organized training programme for farmers on application of fertilizer on October 22, 2019. In this training programme 63 farmers were participated.
- ❖ The Institute's KVK at Shikohpur has organized vocational training programme during November 1-8, 2019 on Production Technology of Button Mushroom. In this training programme 14 rural youths were participated. During training the production technology of button mushroom, oyster mushroom and milky mushroom were advised.
- ❖ The Institute's KVK at Shikohpur has organized a training programme on integrated plant nutrient management on December 20, 2019. In this training programme 24 Agriculture Development Officers of Haryana agriculture department Gurugram were participated.
- ❖ CATAT organized one to

two days eight training programmes each for 25 extension staffs and farmers sponsored by Development Department of NCR, Delhi.

- ❖ CATAT organized seven days training programme during October 10-16, 2019 on Improved Agricultural Practices & Post-harvest Management for 30 farmers from Ajmer (Raj) sponsored by ATMA, Ajmer.
- ❖ CATAT organized five days training from November 17-21, 2019 on Improved Agricultural Technologies for Higher Income for farmers of North Tripura District, Tripura. Twenty five farmers participated in the training.
- ❖ Training Programme on “Value added Products of Pearl millet” was organized by the Division of Agricultural Extension on November 6, 2019 under the DST funded

project in Mukari, Baghpat district, UP. The training programme emphasis was on awareness to the rural women about the nutritional benefits of pearl millet (bajra) for healthy living conditions.

- ❖ Division of Agricultural Extension organized an ICAR-IARI and IFPRI Collaborative Training Programme on “Impact Assessment Methodologies and Techniques” under ICAR network project on New Extension Methodologies and Approaches (NEMA) during October 14-19, 2019. There were 27 participants including 13 scientists, 7 SRFs and 7 postgraduate students. The valedictory session was graced by Dr. Shahidur Rashid, Director, IFARI, South Asia; Dr. Anjani Kumar, Research Fellow, IFARI, South Asia; Dr. J.P.



Chief Guest Dr. Shahidur Rashid being honoured by Dr. J.P.Sharma, Joint Director (Extension)

Sharma, Joint Director (Extension), IARI; and Dr. R.N. Padaria, PI of the project.

- ❖ Division of Agricultural Extension organized an training programme on “An initiative to empower lives of Rural Women” under the DBT funded project during October 16-18, 2019 in Basi village, Baghpat district, U.P. In the training programme, 50 rural women representing various Self Help Groups were participated. This training programme was mainly focused on the importance of nutrition, especially for young women and infants.
- ❖ Division of Agricultural Extension organized a training programme on “Organic Farming” during December 3-7, 2019. A total of 30 farmers from the Hapur, Bulandsher, Muradnagar, Aligarh, and Kanpur districts of UP state were participated. The trainees were also exposed to practical demonstrations about the mass production facilities of bio-fertilizers and other bio-agents at IARI, New Delhi. Further, they visited IARI innovative farmers field and discussed about the importance and success of organic farming under field conditions.

- ❖ Division of Plant Pathology organized a training programme on "Plant Disease Monitoring for Timely Management Options" from December 4-24, 2019. In this training eighteen Scientists/ Assistant Professor from 13 different states were participated. Dr. Rashmi Aggarwal, Head-Plant Pathology and Dean & Joint Director (Edn), IARI was the Course Director. Dr. Parimal Sinha and Dr M S Saharan, Principal Scientist, were the coordinators for this programme.

1st National Agro-chemicals Congress

The Division of Agricultural Chemicals and The Society of Pesticide Science India organized “1st National Agro-chemicals Congress: Country's Status on Various Fronts of Agrochemicals” from November 13-16, 2019. The

congress was inaugurated by Shri Narendra Singh Tomar, Union Minister for Agriculture and Farmers Welfare. He mentioned that agro-chemicals have played a significant role in boosting productivity; but, also emphasized that the pesticide industry should take steps to clear “doubts” about pesticide usage, to small and marginal farmers who comprise 86 per cent of the farm sector. In the concluding remark, Prof. Ramesh Chand, Member Niti Ayog, outlined the importance of responsible use of agrochemicals utilizing precision technologies to reduce wastage of applied chemicals into the environment. Dr. Trilochan Mohapatra, DG, ICAR addressing the session said that agrochemicals will continue to play a major input in agriculture to meet the crop production targets and we must strive to ensure their safe and judicious use.



Release of Publications by Hon'ble Minister of Agriculture and Farmers Welfare, Shri Narendra Singh Tomar

Workshop

- ❖ CATAT organized a workshop on October 3, 2019 under the chairmanship of Dr. A.K. Singh, DDG (AE), ICAR and Director, IARI. The workshop was organized to review the performance of the crops/technologies under demonstrations during *Rabi*, 2018-19 and to decide the future course of action under National Extension programme in collaboration with ICAR Institutes/ SAUs and IARI-VOs partnership outreach programme.
- ❖ Division of Entomology organized a workshop on “Genotyping of whitefly species complex and its associated endosymbionts” on December 5-6, 2019. The inauguration of the workshop was done by Dr. A K Singh, DDG (Ag. Extension), ICAR and Director, ICAR-IARI, New Delhi. Dr. Rashmi Agarwal, Dean and Joint Director (Edn.), ICAR-IARI, Dr. Harish Kumar, Nodal Officer (Animal Sciences), DST-SERB, Dr. Subhash Chander, Professor, Division of Entomology, Dr. S. Subramanian, Course Director of the workshop were present in the inaugural function. Twenty nine participants from sixteen different universities/ institutions belonging to ten states were participated in the workshop.

MISCELLANEOUS

Externally Funded Projects Sanctioned

- ❖ “Diversity of Tortricoidea and Cossioidea (Lepidoptera) from North Eastern India” funded by MoEF&CC. Amount Rs. 39.83 lakhs for 3 Years. Principal Investigator: Dr. P.R. Shashank, Scientist, Division of Entomology.
- ❖ “Use of Modular Green Wall Technologies for the Improvement of the Air Environment of Buildings” funded by DST. Amount Rs. 10.40 lakhs for 2 Years. Principal Investigator: Dr. Ajay Kumar Tiwari, Principal Scientist, Division of F&LS.
- ❖ “Candidate Gene Association Mapping for Tolerance to Iron Toxicity and Low Phosphorus Stress in Rice” funded by DBT. Amount Rs. 27.21 lakhs for 3 Years. Principal Investigator: Dr. Renu Pandey, Principal Scientist, Division of Plant Physiology.
- ❖ “Development of Prolific Baby Corn Hybrids through Marker-assisted Introgression of Teosinte Branched-1 (tb1) Allele from Teosinte Accession (*Zea mays* ssp. *parviglumis*)” funded by DBT. Amount Rs. 61.34 lakhs for 2 Years. Principal Investigator: Dr. Firoz Hossain, Sr. Scientist, Division of Genetics.

- ❖ “Analyses of Genetic Variability and Validation of Candidate Genes for Folate (vitamin B9) Accumulation in Maize Kernel” funded by DST-SERB. Amount Rs. 36.53 lakhs for 3 Years. Principal Investigator: Dr. Rajkumar Uttamrao Zunjare, Scientist, Division of Genetics.
- ❖ “Genome Editing for Imparting PRSV Resistance” funded by NASF. Amount Rs 74.02 lakhs for 3 Years. Principal Investigator: Dr. Anirban Roy, Pr. Sci., Plant Pathology.
- ❖ “Development of Sustainable Management Tools for the Invasive Pest, Fall Armyworm *Spodoptera frugiperda* (J.E. Smith) in Maize” funded by NASF. Amount Rs 29.194 lakhs for 3 Years. Principal Investigator: Dr. Vinay Kalia, Pr. Sci., Entomology.
- ❖ “Molecular Breeding to Develop Resistance against Anthracnose (fruit rot) in Chilli” funded by SERB-DST for 3 Years. Amount 21.50 lakhs. Principal Investigator: Dr Lakshman Prasad and Co-PI- Dr V. Shamungam, Division of Plant Pathology.
- ❖ “Designing and IARI Technology based Eco-friendly Wastewater Treatment Facility for RK Overseas site in Ghilot

Industrial Area, Rajasthan” funded by RK Overseas. Amount Rs. 5.9 lakhs for 12 months. Principal Investigator: Dr. Ravinder Kaur, Principal Scientist, WTC.

- ❖ “Designing an IARI Technology based Eco-friendly STP for Construction of Polishing Unit for the Work of Creation of New Water Body at Sector 25, Rohini STP under EE(NW)-II of Delhi Jal Board” funded by M/s Tirupati Cement Products. Amount Rs. 9.98 lakhs for 15 months. Principal Investigator: Dr. Ravinder Kaur, Principal Scientist, WTC.

Patent Granted

The 3' polymorphic primers for species-specific

detection of begomovirus (Patent No. 201911051754)

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Technologies Commercialized

Four IARI Technologies i.e. Wheat variety HD 3226, HD 3086 and Maize Hybrid- Pusa Jawahar Hybrid Maize-1 (PJHM-1) and STFR Meter Technology were licensed to Eighty Four (84) industry partners, generated a revenue of Rs. 41.75 lakh in this quarter.

Incubation Programs/Events Organized

- **SAMARTH - Review & Planning Workshop**

Z T M & B P D Unit organized a three days workshop, Samarth Phase-4 from November 18-20, 2019

at NASC Complex, New Delhi to review the progress of all RABIs & KPs under RKVY RAFTAAR Project in the last one year and their role in promoting regional agri-preneurship through Startup Incubation. SAMARTH was attended by the agribusiness Incubation Professionals representing 20 agri-incubators pan India.

- **Maitri Indo-Brazil Agri-tech Cross Border Incubation Program**

Pusa Krishi Incubator (IARI) launched a first of its kind Maitri: Indo-Brazil agri-tech cross-border incubation programme on December 09, 2019 at NASC Complex, New Delhi in collaboration with Embassy of Brazil, New Delhi and ICAR-Indian



Maitri Indo-Brazil Agri-tech Cross Border Incubation Program

Institute of Millets Research (IIMR), Hyderabad with support from Department of Science and Technology, GoI. The objective of Maitri was to provide agritech startups access to the global market through mutual benefits and shared learning. The Chief Guest, H.E. Shri Andre Aranha Correa do Lago, Ambassador of Brazil to India and Dr. Trilochan Mohapatra, Secretary (DARE) & Director General (ICAR) regarded the venture as an auspicious occasion highlighted the importance of such programmes for strengthening the partnership with the agriculturally-rich countries like India and promoting the best agricultural practices internationally. Maitri Indo-Brazil Agri-tech Cross Border Incubation Program

- **Agri-preneurship Development Program (ADP)**

Division of Food Science & Postharvest Technology (FS&PHT) and Zonal Technology Management & Business Planning and Development (ZTM & BPD) Unit organized an Agripreneur Development Programme on “A hands-on-training on Valorization of Horticultural and Arable Crops” from December 2-7, 2019.

In this programme sixteen participants from SHGs,

academia, farmers and MSME from PAN India participated to become a successful agripreneurs in near future.

Corporate Membership

In this quarter, a total of 191 members were registered, generating revenue of Rs. 7.41 lakhs.

MOU Signing Ceremony of Partnership Agreement for Ph.D. Programme

Under the approved MoU executed between ICAR and Western Sydney University (WSU), Australia, and as a part of agreed work plan on human resource development and student exchange, a signing ceremony of partnership agreement for dual award and

higher degree research candidature for the student exchange between Western Sydney University (WSU), Australia and IARI, for the academic session 2019-2020 held on November 21, 2019 at 11:30 AM at the ITC Maurya Hotel, New Delhi in the presence of Mr. Dan Tehan, Honourable Minister for Education, Australia. From IARI, New Delhi, Dr. A.K. Singh, Director, and from WSU, Australia, Dr. Barney Glover AO, Vice Chancellor and President, WSU, Australia signed the agreement. Dr. Rashmi Aggarwal, Dean & Joint Director (Education); Dr. K.M. Manjaiah, Associate Dean, short-listed students and their Supervisors from IARI also participated in this signing ceremony.



Signing ceremony of partnership agreement for Ph.D. programme

Visitors from Abroad

During the period October-December, 2019 two delegations from Chile & BIMSTEC visited the Institute. The delegation from Chile was led by Mr Eugenio Aguiló – Agricultural Attaché of Chile in India and BIMSTEC delegation was led by Han Thein Kyaw Director, BIMSTEC Secretariat.



Chile delegation with IARI Team

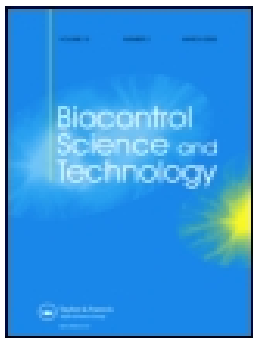


BIMSTEC delegation with IARI Team

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Non-consumptive effects of the zigzag ladybird beetle, *Cheilomenes sexmaculata* (Fab.) on its prey, the cotton aphid, *Aphis gossypii* Glover

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RESEARCH ARTICLE



Non-consumptive effects of the zigzag ladybird beetle, *Cheilomenes sexmaculata* (Fab.) on its prey, the cotton aphid, *Aphis gossypii* Glover

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ABSTRACT

Non-consumptive effects (NCEs) of predators include non-lethal changes in prey altering their behaviour and physiology with increased oxidative stress under predation-risk. Although prey species may exhibit a complex of antioxidant mechanisms, these may be inadequate to result in a reduction of adverse biological consequences in the prey. Reduced prey fitness may result and could contribute to enhancing biological control. Cotton aphid, *Aphis gossypii* is an important polyphagous pest preyed by a variety of coccinellids and very much preferred by zigzag ladybird beetle, *Cheilomenes sexmaculata* (F.). Possible NCEs of *C. sexmaculata* on cotton aphid were studied to understand the underlying anti-oxidative mechanisms. We found that the non-consumptive effect is induced by olfactory cues, and even short-duration exposure could raise antioxidant enzyme levels in the cotton aphid. Aphids antioxidant mechanism uses upregulation of superoxide dismutase (SOD) and catalase (CAT) but not sufficiently to offset the oxidative damage that led to increased Malondialdehyde (MDA) content. Affected aphid biology with reduced reproductive performance as recorded 30.0 ± 4.40 nymphs/female under predation-risk compared to no-predation (41.6 ± 5.60). Further, the induction of winged morphs increased with active predation ($21.53 \pm 1.05\%$) and increased risk of predation ($18.28 \pm 4.86\%$) compared to aphids not exposed to predators ($6.00 \pm 2.99\%$). Antioxidant production costs were compensated by the exposed aphids directly through reduced reproduction and also triggered winged morphs in progeny. Non-consumptive effect might directly contribute to declines in aphid populations through reduced reproduction and may also stimulate dispersal of winged morphs.

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Antioxidant enzymes;
Malondialdehyde; oxidative stress; predation-risk effect; superoxide dismutase; winged aphids

Introduction

Aphis gossypii Glover (Aphididae: Homoptera) is one of the most important sucking insects damaging agriculture and horticultural crops. It is a highly polyphagous pest infesting more than 320 plant species of 46 families, and a major pest of cucurbits and

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cotton. Cotton aphid damages the crops by direct feeding on plant sap, and excretes honey dew that attracts black sooty mould which affects photosynthesis and crop yield. It is also responsible for transmitting about 50 plant viruses in different crops (Blackman & Eastop, 2000). Even though there are many insecticides available for the control of *A. gossypii*, insecticide resistance development and increasing concerns about the environmental hazards created by insecticide use call for more research on eco-friendly options like bio-intensive pest management.

Numerous attempts have been made in the past to suppress aphids by introducing coccinellid beetles across the world (Joshi et al., 2003). The six spotted zigzag ladybird beetle, *Cheilomenes sexmaculata* (F.) is one of the most important native species of India known for its predation on aphids (Reddy et al., 2001). The larvae and adults of *C. sexmaculata* feed voraciously on aphids with high attack rate. It can be reared and handled easily in the laboratory (Venkatesan et al., 2006). Both the adult and larval stages of *C. sexmaculata* have shown high feeding potential on *A. gossypii* (Kumar et al., 2016; Sanghani et al., 2017). In order to have a robust and sustainable bio-control strategy, we must strive to decipher predator-prey relationships at each level, so that the effect of biological control could be optimised. An understanding of a concept like non-consumptive effect/predation-risk effect of predators on prey should be unravel to further develop new tools for pest suppression.

Non-consumptive effects (NCEs) are the changes in prey behaviour and physiology when they are exposed to the predator, but not killed. These predator-mediated changes which do not involve direct consumption are also referred as non-lethal effects, predation-risk effects or trait-mediated interactions. NCEs can help prey to avoid predation, but sometimes pose threats due to increased stress and vigilance which leads to reduced feeding. NCEs could be as strong as consumptive effects in terms of reducing target populations (Preisser et al., 2005). Although overall understanding of NCEs is increasing in general, the knowledge on non-consumptive predation responses in insect pests is very limited and warrants elucidation.

On sensing the presence of predator, the perceived threat produces signals of fear and triggers certain adaptive changes in the prey. Examples of prominent behavioural changes observed in aphids under predation-risk are dropping from the plant to avoid exposure (*Acyrtosiphon pisum* Harris and bird cherry-oat aphid, *Rhopalosiphum padi* L.), release of alarm pheromones, shaking the body vigorously while kicking at the parasitoid with the hind legs (*Aphis nerii* Fonscolombe and *Uroleucon hypochoeridis* Hille Ris Lambers), walking away from the threatened feeding site (*A. pisum*) and clustering together to reduce predation-risk by a satiation effect (*Aphis varians* Patch) (Chau & Mackauer, 1997; Dill et al., 1990; Hartbauer, 2010; Losey & Denno, 1998; Ninkovic et al., 2013; Turchin & Kareiva, 1989; Weisser et al., 1999). NCEs and their associated fitness-costs have the potential to suppress prey population growth and reproduction (Nelson, 2007; Nelson & Rosenheim, 2006; Nelson et al., 2004).

NCEs could also affect irreversible life-history shifts over a long timescale such as changes in timing of reproduction or metamorphosis, quality and quantity of offspring produced and production of winged morphs (Mappes et al., 1997; Relyea, 2007). To achieve a high consumptive effect on a target population, large numbers of

predators are required; however, if NCEs have adequate population impacts, relatively small numbers of predators may be adequate to keep pest numbers below economic injury levels (Carpenter et al., 1987). Understanding the role of NCEs is still in its early stages and requires considerably more work to integrate its impacts further into the biological control arena.

Although, some recent research findings have convincingly demonstrated that the impacts of NCEs alone can reduce crop damage caused by pests (Griffin & Thaler, 2006; Hermann & Thaler, 2018), recommendations to reliably include NCE effects are still under development. Some of earlier work showed that the NCEs can cause oxidative damage in prey and induce negative fitness consequences (Guerra et al., 2013; Janssens & Stoks, 2013; Monaghan et al., 2009). Thus, the enzymes involved in oxidative damage could be used as biological markers for assessing the NCEs on prey. Under oxidative damage, the scavenging mechanism of reactive oxygen species (ROS) involves the antioxidant defence system, where the enzyme superoxide dismutase (SOD) and catalase (CAT) play an important role (Amstad & Cerutti, 1990). Oxidative damage has negative impacts on prey performance because it damages lipids and in turn leads to damaged proteins and further reduced muscle strength and fitness of prey (Halliwell & Gutteridge, 2015). Initially, when prey detects the predator, there is an increase in metabolism with increased vigilance and increased activity as a defence reaction, for which the prey item relocates resources (Hawlena et al., 2012). Over time, this leads to the production of increased ROS and forced activation of the cellular defence system involving antioxidant enzymes which is energetically costly. Hence, the prey allocates less energy to other physiological activities. With this background we studied predator-prey system of *C. sexmaculata* and *A. gossypii* to investigate NCEs on *A. gossypii* and to understand the underlying antioxidant mechanisms.

Materials and methods

Host plant

Seedlings of mesta, *Hibiscus cannabinus* L. (Malvaceae) were used as host plant for rearing of aphids. Seedlings were grown in a Petri dish (10 cm Ø) @ 25 seedlings per plate using absorbent cotton as substrate. Growth conditions of temperature, $25 \pm 1^\circ\text{C}$, RH of $60 \pm 5\%$ and photo-phase of 16 h were maintained by keeping in a controlled environmental chamber.

Rearing of prey, *A. gossypii*

Cotton aphids, *A. gossypii* were collected from the cotton fields of the ICAR-Indian Agricultural Research Institute (IARI), New Delhi. Active parthenogenic adults were transferred on to the mesta seedlings grown in the Petri dishes. A continuous population was maintained on the host seedlings by transferring aphids onto new seedlings after every 20 days. Due care was taken to avoid crowding by observing winged morphs as a crowd indicator. From this stock culture, aphids were used for the experiment and also for the rearing of predators.

Rearing of predator C. sexmaculata

Adult beetles of *C. sexmaculata* were collected from cotton crop grown in the fields of ICAR-IARI, New Delhi. Collected adult beetles were released on to the mesta seedlings having sufficient aphid populations, maintained continuously in the screen cages to avoid escape of beetles and observed for egg laying (Figure 1). Eggs of the beetle were collected along with the leaf and kept separately for hatching. After hatching, larvae were reared in a closed Petri dish by providing aphids on host leaf. After pupation, pupae were separated, and emerged adults were transferred on to host seedlings with aphids. Both the adults and larvae were maintained simultaneously to obtain adequate numbers of adults continuously, which were used for the experiments.

Predation-risk exposure

A container was designed with a double cylinder system using Petri dishes and iron mesh. Petri dishes served as top and bottom of a vertical cylinder with iron mesh providing a curved surface fixed between the two Petri dishes. The inner small cylinder was made with Petri dishes of size 10 cm diameter and kept in a bigger outer cylinder of



Figure 1. Screen cages used to rear aphid and ladybird beetles.

size 18 cm diameter. The inner cylinder held 15-day-old mesta seedlings (25) of 8–10 cm height with two leaves on which aphids were hosted. Mixed populations of aphids comprising different instars of nymphs and wingless adults were exposed to predation-risk by releasing 25-day-old adults of *C. sexmaculata* into the cages. For active predation, beetles were released on to the host seedlings in the inner cylinder of container and allowed to predate on aphids for direct consumption. However, for exposure to perceived risk of predation (non-consumptive), beetles were released into the inter-cylinder space (between the big and small cylinder) where beetles could not consume the aphid as they were separated by the iron mesh which allowed olfactory cues to pass through to the aphids. In yet another set of cylinders, aphids without predators were used as controls (no-predation or predator exposure).

Influence of predation-risk exposure duration on oxidative stress level and its sustainability in cotton aphid, A. gossypii

To assess the responsive dynamics of oxidative stress under varying durations of predation-risk exposure, a mixed population of nymphs and adults of *A. gossypii* was exposed to predation-risk (Non-consumptive) by releasing one hundred 25 days old adult beetles per container into the inter-cylinder space and exposing them for 1, 6, 12 and 24 h. From the exposed populations, a mix of nymphs and adult aphids were collected at 0, 1, 6, 12 and 24 h after removal of predators and analysed for SOD enzyme activity by maintaining three replications. Oxidative stress level was assessed by comparing SOD levels of exposed aphids with that of control (without predator release) at varying time periods. Temperature of $25 \pm 1^\circ\text{C}$ and RH of $60 \pm 5\%$ were maintained as experimental conditions.

Treatments to assess NCEs of C. sexmaculata on A. gossypii

Based on the expression of SOD levels in the above experiment, the treatment duration (predation exposure duration) was fixed as 24 h. A mixed population of nymphs and adults of *A. gossypii* grown on mesta seedlings was used for the treatment. Oxidative stress levels and biological parameters were assessed and compared among the following treatments.

- (1) Active predation (Consumptive + Non-consumptive): Predators were released on to host seedlings in the inner cylinder and allowed to predate on aphids by direct consumption.
- (2) Risk of predation (Non-consumptive): Predators were released into inter-cylinder space and not allowed to consume aphids but, could pass the olfactory cues through the mesh used to separate aphids and host seedlings from the released beetles.
- (3) No predation (Control): Predators were not released.

Immediately after 24 h of treatment, 100 mg of aphids were collected per treatment and stored at -20°C for enzyme analysis. The aphids collected from each treatment were cold homogenised in 1 mL of 50 mM phosphate buffer (pH 7.0) and centrifuged

at 3000 rpm for 15 min at 4°C. The supernatant was analysed for the activity of SOD, CAT and Malondialdehyde (MDA) to assess the oxidative stress levels in the aphids.

Superoxide dismutase activity

SOD was quantified on the basis of its ability to inhibit the photochemical reduction of Nitro Blue Tetrazolium (NBT) following the method given by Beauchamp and Fridovich (1971) modified by Lukasik et al. (2012). A reaction mixture of 3 mL was prepared by adding 0.2 mL each of methionine (200 mM), NBT (1.125 mM), EDTA (1.5 mM) and riboflavin (75 µM) to the 25 µL of enzyme extract added with 50 mM phosphate buffer (pH 7.8) for the remaining volume. After adding riboflavin, the content was incubated under a 15 W fluorescent lamp for 10 min, then taken into the dark and covered with black cloth to halt the reaction. Spectrophotometer readings for absorbance were taken at 560 nm. Standards with the same reaction mixture without enzyme extract and a blank with double distilled water were taken for the calibration. One unit of SOD activity was defined as the amount of enzyme required to inhibit 50% of NBT reduction under assay conditions. The values were given as SOD units/mg protein.

Catalase (CAT) activity

A reaction mixture was prepared with 1000 µL of 0.01 M phosphate buffer (pH 7.8), 475 µL of double distilled water, 25 µL of enzyme extract and 500 µL of 0.2 M hydrogen peroxide. Exactly after 1 min, 2000 µL of dichromate acetic acid (DAA) was added and heated the tubes for 15 min in a boiling water bath. After cooling, absorbance was measured at 570 nm (Aebi, 1984; Sinha, 1972). A standard curve was prepared with the range of 500–850 µL of H₂O₂ without enzyme extract. Activity of CAT was given as µM of H₂O₂ decomposed per minute per mg protein.

Malondialdehyde content

With the standard Malondialdehyde (MDA) solution (1 µM), a series of solutions of concentration ranging from 0.01 nM to 1000 nM was prepared by dissolving in Glacial Acetic Acid (GAA). Thio Barbituric Acid (TBA) of 4 mM was prepared with GAA using as a solvent. One mL of each MDA and TBA was taken in 10 mL test tube and heated in a water bath at 95°C for 60 min. After cooling, absorbance was read at 532 nm. Standard curve was prepared with a serial concentration. For samples, 50 µL of extract was added first to the GAA (950 µL) followed by 1 mL of TBA. Sample values were estimated from the standard curve. Units are expressed as nM/mg protein (Ohkawa et al., 1979; Halliwell & Gutteridge, 1990). The protein content in each sample was determined using Bradford (1976) method and used for calibration of enzyme activity.

Effect of treatments on aphid biology

After 24-hour predation exposure treatments (active predation, risk of predation and no predation), newly developed parthenogenic adult aphids were transferred onto

fresh seedlings of host plants (one aphid per seedling). Transferred adults were allowed 24 h to produce nymphs, after which they were transferred to other fresh seedling and maintained until their death. Adults longevity and reproductive performance were recorded. Nymphs survival, developmental period and type of adult morph into which it developed (winged or wingless adult) were recorded. Mesta seedlings (15 days old) raised on non-absorbent cotton as a substrate in Petri dishes were used as host plant. Experimental conditions were maintained at $25 \pm 1^\circ\text{C}$, $60 \pm 5\%$ RH and photo-phase of 16 h.

Data analysis

Each treatment in the experiments was replicated thrice. In each replication, a mix of nymphs and adult aphids hosted on 25 mesta seedlings grown in a Petri dish were used for predation-risk exposure treatments *viz.*, active predation, risk of predation and no predation. From each replication, 3 samples, each 100 mg of aphids, consisting of a mix of nymphs and adult aphids were drawn for the enzyme analysis. Simultaneously, 75 parthenogenetic adult aphids from each treatment were used for analysing biological parameters considering each individual aphid as a replication. Data were subjected to analysis of variance (ANOVA) using the aov function and multiple comparisons were done using HSD.test function through R software (R Core Team, 2020). Test significance is expressed with F and P-values with the corresponding level of significance.

Results

Influence of predation-risk exposure duration on oxidative stress level and its sustainability in cotton aphid, A. gossypii

Two-way ANOVA of SOD activity in *A. gossypii* at varying post exposure periods under different treatments of predation-risk exposure duration resulted in a significant variance for both treatment duration as well as post exposure period.

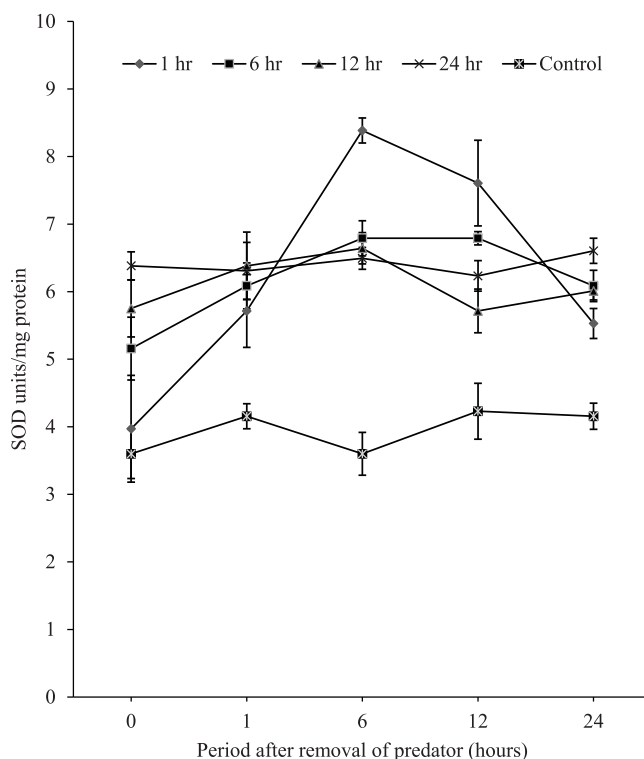
SOD activity levels in the cotton aphid significantly differed among the treatments (predation-risk exposure durations) at all the post exposure durations. SOD levels were significantly different among the different post exposure periods for the treatment for predation-risk exposure durations of 12 and 24 h. The other treatment durations and control did not show any significant effects. All the treatments (predation-risk exposure durations) exhibited a significant higher SOD activity than controls throughout all the post exposure periods except one-hour predation-risk exposure. Predation-risk exposure for one-hour duration did not show significantly higher SOD levels than no-predation (control) at immediate after exposure, but it was increased and remained significantly higher than controls at 1, 6, 12 and 24 h of post exposure period. Among the different treatments of predation-risk exposure duration, 24-hour exposure showed maximum SOD activity immediately after removal of predators (0-hour post exposure period) and remained elevated throughout the post exposure period up to 24 h (Table 1).

Table 1. Superoxide dismutase activity in the cotton aphid *A. gossypii* under predation-risk of *C. sexmaculata*.

Treatment (duration of exposure)	SOD units/mg protein $\pm$ SE in cotton aphid at post exposure period (after removal of predator)					P-value
	0 h	1 h	6 h	12 h	24 h	
Control (0-hour)	3.60 $\pm$ 0.63 ^b	4.16 $\pm$ 0.32 ^b	3.67 $\pm$ 0.55 ^c	4.23 $\pm$ 0.75 ^c	4.19 $\pm$ 0.33 ^c	0.456NS
1-hour	3.97 $\pm$ 1.37 ^b	5.71 $\pm$ 0.93 ^{ab}	8.39 $\pm$ 0.32 ^a	7.61 $\pm$ 1.09 ^a	5.53 $\pm$ 0.39 ^b	0.049*
6-hour	5.16 $\pm$ 0.80 ^{ab}	6.08 $\pm$ 0.59 ^a	6.79 $\pm$ 0.45 ^b	6.79 $\pm$ 0.17 ^{ab}	6.08 $\pm$ 0.40 ^a	0.003**
12-hour	5.75 $\pm$ 0.73 ^{ab}	6.38 $\pm$ 0.86 ^{ab}	6.64 $\pm$ 0.40 ^b	5.7 $\pm$ 0.56 ^{ab}	6.01 $\pm$ 0.23 ^{ab}	0.096NS
24-hour	6.38 $\pm$ 0.36 ^a	6.31 $\pm$ 0.72 ^a	6.49 $\pm$ 0.28 ^b	6.23 $\pm$ 0.39 ^{bc}	6.60 $\pm$ 0.32 ^{ab}	0.349NS
F value	5.779	4.848	51.36	10.86	21.81	-
P-value	0.011*	0.019*	<0.001***	0.001**	<0.001***	-
MSD	2.274	1.938561	1.105824	1.788099	0.9138463	-

Note: Values with the same letter for the columns are not significantly differ. F and P-values for test significance of ANOVA within the column at 4,10 d.f. MSD: Minimum Significant Difference. NS: Non-significant. *Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$.

Among all the treatments, 24-hour exposure resulted in sustained increased SOD level throughout the post exposure period (linear $R^2 = 0.36$) whereas the, 1-hour treatment displayed less consistency (linear $R^2 = 0.003$) (Figure 2). Based on the high SOD activity and its stability throughout the post exposure period after removal of predators, 24-hour predation exposure was adopted as the treatment duration for conducting experiments under active predation and risk of predation.

**Figure 2.** Superoxide dismutase level in the cotton aphid, *A. gossypii* under varying durations of predation-risk exposure to the six spotted zigzag ladybird beetle, *C. sexmaculata* and its sustainability over the period after removal of predator.

NCEs of *C. sexmaculata* on oxidative stress level in *A. gossypii*

SOD activity was significantly higher for active predation (9.75 ± 0.60 units/mg protein) and risk of predation (9.30 ± 0.40 units/mg protein) over controls (5.39 ± 0.13 units/mg protein). This clearly indicated oxidative stress under active predation, and the same magnitude of effect was also induced through the olfactory cues as expressed in risk of predation without significant differences between active- and risk of predation. Catalase (CAT) showed highest activity in the aphids exposed to active predation (78.12 ± 5.26 $\mu\text{M}/\text{min}/\text{mg}$ protein) followed by risk of predation (62.62 ± 5.22) and no-predation (26.48 ± 5.99) with significant differences between each other. A similar trend was also observed for Malondialdehyde (MDA) content as recorded 3.41 ± 0.30 , 2.73 ± 0.39 and 1.40 ± 0.04 nM/mg protein in aphids exposed to active predation, risk of predation and no-predation, respectively (Table 2, Figure 3).

NCEs of *C. sexmaculata* on the biology of *A. gossypii*

Summary data for different biological parameters measured for adult aphids and their progeny under active- and risk of predation is presented in Table 3. NCEs did not affect the longevity, mortality and developmental period of aphids as there is no significant difference between the treatments and control (Table 3). NCEs did have adverse effects on the reproduction of aphids exposed to the treatments, with reduced reproductive period and reduction in the number of nymphs produced per female during the total life span (Table 3). A significant decrease in reproduction was recorded in aphids under active predation (32.35 ± 4.65 nymphs/female) as well as risk of predation (30.00 ± 4.40 nymphs/female) compared to the no-predation treatment (41.62 ± 5.60 nymphs/female). Both the active predation and risk of predation treatments resulted in a similar trend in lower aphid reproduction, albeit with no significant difference between them. There was no significant difference between treatments for the number of nymphs produced in the first 24 h after treatment (Table 3).

Despite the fact that there was no influence of NCEs on mortality and developmental period of nymphs produced by the parthenogenic adults of *A. gossypii* which were exposed to predators, exposure clearly paved the way for the production of winged

Table 2. Activity levels of antioxidant enzymes (SOD and CAT) and MDA content in the cotton aphid, *A. gossypii* under predation-risk of *C. sexmaculata*.

Treatment	Superoxide Dismutase (SOD) [SOD units/mg protein $\pm$ SE]	Catalase (CAT) [H ₂ O ₂ decomposed $\mu\text{M}/\text{min}/\text{mg}$ protein $\pm$ SE]	Malondialdehyde (MDA) [nM/mg protein $\pm$ SE]
Active predation	9.75 ± 0.60^a	78.12 ± 5.26^a	3.41 ± 0.30^a
Risk predation	9.30 ± 0.40^a	62.62 ± 5.22^b	2.73 ± 0.39^b
No predation	5.39 ± 0.13^b	26.48 ± 5.99^c	1.40 ± 0.04^c
F value	94.94	69.45	13.07
P-value	$2.87\text{e-}05^{***}$	$7.1\text{e-}05^{***}$	0.0065^{**}
MSD	1.068464	13.79682	1.2247

Note: Values with the same letter for the columns are not significantly differ. F and P-values for test significance of ANOVA within the column at 2,6 d.f. MSD: Minimum Significant Difference. *Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$.

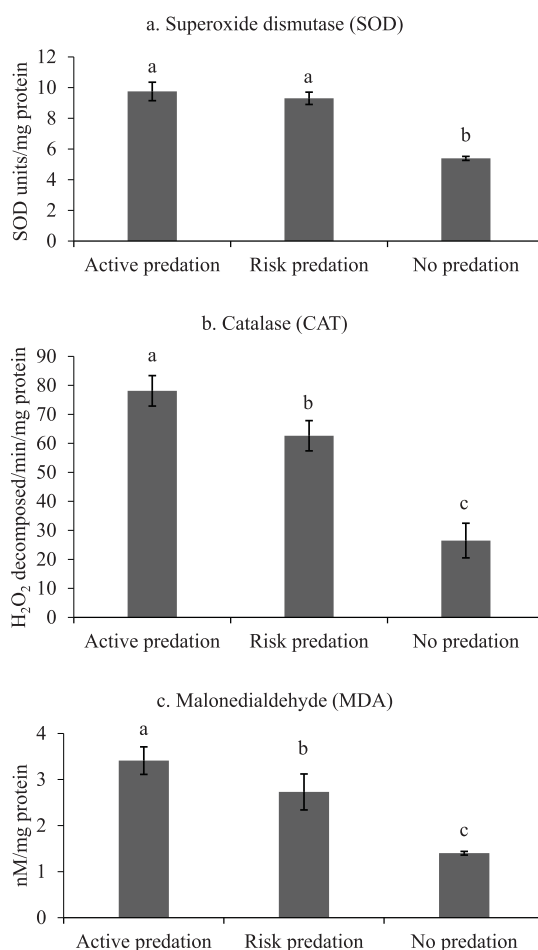


Figure 3. Levels of oxidative stress markers (SOD, CAT and MDA) expressed in cotton aphid, *A. gossypii* under predation-risk of six spotted zigzag ladybird beetle, *C. sexmaculata*.

Table 3. Biological parameters of the cotton aphid, *A. gossypii* under predation-risk of *C. sexmaculata*.

Biological parameters	Treatments			F value	P-value	MSD
	Active predation	Risk predation	No predation			
Adult longevity (days $\pm$ SE)	17.03 $\pm$ 4.19	16.63 $\pm$ 3.25	18.31 $\pm$ 3.38	1.70	0.188NS	-
Reproductive period (days $\pm$ SE)	13.48 $\pm$ 1.61 ^b	13.20 $\pm$ 1.56 ^b	14.93 $\pm$ 1.19 ^a	11.71	3.14e-05***	1.372
Number of nymphs produced per adult $\pm$ SE	32.35 $\pm$ 4.65 ^b	30.00 $\pm$ 4.40 ^b	41.62 $\pm$ 5.60 ^a	46.12	2.21e-14***	3.372
Number of nymphs produced/day/adult in first 24 h after treatment $\pm$ SE	3.12 $\pm$ 1.15	2.99 $\pm$ 1.05	2.81 $\pm$ 0.95	1.56	0.21NS	-
Developmental period of nymph (days $\pm$ SE)	4.62 $\pm$ 0.45	4.63 $\pm$ 0.48	4.61 $\pm$ 0.46	0.052	0.95NS	-
Mortality of nymphs (% $\pm$ SE)	6.74 $\pm$ 1.16	5.70 $\pm$ 1.91	4.91 $\pm$ 1.037	1.18	0.31NS	-
Winged morphs to the total adults (% $\pm$ SE)	21.53 $\pm$ 1.05 ^a	18.28 $\pm$ 4.86 ^a	6.00 $\pm$ 2.99 ^b	17.91	0.00295**	8.396

Note: Values with the same letter for the columns are not significantly differ. F and P-values for test significance of ANOVA within the column at 2,87 d.f. MSD: Minimum Significant Difference. NS: Non-significant. *Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$.

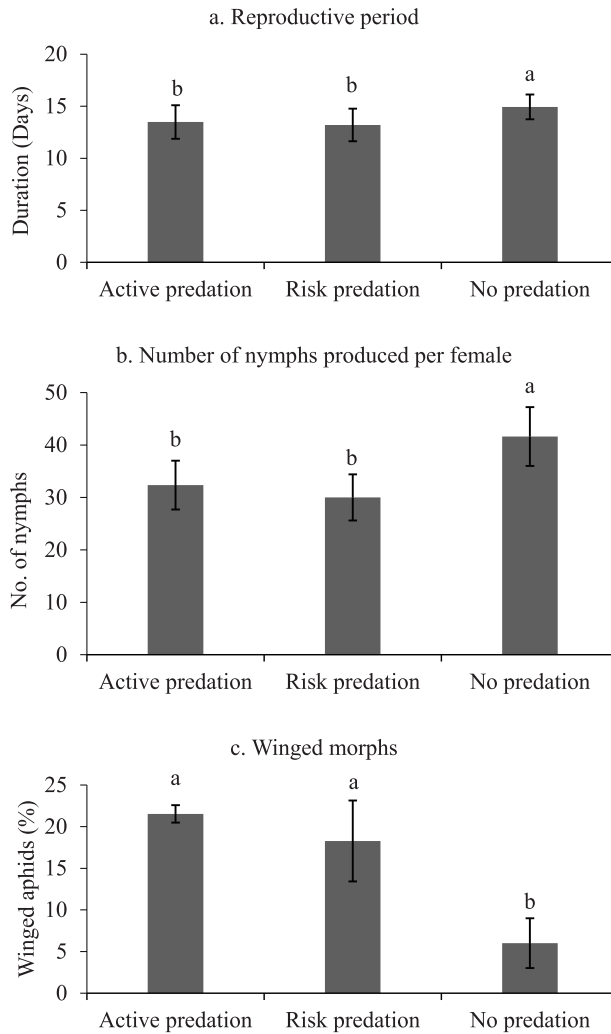


Figure 4. Biological parameters differentially expressed by cotton aphid, *A. gossypii* under predation-risk of six spotted zigzag ladybird beetle, *C. sexmaculata*.

morphs. There was more than three-fold increase in the per cent of winged morphs produced by the aphids under predation-risk compared to the control (Table 3). However, there was no significant difference observed for winged morphs between the active predation and risk of predation treatments (Figure 4).

Discussion

Elevated SOD levels in *A. gossypii* under predation-risk of *C. sexmaculata* confirmed oxidative stress in the prey. Predator-induced oxidative stress was most probably induced through the olfactory and visual cues perceived by the aphids from the adults *C. sexmaculata*.

Our findings revealed that there was a sustained oxidative stress in the aphids even after removal of predators from the arena, since the higher SOD levels were expressed

consistently up to 24 h after removal of the predator. Interestingly, exposure duration as brief as one hour could induce significantly higher oxidative stress for a prolonged period. However, a peak in SOD level for the 1-hour exposures measured at 6 h after removal of predators might be due to high stress upon the aphids during the initial period, and stabilisation in over of time, but higher SOD levels than the control aphids exhibited. This indicates that the effect of short-term exposure was similar to long-term exposures. SOD is reported as a major antioxidant enzyme in many insects as a first line of defence. SOD converts superoxide into oxygen and less toxic hydrogen peroxide (Aucoin et al., 1991; Lomate et al., 2015; Wang et al., 2001; Weirich et al., 2002). Upregulation of SOD activity in response to other stresses has also been reported in several insects (Krishnan & Kodrik, 2006; Wang et al., 2012).

Similarly, the increased SOD and CAT activity under both the active predation and risk of predation treatments did confirm the prime role of olfactory cues in inducing predation-risk effects. It also indicates the existence of an underlying antioxidant mechanism in cotton aphid through upregulation of these two antioxidant enzymes, particularly under predation-risk. Absence of upregulation of either SOD or CAT might lead to increased oxidative damage, and reduction in growth rate and fecundity of the aphids. In larvae of the damselfly, *Enallagma cyathigerum* (Charpentier) predation-risk induced oxidative stress led to reduced growth due to insufficient CAT levels (Slos & Stoks, 2008). It further resulted in higher lipid peroxidation as a result of ineffective antioxidation mechanisms, attributable to lower levels of SOD (Janssens & Stoks, 2013).

In the present studies, elevated MDA content in cotton aphid clearly indicates lipid peroxidation due to incomplete stabilisation of superoxide and other ROS due to insufficient production of SOD and CAT. The maintenance of antioxidant enzymes is also a costly physiological response, and requires energy inputs as shown earlier in damselflies (De Block & Stoks, 2008; Slos et al., 2009). This suggests possible lipid damage in aphids under predation-risk, which might lead to adverse biological consequences, expressed as NCEs. Oxidative stress induced by plant allelochemicals causes lipid damage in aphids, which ultimately influenced the growth and reproduction (Ahmad, 1992).

The present findings in cotton aphid showed a significant reduction in reproductive performance as reduced fecundity and reduced reproductive period, without any effect on the longevity and mortality of aphids exposed to predation-risk. Production and maintenance costs of antioxidant enzymes might have been compensated for by reduced reproductive output. However, there was no induction of growth reduction in the prey when exposed to predation (Benard 2004).

Although, predation-risk did not affect either the longevity of exposed adults or the developmental period of progeny, it strongly affected the development of winged polyphenism in the progeny. A three-fold increase in the number of nymphs that developed to alate adults under predation-risk confirmed that alate polyphenism is an important biological indicator of NCEs in *A. gossypii*. Our findings are consistent with earlier works which proved that predation-risk could induce winged offspring in the pea aphid, *A. pisum* (Dixon & Agarwala, 1999; Kunert & Weisser, 2003; Sloggett & Weisser, 2002; Weisser et al., 1999) and the cotton aphid, *A. gossypii* (Mondor et al., 2005). Predator-induced production of winged morphs could be a beneficial adaptation, facilitating dispersal of aphids to seek reduced risk space (Weisser et al., 1999).

Some other aphid species exhibit different anti-predation behaviours *viz.*, dropping, kicking, moving etc., which is lacking in cotton aphid. Hence, the cotton aphid showed a 'sit and wait' strategy, and the resulting stress was reduced through the production of antioxidant enzymes like SOD and CAT. The cost of production of antioxidant enzymes in cotton aphids might have affected its physiology, resulting in reduced reproductive performance. While the aphids exhibited a 'sit and wait' strategy in the adult stage, they showed a 'fight or flight' strategy through their progeny by the induction of winged morphs. The underlying mechanism of winged polyphenism could be debatable because the factors *viz.*, aphid crowding and low nutritional status of food plants are considered as prelude to the production of winged morphs.

NCEs (predation-risk effect) might cause aphid populations to decline directly through reduced reproduction, and indirectly through the induction of winged morphs for dispersal. Production of winged morphs as a result of predation-risk in the pea aphid and its dispersal from predator risk has been explained as phenotypic plasticity (Kunert & Weisser, 2003; Mondor et al., 2005).

Of late, field-level experiments on the potential role of NCEs showed the contribution of non-consumptive predator-prey interactions in biological control of insect pests. The NCEs induced by *Podisus maculiventris* (Say) on *Manduca sexta* (L.) affected the growth of caterpillar due to reduced feeding, resulting in reduced plant damage by 35% (Griffin & Thaler, 2006). Similarly, field cage experiments revealed that the NCEs reduced caterpillar growth since less time was spent feeding under predation-risk (Thaler & Griffin, 2008). Recent studies on Colorado Potato Beetle (CPB), *Leptinotarsa decemlineata* Say, has shown that the NCEs induced by *P. maculiventris* reduced crop damage by 23%, which led to 33% reduction of the CPB population, and a further 36.6% reduction in its oviposition (Hermann & Thaler, 2018). Due to the lack of anti-predation behaviour in *A. gossypii*, it could be more susceptible to predator-induced NCEs with high and prolonged oxidative stress, which reduce aphid populations at the field level. To make use of NCEs in the biological control of *A. gossypii*, it is necessary to understand the influence of NCEs on colonisation, dispersal and population build-up under field conditions. Identification and study of volatile chemicals which mimic predator's olfactory cues could lead to development of behaviour modifying formulations for environmentally sound methods of pest management.

In the present investigations, we found that the NCEs resulting from predation-risk by *C. sexmaculata* on cotton aphid, *A. gossypii* was likely transmitted through olfactory cues, and even short exposure could raise the antioxidant enzyme levels in the prey aphids. The adult aphids exhibited antioxidant mechanisms through the upregulation of SOD and CAT but not sufficient to avoid oxidative damage that led to increased MDA content. As a result, the cost of production of SOD and CAT might have been compensated for with reduced reproduction in cotton aphid by adopting a 'sit and wait' strategy, and also by triggering winged morphs production for resorting to a 'fight or flight' strategy.

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sexmaculata* (Fab.) and its predation risk on prey**

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**DIVISION OF ENTOMOLOGY
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NEW DELHI -110 012**

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BY

VENKANNA. Y

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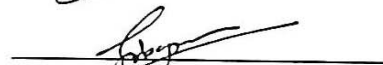
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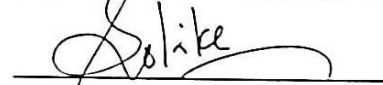
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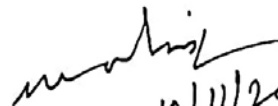
CERTIFICATE

This is to certify that the thesis entitled “**Predatory behaviour of coccinellid beetle, *Cheilomenes sexmaculata* (Fab.) and its predation risk on prey**” submitted to the Faculty of the Post-Graduate School, ICAR-Indian Agricultural Research Institute, New Delhi, in partial fulfilment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY in ENTOMOLOGY**, embodies the results of *bona fide* research work carried out by **Mr. Yana Venkanna** under my guidance and supervision, and that no part of this thesis has been submitted for any other degree or diploma.

It is further certified that any assistance and help availed during the course of investigation as well as sources of information, have been duly acknowledged by him.

Place: New Delhi

Date: 10th November 2020


10/11/2020
(Dr. Sachin Suresh Suroshe)

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LIST OF ABBREVIATIONS

Abbreviation	Expansion
NR	Numerical Response
FR	Functional Response
TR	Total Response
FP	Feeding Potential
SOD	Superoxide Dismutase
CAT	Catalase
MDA	Malonedialdehyde
HIPVs	Herbivore Induced Plant Volatiles
mm	Millimeter
cm	Centimeter
h	Hour
Ø	Diameter
a'	Coefficient of attack
Th	Handling time
°C	DegreeCentigrade
RH	Relative Humidity
@	At the rate of
DCM	Di Chloro Methane
WA	With Aphids
WOA	With Out Aphids
AO	Aphids Only
UL	Un-infested Leaf
ml	Millilitre
mg	Milligram
µM	MicroMole
nM	NanoMole
%	Per cent
N0	Initial prey density
Ne	Number of prey eaten

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1. INTRODUCTION

Among the different natural enemies of crop pests, predacious entomophagous insects play an important role in biological control of many insect pests by keeping their population under general equilibrium position. Coccinellid beetles are the most important predators which are being used against many soft bodied pests due to their voracious feeding potential (FP) and rapid numerical response (NR) in many agricultural and horticultural ecosystems (Honek and Hodek, 1996). Coccinellids (Coleoptera: Coccinellidae) are small insects ranging in size from 0.8 to 18 mm, comprising about 6000 described species under 360 genera and 42 tribes (Hodek *et al.*, 2012). Coccinellids are generally termed as ladybird beetles that named after the Virgin Mary and hence, called “beetle of Our Lady”.

Ladybird beetles have a distinctive convex, crescent to elliptical shaped body with clavate antennae, and often brightly coloured elytra with red, orange or yellow markings. The grub pass through the four stages, which are usually gray with blue, green, red, or black spots. Coccinellids have varied feeding habits as the large number of species are predaceous on different stages of harmful and thus, extremely useful in biological control programmes. These ladybird beetles are important predators because both the adults and grubs feed voraciously on their preys (Srikanth and Lokkundi, 1990; Dixon and Dixon, 2000; Hodek *et al.*, 2012; Omkar and Pervez, 2016; Rakshith *et al.*, 2018). However, the members of a relatively small sub-family Epilachninae are herbivorous and infests agriculturally important crops.

These predaceous Coccinellids have been successfully used in the biological control programmes. Many attempts of Classical Biological Control (CBC) programmes were made through the introduction of these coccinellid beetles across the world. Introduction of *Rodolia cardinalis* (Mulsant) in 1889 in to California for the control of cottony cushion scale, *Icerya purchasi* Maskell on citrus and pear is still considered as a great success story. Later, it was introduced in to India in 1929 from California and successfully kept the pest

under check (Kapur, 1942). Mealybug destroyer, *Cryptolaemus montrouzeri* Mulsant is another well known coccinellid beetle which was first introduced into California in 1891 from Australia and subsequent to Hawaii in 1893 and later has been introduced into more than 40 countries throughout the world to control citrus mealybug, *Planococcus citri* (Risso). These predatory beetles continue to provide important ecosystem services as an efficient bio-control agents and ecological indicators all over the world.

The six spotted zigzag ladybird beetle, *Cheilomenes sexmaculata* (Fab.) is an important versatile predator that suppresses the aphid and other soft bodied pest population to a great extent in nature (Reddy *et al.*, 2001). It is the native coccinellid species remains active throughout the year with wide geographic distribution throughout the country and a vast host range in India. It is reported from many Asian countries and also from Australia (Poorani, 2002). Of late, it has been reported from South America (Gonzalez, 2016) and Colombia (Ramirez *et al.*, 2017). It has high searching ability, voracious feeding, resistance/tolerance to certain pesticides and easy to rear in the laboratory (Venkatesan *et al.*, 2006).

With high developmental rate, immature survival, fecundity, egg viability, reproductive rate and food assimilation efficiency, it has the intrinsic advantage over two coexisting species, *Coccinella septempunctata* L. and *Coccinella transversalis* (Fab.) (Omkar *et al.*, 2005). Worldwide, it has been observed to prey on more than 70 species of aphids, as well as many species of mealybugs, psyllids, whiteflies, lace bugs, leafhoppers, plant hoppers, spider mites, and early instars of lepidopteron larvae. It was reported that, out of 21 species of Coccinellid beetles, *C. sexmaculata* ranked first (46%) in position and recorded as dominant species feeding on aphids and mealybugs of different host plants, followed by *C. septempunctata* (30.58%) (Kumar *et al.*, 2017). The *C. sexmaculata* has also been reported to feed on other important pest, *Phenacoccus solenopsis* (Tinsley) in cotton crop ecosystem (Suroshe *et al.*, 2013).

Cotton aphid, *Aphis gossypii* Glover has been a key pest of agri-horticultural crops due its economic importance. It is having a wide host range of about 320 plant species covering 46 families which have been documented as a favoured hosts (Blackman and

Eastop, 2000). It is known worldwide as a key pest of cotton, *Gossypium hirsutum* L. due to susceptibility of both Indian & American cotton (Ebert and Cartwright, 1997). In India, *A. gossypii* is widely distributed to its tropical, sub-tropical and temperate regions and has become severe due to its parthenogenetic mode of reproduction (Basu and Raychaudhuri, 1980). Different morphs of *A. gossypii* exist simultaneously throughout the year on the primary host, it can also migrate to secondary hosts viz., perennial shrubs and trees (Banziger, 1980). The growing emphasis on eco-friendly approaches such as biological control warrants deep understanding of bionomics of aphids and their natural enemies in particular and the coccinellid predators in general.

Aphids are considered as the most preferred prey compared to other soft bodied insects. So, the interactive biological parameters may vary with the combination of ladybird beetle and the aphid species to be targeted. Among the various interactive parameters FP and the foraging behavior are vital to govern predator's efficiency against its prey. Hence, the study of biology of *C. sexmaculata* on *A. gossypii* is a pre-requisite to assess different biological parameters viz., developmental period of grubs, longevity of adults, fecundity, hatching and survival. These parameters differ with the prey species and can influence total FP of all stages and reproductive potential of predator. The deep knowledge on biology of *C. sexmaculata* on various hosts could also facilitate laboratory rearing, maintenance and possible mass multiplication for its augmentative release in biological pest suppression programmes for sustainable crop production.

FP is the number of preys consumed per unit time when the availability of prey is abundant and continuous. It shows the rate and trend of prey consumption with specificity to age and different stages of a predator. Hence, it is considered as an important criterion for the selection of a predator as it ultimately decides the reproduction of predator.

Foraging behavior describes the way of predator responds to the changing prey density which results in to two kinds of responses namely, Functional response (FR) and NR (Price, 1975). FR describes the rate of predation of a predator at different prey densities whereas, the NR is the progressive change of predator population in relation to increasing prey density. Bio-ecology experts have propounded three types of FR viz., Type-I, II and III

which, represents increasing linear relationship, a decelerating curve and a sigmoid curve, respectively (Holling, 1966; Trexler *et al.*, 1988). The type of FR can be assessed by a polynomial logistic regression analysis of the proportion of prey eaten (N_e/N_0) in relation to the initial prey density (N_0) (Trexler and Travis, 1993; Juliano, 2001). Foraging behavior studies are important for getting deep understanding of predator-prey interactions and for advocating predator's release rate based on attack rate, handling time and rate of predation (Solomon, 1949; Holling, 1966; Hughes, 1990).

In the predator-prey interaction, initial prey density is a key driving factor for population change of both predator and prey. As the prey tends to increase at finite rate of increase (λ), the predator feed the prey at the rate of total response (TR), and the difference would be the remaining prey population for its further buildup.

Even though, there are many insecticides available for the control of *A. gossypii*, insecticide resistance development and the increasing concerns about the environmental hazards is calling for more research on eco-friendly options like bio-intensive pest management. Hundreds of attempts were made in past to suppress aphids by introducing coccinellid beetles across the world (Joshi *et al.*, 2003). The six spotted ladybeetle, *C. sexmaculata* is one of the important native species of India known for having appetite of aphids (Reddy *et al.*, 2001). It feeds voraciously on aphids with high attack rate and could be handled easily in the laboratory (Venkatesan *et al.*, 2006). Both the adult and grub stages of *C. sexmaculata* have shown high FP on *A. gossypii* (Sanghani *et al.*, 2017; Kumar *et al.*, 2016).

In order to have a robust and sustainable biocontrol strategy, we must strive to decipher predator-prey relationship at each level, so that, the effect of biological control could be enhance to maximum fold. To realize the same deep understanding of a concept like predation risk effect/non-consumptive effect of predator on prey must be worked up on for developing new tools for the insect pest suppression.

Predation risk effects are the changes in prey that alters their behavior and physiology when get exposed to predator. These predator-mediated changes which do not

involve direct consumption are also referred as non-lethal effects, risk effects, trait-mediated interactions or Non-Consumptive Effects (NCEs). The predation risk effects can help prey to avoid predation, but sometimes pose threat due to increased stress and reduced feeding. Predation risk effects could be as strong as consumptive effects or even more with the contribution of over 50 per cent to the total predator effect on prey population (Preisser *et al.*, 2005). Although, the concept of predation risk effects is growing, the knowledge on non-consumptive predation responses in the insect pests is very limited and must be elucidated.

On sensing predation risk by perceiving threat signals trigger adaptive changes in aphids for their further establishment and reproduction. Such prominent behavioral changes noticed in aphids under predation risk are *viz.*, dropping off the plant to avoid exposure (*Acyrtosiphon pisum* Harris and bird cherry-oat aphid, *Rhopalosiphum padi* L.), releasing of alarm pheromone, shaking the body vigorously while kicking at the parasitoid with the hind legs (*Aphis nerii* Boyer and *Uroleucon hypochoeridis* Hille), walking away from the threatened feeding site (*A. pisum*) and clustering together to reduce predation risk by dilution effect (*Aphis varians* Patch) (Turchin and Kareiva, 1989; Dill *et al.*, 1990; Chau and Mackauer, 1997; Losey and Denno, 1998; Weisser *et al.*, 1999; Ninkovic *et al.*, 2013). Predation risk induced responses and their associated fitness-costs have the potential to suppress prey population growth and reproduction (Nelson *et al.*, 2004; Nelson and Rosenheim, 2006; Nelson, 2007).

Although, the recent research findings convincingly infer that the predation risk effects alone can reduce the crop damage by the pests, but management recommendations are still under development. One of the potential areas for aphid management is the use of olfactory cues to induce predation risk effect which results in reduced growth of population, colonization and reproduction thereby, reduction of plant damage. Before exploring such cues, a better understanding of the dynamics of predation risk effects on prey is necessary.

Despite of their importance, the studies on predation risk effect on cotton aphid, *A. gossypii* are lacking that might be due to difficulties faced in recording observations as

there is no prominent anti-predation behavior observed in this pest. Some of the work done earlier revealed that predation risk can cause oxidative damage in prey and induce negative fitness consequences (Monaghan *et al.*, 2009; Guerra *et al.*, 2013; Janssens and Stoks, 2013). Thus, the enzymes involved in oxidative damage could be used as biological markers for assessing the predation risk effect on prey. Under oxidative damage, scavenging mechanism of reactive oxygen species involves antioxidant defense system where the enzymes Super Oxide Dismutase (SOD) and Catalase (CAT) plays an important role (Amstad and Cerutti, 1990).

Oxidative damage shows negative impact on prey performance because it damages lipids and in turn leads to damaged proteins and further reduced muscle strength and fitness of prey (Halliwell and Gutteridge, 2010). Initially, when prey detects the predator there is an increase in metabolism with increased vigilance and fast activity as a defense reaction for which it relocates resources (Hawlena and Schmitz, 2010). Over the time, it leads to production of increased Reactive Oxygen Species (ROS) and forced to activate cellular defense system involving anti-oxidant enzymes which is energetically costly hence, the prey allocates less energy to other activities.

Predator-prey interactions are strongly influenced by allelochemicals or herbivore induced plant volatiles (HIPVs) released from the host plant attacked by pest plays a crucial role in attraction of predators towards the habitat and pest's (prey) colonies. The chemicals involved in the tri-tropic interactions can alter the behavior, physiology and population buildup of prey and might be exploited to develop formulations to attract predatory beetles to a particular crop ecosystem for their further conservation.

Keeping this in view and the availability of scanty information, the present studies were conducted using a predator-prey system of six spotted zigzag ladybird beetle, *C. sexmaculata* and the cotton aphid, *A. gossypii* with the following objectives.

- i. To study the predatory behaviour of *C. sexmaculata* against cotton aphid, *A. gossypii*.

- ii. To assess the predation risk induced by *C. sexmaculata* on the cotton aphid, *A. gossypii*.
- iii. To study the orientation response of *C. sexmaculata* towards the prey and host plant extracts.

2. REVIEW OF LITERATURE

2.1 Biology and morphometrics of six spotted zig-zag ladybird beetle, *C. sexmaculata*

Hodek (1960) has studied the biological performance of *C. septempunctata* and *Adalia bipunctata* L. on different aphid species among which, *Aphis craccivora* Koch was found the best. Six spotted zig-zag ladybird beetle, *C. sexmaculata* has also shown high fecundity of 717.7 eggs per female on *A. craccivora* (Rajamohan and Jayaraj, 1973). Reproductive performance of ladybird beetles declined with the age for three coccinellid predators (*C. sexmaculata*, *C. transversalis* and *Harmonia axyridis* (Pallas) as evidenced a triangular fecundity function (increase initially to reach peak then declined gradually) when fed on *A. craccivora* (Dixon and Agarwala, 2002).

Rai *et al.* (2003) have studied the morphometrics of *C. sexmaculata*, length and breadth for egg, pupa and adult was recorded as (1.05 ± 0.011 and 0.49 ± 0.009), (4.05 ± 0.03 and 2.66 ± 0.033) and (5.56 ± 0.039 and 4.64 ± 0.038 mm), respectively.

Omkar and Bind (2004) have reported that among the seven species of aphids, *C. sexmaculata* preferred *A. gossypii* followed by *A. craccivora*. *C. sexmaculata* showed an incubation period of 3.4 ± 0.6 days with a developmental period of 1.75 ± 0.4 , 1.67 ± 0.36 , 2.55 ± 0.32 and 3.17 ± 0.54 days for I, II, III and IV instar grubs, respectively on *A. gossypii*.

Omkar *et al.* (2005) have reported that the biological performance of ladybird beetles varies with the predator-prey combination. Among the different predator (*C. sexmaculata*, *C. transversalis* and *C. septempunctata*) and prey (*Lipaphis erysimi* (Kaltenbach), *A. craccivora* and *A. gossypii*) combinations, *C. sexmaculata* showed an intrinsic advantage over other two coexisting species with a shorter developmental period (12.31 ± 1.06 days), high survival per cent (85.00 ± 7.07), ovipositional period (53.3 ± 3.9 days) and fecundity (1267.6 ± 71.9 eggs).

Preference–performance correlation study has shown highest performance of *Propylea dissecta* (Mulsant) to *A. craccivora* provided with different aphids viz., *A. craccivora*, *A. gossypii*, *A. nerii*, *L. Erysimi*, *Myzus persicae* (Sulzer), *Rhopalosiphum maidis* Fitch (Omkar and Mishra, 2005).

Tank and Korat (2007) have reported that the longevity of female of *C. sexmaculata* was higher (34.15 ± 2.54 days) than male (29.72 ± 2.20) and also showed high fecundity (382.7 ± 16.17) and hatching (85.96%) with an incubation period of 1.40 ± 0.66 days on *A. craccivora*. Duration of first, second, third and fourth instar grub was 1.80 ± 0.50 , 1.72 ± 0.46 , 1.88 ± 0.53 and 1.96 ± 0.73 days, respectively. Total developmental period was 7.36 ± 1.22 days. Length and breadth for egg (1.01 ± 0.11 mm and 0.39 ± 0.03 mm), I (1.41 ± 0.16 and 0.42 ± 0.02), II (4.25 ± 0.18 and 0.75 ± 0.17), III (5.83 ± 0.29 and 0.83 ± 0.05), IV instar grub (7.17 ± 0.20 and 1.29 ± 0.14) and pupa (3.88 ± 0.19 mm and 2.30 ± 0.45) was also recorded.

Singh *et al.* (2008) has studied the biology of *C. sexmaculata* on mustard aphid, *L. erysimi*. Incubation, larval and pupal period was recorded as 2.68 ± 0.12 , 11.08 ± 0.21 and 4.48 ± 0.16 days, respectively. The longevity of adult was 92.25 ± 1.61 days.

Omkar *et al.* (2009) have studied the biology of ladybird beetle, *Anegleis cardoni* (Weise) on three different host species viz., *A. gossypii*, *A. craccivora* and *L. erysimi*. Development was quick on *A. gossypii* (25.71 ± 0.25 days) followed by *A. craccivora* (28.75 ± 0.27 days) and *L. erysimi* (30.56 ± 0.21). Longevity of adult was highest on *A. gossypii* (75.70 ± 1.55 days) and lowest on *L. erysimi* (43.95 ± 1.47 days). Similarly, fecundity and egg viability was noticed maximum on *A. gossypii* (397 ± 10.84 eggs and $78 \pm 1.60\%$, respectively) and minimum on *L. erysimi* (136.60 ± 10.13 eggs and $61.86 \pm 1.22\%$). Life table parameters proved *A. gossypii* as most preferred host followed by *A. craccivora* and *L. erysimi*.

Ladybird beetles, *C. sexmaculata* and *Micraspis discolor* (Fabricius) developed faster and showed higher survival on *A. gossypii* reared on *Solanum xanthocarpum* Schrad than *Chromolaena odorata* (L) (Rattanapun, 2012).

Rakshan and Ahmad (2013) have studied the morphometrics of *C. sexmaculata* grubs. Length was reported as 1.41 ± 0.09 , 4.24 ± 0.14 , 5.83 ± 0.12 and 7.16 ± 0.01 mm for I, II, III and IV instar grubs, respectively. Whereas, the breadth was recorded as 0.44 ± 0.01 , 0.78 ± 0.03 , 0.85 ± 0.04 and 1.32 ± 0.06 , respectively.

Rakshan and Ahmed (2015) have investigated the host preference of *C. sexmaculata* for *A. craccivora* reared on different host plants. Grub and adult consumed highest number (717.0 ± 10.33) of aphids reared on *Vigna sinensis* (L.) followed by aphids reared on *Lablab purpureus* (L.), *Vigna radiata* (L.) and *Vigna mungo*. Adult beetle has shown some morphological variations between the populations reared on different host plants.

Priyadarshani *et al.* (2016) have studied the biology and feeding potential of *C. sexmaculata* on *A. craccivora*. Incubation period, developmental period of grub, longevity of male and female was recorded as 3.0 ± 0.4 , 7.1 ± 0.5 , 38.0 ± 0.6 and 47.3 ± 1.0 days, respectively.

With high consumptional preference, *C. sexmaculata* has shown faster development of grub (12.50 ± 0.83 days), whereas adult longevity ranged between 32.77 to 37.26 days when reared on *A. gossypii* (Sanghani *et al.*, 2017).

2.2 Feeding potential of *C. sexmaculata*

Parmetric values of feeding potential (FP) differs as predator species predation ability is enhanced through age, stage, physical strength, walking speed, voracity, digestion ability and learned behaviour of predation *etc.* (Mills, 1982). Among different preys, *A. gossypii* resulted faster development of *C. sexmaculata* in just 5.2 days and pre-adult period in 10.2 days (Agarwala and Saha, 1986; Saha, 1987).

Das (1991) has studied the feeding potential of *C. sexmaculata* grub on adult of *A. craccivora* and reported that the consumption rate of 10.95 ± 0.32 aphids per day on first day of hatching, got increased gradually by 8th day (53.05 ± 0.93 aphids/day) and there was sharp

decline during pre-pupation period. Mean consumption of 312.90 ± 6.91 aphids was recorded for the entire grub period.

Studies on foraging behaviour of *C. sexmaculata* revealed that the grub and adult had different foraging strategies. Grubs have shown poor power of dispersal and could not recognise prey from distances and had limited time of development. In contrast, adults have displayed more power of dispersal and could recognise prey from distance and had long life. That's why growing larvae seeked high prey density for its development and did not leave aphid patch once found. Whereas, females seeked growing aphids for oviposition which would last long to support the development of neonates (Agarwala and Yasuda, 2000).

All the stages of *C. transversalis* preferred *A. gossypii* the most for the consumption. It has shown consumption of 665.30 ± 5.75 , 4831.10 ± 123.54 and 5412.30 ± 94.51 aphids by the grub, male and female, respectively. However, it has shown least preference for *Aspidiotus nerii* (Bouche) and recorded consumption of 434.80 ± 4.03 , 802.80 ± 34.37 and 905.20 ± 52.48 scales by grub, male and female, respectively. It has exhibited a positive correlation between the daily prey consumption and relative growth rate (Omkar and James, 2004). Similar findings were reported with choice and no choice condition where, *C. sexmaculata* preferred nymph of *A. gossypii* as compared to adult. Developmental period, total consumption and per day consumption of grub recorded as 11.50 ± 0.69 days, 335.75 ± 25.40 and 100.92 ± 2.56 aphids, respectively (Patro, 2004).

Yu *et al.* (2005) have studied the predation rate of ladybird beetle, *Lemnia biplagiata* (Swartz) on *A. gossypii*. Adult consumed 1548 ± 118 aphids during the first 25 days with an age-specific survival rate of 0.07 to 0.89. Age-specific fecundity curve displayed irregular peaks during active ovipositional period.

Solangi *et al.* (2007) who have studied the feeding potential of *C. sexmaculata* on *A. gossypii*, reported the mean number of cotton aphids fed by I, II, III and IV instar grubs of *C. sexmaculata* on *A. gossypii* as 6.4, 20, 30.5, and 43.27 aphids, respectively. Mean fecundity was reported as 530 eggs and egg hatchability of 64.33 per cent for predator.

Adult of seven spotted ladybird beetle, *C. septempunctata* has shown a mean daily consumption of 77.8 ± 5.15 cotton aphids, *A. gossypii* whereas, the total consumption of 21.9, 55.9, 107.4 and 227.3 aphids was shown by I, II, III and IV instar grub, respectively (Sattar *et al.*, 2008).

Xue *et al.* (2009) have reported that the third instar grub and females of *C. septempunctata* and *H. axyridis* fed more number of soybean aphids than males. Both the predatory species have shown high FP for soybean aphid.

Feeding potential study on *C. septempunctata* using mustard aphid, *L. erysimi* as prey has shown a mean feeding potential of grub and adult as 50.38 ± 1.56 and 83.54 ± 1.15 aphids per day per individual, respectively (Singh *et al.*, 2008).

Positive foraging parameters and feeding potential of a predator indicate its efficiency of bringing down prey population. Survival and reproduction ability of predator at low prey densities is important attribute which determine sustainable predator population in ecosystem. Kajita *et al.* (2009) have worked out reproductive performance of two species of ladybird beetles, invasive spp., *C. septempunctata* and native spp., *C. transversoguttata richardsoni* (Brown), in alfalfa fields to varying densities. Both the invasive and native predators responded by laying few and less viable eggs at low prey densities. However, *C. septempunctata* could maintain body weight at limited prey availability than *C. transversoguttata* due to former's higher physiological ability than later's. This might be the reason for the numerical dominance of introduced spp., *C. septempunctata* in alfalfa fields which has led to less numbers of aphids recently.

Borah and Dutta (2010) have reported that the voracity of *C. transversalis* has increased with the advancement of age and stage of grub. The fourth instar grub has consumed 65.67 apterous adults of *L. erysimi* in 24 h and the total consumption by the fourth instar grub was 252.90 apterous adults.

Sharma and Joshi (2010) have studied the feeding potential of *C. septempunctata* under starved and unstarved conditions on two prey species viz., *A. gossypii* and *L. erysimi*. It was observed that the the fourth instar grub of *C. septempunctata* showed high feeding

efficiency on mustard aphid, *L. erysimi* than *A. gossypii*. The hourly consumption on *L. erysimi* was 6.50 ± 0.80 , 6.10 ± 0.73 and 6.40 ± 0.96 aphids for the I, II and III hour, respectively under unstarved condition. But when the beetles were starved the hourly predation was 11.2 ± 0.9 , 8.3 ± 0.9 and 8.0 ± 1.1 aphids for I, II and III hour, respectively. Whereas, the hourly consumption of *C. septempunctata* on *A. gossypii* under unstarved condition was 2.60 ± 0.69 , 2.20 ± 0.78 and 2.00 ± 0.66 aphids for first, second and third hours, respectively, while for starved condition, the hourly consumption was 3.30 ± 0.67 , 2.70 ± 0.67 and 2.30 ± 0.67 for first, second and third hours, respectively.

Shera *et al.* (2010) have studied the feeding potential of *C. septempunctata* against cotton aphid and mealybug in a free choice and no-choice test. Aphids were more preferred (53.8%) compared to mealybugs (46.2%) by *C. septempunctata* in free choice test. Under no choice test, the predation on mealybugs was 80 per cent after 24 hours, which increased to 84.7 per cent after 96 hours. The overall consumption ranged from 24.0 ± 0.77 to 25.40 ± 0.98 mealybugs per day. Predation on aphids ranged from 26.00 ± 0.81 to 28.00 ± 0.71 per day with the per cent predation of 88.7 after 24 hours which rose to 93.3 per cent after 96 hours.

Grub of *C. sexmaculata* showed high feeding potential on *A. gossypii* with a consumption of 46.6 ± 2.50 , 56.4 ± 1.03 , 152.4 ± 6.89 and 159.4 ± 7.53 aphids by I, II, III and IV instars, respectively. Developmental period was also recorded for I, II, III and IV instar grubs as 2.8, 3.0, 3.4 and 3.2 days, respectively (Bukero *et al.*, 2014).

Kumar *et al.* (2016) have studied the feeding performance and post embryonic development of *C. sexmaculata* on three economically important aphid species viz., *A. craccivora*, *A. gossypii* and *L. erysimi*. The grubs of *C. sexmaculata* showed greater preference for *A. craccivora* (141.4 ± 6.67 aphids) followed by *A. gossypii* (122.25 ± 7.44 aphids) and *L. erysimi* (106.95 ± 7.73 aphids). The higher development period was observed on *L. erysimi* (17.50 ± 0.72 days) than *A. gossypii* (16.0 ± 0.5 days) and *A. craccivora* (15.6 ± 0.24 days). Similarly, *C. sexmaculata* responded with higher pre-pupal and pupal period on *L. erysimi* than *A. gossypii* and *A. craccivora*. The higher longevity of the *C. sexmaculata* was observed on *A. craccivora* (39.6 ± 1.21 days) followed by on *A.*

gossypii (35.25 ± 0.47 days) and *L. erysimi* (33.25 ± 0.47 days). It is probably due to more preference of *A. craccivora* than *A. gossypii* and *L. erysimi*. Hence, this predator could consume more number of *A. craccivora* than *A. gossypii* and *L. erysimi*.

Sanghani *et al.* (2017) have conducted a laboratory experiment to determine the feeding potential of *C. sexmaculata* on *A. gossypii*. Grub has shown faster development (12.50 ± 0.83 days) with a total and per day consumption of 391.70 ± 34.91 and 118.90 ± 4.14 aphids, respectively. Whereas, adult consumed a total of 1164.00 ± 179.95 aphids with mean daily consumption of 34.24 ± 1.36 aphids during adult life of 33.90 ± 4.27 days.

Ugine *et al.* (2018) have studied the role of aphid density on the retention time and foraging behavior of imported beetle *C. septempunctata* versus native beetle, *Coccinella novemnotata* Herbst on fava bean plants in North America. It was found that prey density did not affect the retention time of *C. novemnotata*, but showed significantly faster dispersal of *C. septempunctata* in the absence of prey. The females of *C. septempunctata* left the plants significantly faster than males, whereas there was no difference between the two sexes for *C. novemnotata*. It was noted that the *C. novemnotata* spends more time on foraging than *C. septempunctata* when aphids are scarce.

Beltra *et al.* (2018) have studied the predation efficiency of three ladybird species, *A. bipunctata*, *Hippodamia variegata* (Goeze) and *Scymnus interruptus* Goeze on aphid, *M. persicae* infesting sweet pepper. When 150 aphids were offered daily, the female of *A. bipunctata* was found most voracious with 122.7 aphids/day followed by *H. variegata* (87.7) and *S. interruptus* (27.3). It was found that *A. bipunctata* completely eradicated the aphid population (50/plant) within 2 days of release but *H. variegata* and *S. interruptus* could not. Adults of *A. bipunctata* could survive on the plants even in the absence of prey. but *H. variegata* and *S. interruptus* could not.

Zahedi *et al.* (2019) have investigated the influence of cucumber cultivars on development and reproduction of melon aphid, *A. gossypii* and the coccinellid predator, *H. variegata*. The aphid population increased faster on Khasib cultivar than on Storm cultivar of cucumber. However, the predator number got increased on aphids present on Storm

cultivar rather than Khasib cultivar. The intrinsic rate of increase and net reproductive rate for aphids was noticed more Khasib leaves. On the contrary, the same parameters got reduced for predator feeding on this aphid. Thus, the resistance of cultivar to aphid and the consumption efficiency of predator has shown synergistic effect on the cotton aphid, *A. gossypii* in cucumber system.

Jalali *et al.* (2019a) have studied the effect of two different prey species, *Agonoscena pistaciae* Burckhardt and Lauterer (psylla) and *A. gossypii* on the biology, reproduction, and conversion efficiency of ingested food of ladybird beetle, *C. septempunctata*. Both the grub and adult readily preferred to feed on both the prey. The successful development of grub is seen on psylla, but adult could not lay eggs when fed on psylla. However, *A. gossypii* proved to be the best prey for predator as it supported development and reproduction. The aphid was seen superior to the psylla in terms of food conversion efficiency as grubs of coccinellid consumed aphid more efficiently than psylla. Thus, the results indicate the importance of prey suitability for different instars of grub and adults.

Jalali *et al.* (2019b) have studied the efficiency of five predatory coccinellids against two prey species. Coccinellid species, *Oenopia globata contaminata* (Menetries) and *A. bipunctata* performed well on pistachio psylla with higher net reproductive rate, whereas *C. undecimpunctata* and *Exochomus nigripennis* (Erichson) have preferred aphid resulting better reproduction. Whereas, *E. nigripennis* has showed better specificity towards aphid. However, *H. variegata* has displayed acceptance of both the prey species which indicates its potential as a biocontrol agent in pistachio ecosystem.

Boopathi *et al.* (2020) have worked out the life table and consumption rate of ladybird beetle, *Harmonia sedecimnotata* (F.) on *A. gossypii*. The net consumption rate (C_0), the transformation rate (Q_p) and the total consumption of the cohort were 1458.9, 33.8 and 36473 nymphs, respectively. The age-specific fecundity (m_x), age - specific survival (l_x), and age - specific maternity ($l_x m_x$) ranged from 0.04 to 0.88, 1.62 to 10.71 and 0.84 to 6.00, respectively.

2.3 Functional response (FR) of *C. sexmaculata*

The functional response (FR) of III and IV instar grub, female and males of a coccinellid predator, *Adalia fasciatopunctata* (Mulsant) was worked out against walnut aphid, *Callaphis juglandis* (Goeze) which showed a Type-II FR for all the predator stages without any significant differences between stages for attack rate (a). Foraging performance of different stages got differed mainly due to variations in handling time (h). Among all the stages studied, female has shown the highest predation of *C. juglandis* followed by fourth instar grub, males and third instar grub (Amstad and Cerutti, 1990).

Prey consumption by predators was assessed through FR of coccinellid beetles, *C. transversalis* and *C. sexmaculata* on *A. gossypii*. Consumption rate increased with increasing aphid density thus, showing the Type-II FR. Even though coefficient of attack is an important parameter, the handling time explained the consumption rate precisely (Veeravel and Baskaran, 1997).

Fourth instar grub and adult of *Hippodamia convergens* Guerin-Meneville displayed a Type-II FR to the tobacco aphid, *Myzus nicotianae* Blackman. The IV instar grub showed higher search rate and a longer handling time than adult. Owing high predation, *H. convergens* was found abundant on flue-cured tobacco, *Nicotiana tabacum* L. than *C. septempunctata* and *H. axyridis* (Wells and McPherson, 1999).

Agarwala *et al.* (2001) have studied the ladybird beetles viz., *C. sexmaculata* and *C. transversalis* performance against the aphid, *A. craccivora*. Increase in aphid population was found directly related to increase in fecundity and number of predator irrespective of species. Predator species also showed increase in NR corresponding to increasing prey density. However, *C. transversalis* fared better and showed enhanced fecundity and NR even at lower density of aphids.

Omkar and James (2004) have studied the foraging behaviour of *C. transversalis* on aphid, *A. gossypii* using varying prey-predator densities. Higher grub density led to decreased rate of prey consumption due to mutual interference (0.90). At a constant prey density (200), the fourth instar grubs displayed a decreased searching efficiency with

increase in number of predators. Similarly, the searching efficiency of fourth instar grub got reduced from 1.723 to 0.47 with increasing prey density from 50 to 800. Whereas, the prey consumption of grub was increased with increase in prey density but the percent prey consumption was decreased. A predator-prey ratio of 1:50 for *C. transversalis* was suggested for the augmentative biocontrol of cotton aphid.

The conversion efficiency of ingested prey into eggs by the ladybird female was higher at lower densities of prey which further reduced with increase in prey numbers. A Type-II FR was reported. It was observed that female invested in its maintenance after reaching maximum rate of oviposition (Omkar and Pervez, 2004).

Lee and Kang (2004) have investigated the stage specific FR of *H. axyridis* to varying densities of *A. gossypii*. The predator showed a Type-II FR with the attack rates of 0.0037, 0.0442, 0.3590, 0.3228, and 0.1456 h⁻¹ and handling times of 4.1001, 2.4575, 0.7500, 0.2132, and 0.1708 h for the I, II, III and IV instar, and female, respectively.

Pervez and Omkar (2005) have investigated the FR of *C. sexmaculata*, *P. dissecta*, and *C. transversalis* on two different prey species viz., *A. craccivora* and *M. persicae*. All three predators showed a Type-II FR by exhibiting a decelerating curve determined by a logistic regression model. Among the three beetles, *C. sexmaculata* responded nicely followed by *C. transversalis* and *P. dissecta* with high coefficient of attack rates by adult and low handling times by IV instar grub.

FR of coccinellid predator, *Cydonia vicina nilotica* Mulsant against nymphs of aphid, *A. craccivora* confirmed Type-II FR for all four grub instars, male and female. Among all the stages of predator, female showed highest number of prey consumption. FR parameters estimated through Holling's disc equation noted the maximum predation of 144.9, 116.3, 86.2, 80.0, 72.5 and 20.0 nymphs/day by the female, fourth instar grub, male, third, second and first instar grub, respectively (Nasser *et al.*, 2006).

Farhadi *et al.* (2010) have studied the foraging behaviour of all the stages of ladybird beetle, *H. variegata* on *Aphis fabae* (Scolpoli). Increasing densities of aphids were provided on leaf discs kept in the Petri plates. All the grub stages, male and females

showed Type-II FR by depicting decelerating curve of consumption as a function of prey density. The attack rate (a) and handling time (T_h) were obtained as 0.06 h^{-1} and 6.9 h for I instar, 0.059 and 3.343 for II instar, 0.103 and 1.909 for III instar, 0.114 and 0.455 for IV instar grub, 0.159 and 1.194 for male, 0.093 h^{-1} and 0.409 h for female, respectively. Feeding efficiency was noticed highest for females followed by fourth instar grub and male. Highest consumption of females was three times greater than that of males. Maximum predations for I, II, III, IV instar grub, male and female was noticed as 2.93, 5.85, 12.13, 45.13, 18.33, and 44.60 aphids per day, respectively.

Sabaghi *et al.* (2011) have studied the FR of *Scymnus syriacus* Marseul to different densities of third instar *A. fabae* nymphs. Prey density was ranged between 5 to 80 on the host plant, *Vicia faba* L. at 25°C , $65\pm 5\%$ RH and a photoperiod of 16L: 8D h. Both the male and female have shown a Type-II FR determined by nonlinear least-square regression analysis. FR parameters drawn through the Roger's model resulted searching efficiency (a) and handling times (T_h) for the male as 0.115 ± 0.008 and $0.514\pm 0.016 \text{ h}$, and for the female was 0.123 ± 0.006 and $0.434\pm 0.012 \text{ h}$, respectively. The maximum theoretical predations (T/T_h) for male and female were obtained as 46.64 and 55.18 aphids per hour, respectively.

Bayoumy (2011) has assessed the FR of ladybird beetle, *Nephus includens* (Boheman) on, *A. gossypii* at prey densities ranging from 10 to 80. Fourth instar grub and female showed a Type-III FR. However, second instar grub has displayed Type-II FR. Harlequin ladybird beetle, *H. axyridis* also exhibited Type-III FR against increasing densities of aphid, *Cinara* spp. Harlequin ladybird beetle showed the maximum consumption of 132.82 aphids with a handling time of 10.8 min. (Hu *et al.*, (1989)

Osman and Bayoumy (2011) have reported a modified Type-II FR for the coccinellid beetle, *Stethorus gilvifrons* Mulsan against two spotted spider mite, *Tetranychus urticae* Koch. Polynomial logistic regression line of proportion of prey consumed to the initial prey density exhibited a composite waving form that appeared to be a decelerating response (Type-II) at low to medium prey densities and an accelerating response (Type-III) at high prey densities. However, the best fit was obtained with Type-II FR equation.

Gupta *et al.* (2012) have studied FR of all instars, male and female of *C. septempunctata* against two species of aphid viz., *L. erysimi* and *Brevicoryne brassicae* L. All the stages of *C. septempunctata* exhibited a Type-II FR on both the aphids. The attack rate was highest (0.0192) with the lowest handling time (7.9 min) for female beetle.

Other species of *Cheilomenes* viz., *Cheilomenes propingua* (Mulsant), *Cheilomenes lunata* (F.) and *Cheilomenes sulphurea* (Olivier) also exhibited Type-II FR against *A. gossypii* with high coefficient of attack and low handling time for female beetle (Mrosso *et al.*, 2013).

Davoodi and Sahragard (2013) have studied the FR of *H. variegata* female to varying densities viz., 5, 10, 20, 40, 60 and 80 third instar *A. gossypii* nymphs at the laboratory conditions of 25°C, 65± 5% RH and a photoperiod of 16L: 8D h in a growth chamber using black eyed bean, *Vigna unguiculata* (L.) as host plant. FR data confirmed a Type-II FR when analysed through logistic regression analysis. Coefficient of attack rate (a) was obtained as 0.083±0.011 with handling time of 0.197±0.040 hour using Roger's model. The maximum theoretical predation (T/T_h) of female was 121.5 aphids per hour.

Foraging behaviour and FR of convergent lady beetle, *H. convergens* was worked out with cotton aphid, *A. gossypii* at the densities of 25, 50, 100, and 200 in a Petri dish. Consumption indices were recorded at 1, 4, 8, 16, 24, and 48 h after the release of predator. It was noticed that the consumption rate increased with the increase in prey density. However, at maximum density of prey (200), the consumption rate was recorded as 49, 99, 131, 163, 183, and 200 aphids at 1, 4, 8, 16, 24 and 48 h, respectively. Age-specific mortality of coccinellid in the absence of aphid revealed that adults of *H. convergens* survived for more than 2 weeks (Shrestha and Parajulee, 2013).

Shah and Khan (2013) have assessed the FR of IV instar grub, female and male of coccinellid predators viz., *Adalia tetraspilota* (Hope) and *H. variegata* at five densities of *Aphis pomi* De Geer, *B. brassicae* and *A. craccivora*. All the stages of predator exhibited a Type-II FR. Female of coccinellids showed highest attack rate compared to male and fourth instar grub on *A. craccivora*. Two predator species, *Coccinella undecimpunctata* L. and

Hippodamia tredecimpunctata L. showed high efficiency of predation on *A. gossypii*. Adult of both the predators has shown the maximum predation with lower handling time (Al-Deghairi *et al.*, 2014).

Kumar *et al.* (2014) have studied the foraging behaviour of *C. septempunctata* and *C. transversalis* against the pea aphid, *A. pisum* at extremely scarce, scarce, sub-optimal and abundant prey population. A decelerating (Type-II) FR was obtained for an extremely scarce to abundant prey followed by an accelerating (Type-III) response against an optimal to abundant supply of prey.

Alexander *et al.* (2014) have tested the FR of invasive and native fish predators by providing tadpoles (*Hyperolius marmoratus* Rapp) as a prey. No significant differences was noted for attack rate at low prey densities for both invasive and native predators. Invasive species viz., Largemouth bass, *Micropterus salmoides* (Lacepede) and sharptooth catfish, *Clarias gariepinus* (Burchell) displayed higher predation efficiency with Type-II FR compared to native species, Cape kurper, *Sandelia capensis* (Cuvier) and river goby, *Glossogobius callidus* (Smith). Thus, the comparative FR would work as a authentic method for evaluating impacts of invasive and native species.

The FR of ladybird beetle, *Propylea quatuordecimpunctata* (L.) adults was studied on *A. fabae* with long and short duration foraging periods. Although, predator exhibited Type-II FR at both the foraging durations, the coefficient of attack rate (a) and handling time (h) got differed significantly between long and short-term experiments (Nikos *et al.*, 2014).

Although, the Type-II FR is predominantly exhibited by most of the coccinellid species, Type-III FR is also seen in some species. Sara *et al.* (2016) have reported the variations in type of FR being displayed by different stages of *Nephus arcuatus* Kapur. The I and II instar grub and males exhibited a Type-II FR, while III and IV instar grub and females exhibited a Type-III FR.

Costa *et al.* (2017) have carried out experiments on functional and NR of female ladybird beetle, *Stethorus tridens* Gordon against mite *Tetranychus bastosi* Tuttle, Baker &

Sales a pest of physic nut, *Jatropha curcas* L. The female predator preferred eggs and nymphs than other stages. Type-III FR was seen against eggs and nymphs while, a Type-II response to adults. High predation efficiency was seen against eggs of mite which goes well with the highest attack rate and the lowest handling time. The NR of *S. tridens* was found positively correlated with the adult mite.

Hosseini *et al.* (2018) have studied the impact of nitrogen (N) fertilization on predator's foraging behavior and FR by applying N @ 110, 160, and 210 ppm. Cotton aphid, *A. gossypii* was reared on nitrogen applied plants and used as prey for *H. variegata*. Increase in N fertilization increased the nutrient content (total energy) of aphids which contributed to decline in predation of aphid at higher densities. Predator's female in the 110 ppm N fertilization, and males in all treatments showed a Type-II FR, whereas, females in the 160 and 210 ppm N fertilization exhibited a Type-III response. Searching time, handling time, and duration of digestive pauses increased with increasing N fertilization, especially for females. Results indicate that high N fertilization can increase nutritional quality of prey which might changes foraging behaviour of predator by reduced feeding.

Crookes *et al.* (2019) have analysed the FR of an introduced Asian multicoloured ladybird beetle, *H. axyridis* and a trophically-analogous, native convergent ladybird beetle, *H. convergens* on pea aphid using a prey-replacement FR design. Both the species displayed Type-II FR, as both depicted a decelerating curve for consumption of prey with increasing prey density. Introduced spp. *H. axyridis* displayed high attack rate with lower handling time. It indicates the increased occurrence and abundance of the introduced species rather than native predators.

Coccinellid predator, *Hyperaspis polita* Weise was studied for determining foraging behaviour on cotton mealybug *Phenacoccus solenopsis* Tinsley. Fourth instar grub, male and female of predator exhibited Type-II FR against all the stages of prey. However, the female has shown Type-III response against the first instar nymphs of cotton mealybug. All the prey and predator combinations did not show significant differences for the attack rates. However, handling times showed significant differences particularly against I instar

nymphs of mealybug. Interestingly, the maximum predation rate (T/T_h) decreased with the advancement of nymphal stage of *P. solenopsis* (Seyfollahi *et al.*, 2019).

2.4 Techniques for continuous rearing of cotton aphid, *A. gossypii*

Norman *et al.* (1968) have found Kenaf, *Hibiscus cannabinus* L. as a suitable alternate host for rearing the melon aphid, *A. gossypii* under laboratory conditions for subsequent transfer to citrus in viral transmission studies.

Life table experiments for *A. gossypii* was conducted on host plant, *Cucumis sativus* L. at different temperatures. It was observed that the reproduction period did not get affected by the temperature. However, 25 and 30 °C led more to nymphs than at 20 °C. Among the temperatures tested, 25 °C was noticed most suitable as it led to highest intrinsic rate (0.556) of increase (Steenis and El-Khawass, 1995).

Developmental period of *A. gossypii* varied from 7.4 days at 20°C to 5.9 days at 30°C. The Mean generation time reduced with increasing temperature. Intrinsic rate of natural increase was recorded as 0.184, 0.302 and 0.045 at 20, 25 and 30°C, respectively. Intergenerational variations was noticed for the developmental periods when transferred and reared on citrus plant (Satar *et al.*, 1998).

Satar *et al.* (2005) have reported that the optimal range of temperature for population growth of *A. gossypii* on cucumber was very broad *i.e.*, 22.5°C to 30°C. Developmental period ranged from 4.1 days at 27.5°C to 8.1 days at 17.5°C and 10.8 days at 15°C. The highest net reproductive rate was obtained at 25°C. Constant higher temperature was found lethal to the immature stages. The highest per capita growth rate (0.526) obtained at 25°C, and the lowest (0.132) at 32.5°C.

Parajulee (2007) has conducted the laboratory experiment in clip-cage to quantify the temperature-dependent development, survivorship and reproduction of the cotton aphid, *A. gossypii*. Finite rate of population growth was found highest at 25°C and lowest at 10°C. Although, the stage-specific developmental maxima occurred between 30 and 32°C,

a nonlinear regression model revealed 28.6°C as the optimum temperature for overall cotton aphid development, reproduction, and population increase.

For the rearing of cotton aphid, *A. gossypii* potted plants are being used extensively. Cotton plants grown in potted plants at 25±1°C supported more population of cotton aphids when the migration and infestation of other aphid species and their natural enemies was prevented (Oliveira *et al.*, 2010).

At 25 °C, *A. gossypii* has developed with a mean development cycle of 4.38±0.067 days. The doubling time of aphid population was recorded as 2.25±0.02 days with an intrinsic rate of increase of 0.31 (Elegbede *et al.*, 2014).

Similarly, *A. gossypii* has shown better performance on *Hibiscus syriacus* L. under laboratory conditions of 25±1°C, 65±5% RH and 16 h photoperiod. Higher survival rate, fecundity and longevity was noticed than those reared under field conditions of 23-43 °C and 27-95% RH (Hosseini-Tabesh *et al.*, 2015).

Under the laboratory conditions, *A. gossypii* reared on *H. syriacus* had a higher survival rate, fecundity, and longevity than those reared under field conditions. Among different instars, first instar nymph has shown longer developmental period under laboratory conditions (Hosseini-Tabesh *et al.*, 2015).

Nagrare *et al.* (2019) have investigated the effects of temperature on the biological parameters of *A. gossypii* in cotton. Development rate increased linearly until 27 °C, after which it became non-linear. The lower and upper development threshold temperatures predicted for nymphal development was 6.24 °C and 34 °C, respectively. The range of 22 to 27 °C was found as optimum for the cotton aphid.

2.5 Predation risk effect of *C. sexmaculata*

Mackay and Wellington (1975) have investigated the predation risk effect of natural enemies on *A. pisum* in broad bean, *V. faba*. Presence of natural enemies led to more

scattering by aphids. Subsequently aphids began to produce alatae over a longer period than did apterae, but did not live longer duration.

Roitberg *et al.* (1979) have examined the influence of predators on the dispersal ability of apterous pea aphids in the laboratory and field. Pea aphid got dispersed at ease to new host plants kept in field cages in the presence of predators. However, dispersal of apterous aphids was rare in the absence of active predators. It was observed that fecundity got reduced the day after an aphid disperse. The distance dispersed by aphid nymphs was observed positively correlated with the density of aphids on the plant which aphid leaves.

Kawada (1987) has reported that the winged and wingless phenotypes in aphids differ with respect to morphological, physiological, life history and behavioural features. Crowding and nutritional stress induces winged morphs in aphids. Winged morphs possess an efficient sensory mechanism for locating host plant. It was noticed that winged forms could withstand starvation.

Aucoin *et al.* (1991) have reported a high level of antioxidant enzyme activity in *Anaitis plagiata* (L.), a specialist feeder of *Hypericum perforatum* (L.), which contains high levels of phototoxin, hypericin. Phototoxic plant feeders might adopt to toxic plants with antioxidant enzymes such as Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPOX), and Glutathione Reductase (GR).

The major defence mechanism includes a group of antioxidant enzymes represented by superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase's peroxidative activity (GSTPX), glutathione reductase (GR), and DT-diaphorase (Ahmad, 1992). Therefore, these enzymes are crucial for insect herbivores for removing the free-radical of oxygen, and terminating the lipid peroxidation, in response to the endogenous and potential exogenous oxidant-induced injury.

Natural enemies use different info-chemicals to locate their host, for which it may use cues present either in host or plant alone or from both. Basic information from the first (plant) and second (insect) trophic levels differs in quantity and composition, a difference

that shapes the way info-chemicals are used by the natural enemy species (Vet and Dicke, 1992).

Studies on insect-plant interactions must consider the third trophic level *i.e.*, natural enemies of insect pests, since natural enemies could get influenced by multi-trophic level as these are sensitive to chemical aspects related to locating prey and mates (Poppy, 1997).

Pea aphid, *A. pisum* responded to the tracks left by ladybird grubs by producing more number of proportion of winged offspring. This led to a short-term increase in their activity and reduction in fecundity. However, the black bean (Sc.name) and vetch aphid () which enjoy some protection because they are ant attended and/unpalatable, did not respond to the presence of ladybird grubs (Dixon and Agarwala, 1999).

Aphids are attacked by a array of specialized predators and predation has a strong association with the growth and persistence of aphid colonies. Two clones of pea aphid, *A. pisum* were reared in the presence and absence of predatory ladybirds (*C. septempunctata* or *A. bipunctata*). The presence of a predator enhanced the number of winged morphs. A treatment that simulated disturbance due to predators did not enhance winged offspring number. It inferred that the aphids respond to the presence of a predator by producing the dispersal morph to colonize other plants and locality (Weisser *et al.*, 1999).

Fall army worm, *Spodoptera frugiperda* (J.E.Smith) and cabbage looper, *Trichoplusia ni* (Hubner) cell lines were found to contain unique assemblages of antioxidant enzymes. Insect cell line contained Superoxide dismutase (MnSOD and CuZnSOD) for reducing the superoxide radical to hydrogen peroxide and ascorbate peroxidase for reducing the resulting H₂O₂ to H₂O (Wang *et al.*, 2001).

Spatial and temporal distribution of prey and their land use pattern is driven by the fear of predation (Laundre *et al.*, 2001).

Pea aphid, *A. pisum* under the predation risk of parasitoid *A. ervi* responded with the antipredation behaviour *i.e.*, walking away and dropping responses to kicking behavior

which led to decrease in aphid fitness. As a result, dropping and walking away by the aphids reduced the parasitization from 50 to 33 per cent (Villagra *et al.*, 2002).

Sloggett and Weisser (2002) have examined the response of pea aphid, *A. pisum* to the parasitoid *Aphidius ervi* (Haliday). Aphid clones produced the highest proportion of winged offspring when exposed to natural enemies.

Williams and Wise (2003) have studied the predation risk of wolf spider, *Rabidosa rabida* (Walckenaer) on striped cucumber beetle, *Acalymma vittatum* (F.). The presence of spider badly affected the feeding in beetle. Presence of spider within 15 cm in the vicinity of beetle has increased its dispersal by 1.6 times and also reduced the feeding rate of beetles. Beetle was also observed to consistently moved away from the plant having predator.

Kunert and Weisser (2003) have investigated the trait-mediated effect of predators in their prey. Pea aphid, *A. pisum* produced winged morphs in response to the presence of ladybirds and parasitoids. Both hoverfly maggots and lacewing grubs caused a significant increase in the percentage of winged morphs in pea aphid. Wing induction has further influenced the aphid population as winged aphids dispersed to new location.

Predator-induced phenotypic plasticity is experienced by many species in nature and includes predator-induced changes in prey which may not compulsorily affect its growth and development. Physiological effects may pass to the succeeding stages of preys through metamorphic plasticity and to succeeding generations through their offsprings (Benard, 2004).

Nelson *et al.* (2004) have studied the predation risk effect on *A. pisum* by release of disturbance-only predators (surgically manipulated predators but unable to predate) and normal predator. The highest reduction in aphid population growth was due to normal predators which consumed and disturbed aphids. However, the growth was also reduced by non-consumptive and disturbance-only predators.

Mondor *et al.* (2005) have stated that the predation risk induced the transgenerational phenotypic changes in cotton aphid. Cotton aphid exposed to search tracks left by ladybird beetle, *H. convergens* produced more number of winged offspring. Then, in subsequent generations, produced more number of dwarf and winged morphs for dispersal. Thus, elevated predation risk might cause phenotypic changes in aphids over the multiple generations.

Predation risk effect triggers anti-predation behaviour which is transmitted from adults to the offsprings for the production of winged morphs. It further moves away to safer locality through dispersal (Dixon and Agarwala, 1999; Mondor *et al.*, 2005).

Predation risk induced effects and its associated costs have the potential to suppress prey population growth (Nelson and Rosenheim, 2006).

Krishnan and Kodrik (2006) have studied the antioxidant enzyme levels in the gut of *Spodoptera littoralis* (Boisduval) fed on the plants belonging to *Solanaceae* and *Cruciferae* families. A significant up-regulation of antioxidant enzymes viz., SOD (in midgut tissues), CAT (in foregut, midgut tissue and contents), APOX (in foregut contents, midgut tissue and contents) and GSTpx (in foregut tissues) was recorded when reared on the plant diet in comparison to the semi-artificial diet.

Nelson (2007) has studied the predation risk effect of different predators viz., grub and adult of coccinellids and grubs of chrysopids on pea aphid, *A. pisum* in alfalfa fields. The predation risk effect on aphid was stronger when the cost incurred on defense by the aphid was high. Predation risk induced changes in aphid behavior led to reduced birth rates by 2-35 per cent. It was observed that significant reproductive cost was noticed due to reduction in time of feeding. It was confirmed through manual removal of aphids from host plants for few hours per day led to reduced reproduction. Similarly, starvation periods imposed on pea aphids also revealed reductions in fecundity (Kouame and Mackauer, 1992). The primary constraint on the growth and reproduction of aphid under predation risk was the amount of time spent towards feeding (Risebrow and Dixon, 1987; Dixon, 1998).

Lukasik (2007) has analysed the Superoxide dismutase (SOD) and Catalase (CAT) activity in the bird cherry oat aphid, *R. padi* and the grain aphid, *Sitobion avenae* (F.). Among the different aphid morphs, the highest activity of the antioxidant enzymes was recorded for winged aphids and the lowest for wingless.

De Block and Stoks (2008) have assessed the oxidative stress in naids of damselfly, *Lestes viridis* (Vander Linden) by exposing it to a transient starvation period followed by ad libitum food. Compensatory growth in the larval stage was associated with higher oxidative stress. Costs of compensatory growth was found directly related to free-radical-induced trade-offs and not due to resource-induced trade-offs.

Under predation risk, larvae of damselfly, *Enallagma cyathigerum* (Charpentier) has shown the reduced growth with an increase in oxygen consumption indicating of the fight or flight response. Levels of SOD remained constant and CAT decreased. Together with the increase in respiration, this should inevitably result in oxidative stress. Creation of oxidative stress is a novel cost of predation risk that might leave long term negative fitness consequences on the prey (Slos and stocks, 2008).

Under predation risk of damselfly naids *L. viridis* did not decrease its growth rate or immune defence, and only slightly reduced food intake in the high food treatment, probably because of time stress, i.e. little time available to complete the larval development. However, naids facing predator stress did show an upregulation of antioxidant enzymes. This upregulation was dependent upon food level for CAT and both food level and sex for SOD, consistent with energetic constraints and sex differences in the link between longevity and adult fitness (Slos *et al.*, 2009).

Predation is a natural phenomenon which has got evolved over the years through a series of predator-prey combinations being from simple to complex population dynamics and survival of prey species across the natural and agricultural ecosystems. Impacts of predators mainly acts through the direct consumption and non-consumptively *via* fear of predation. (Lima, 1998; Preisser *et al.*, 2005; Zottl *et al.*, 2013; Kersch-Becker and Thaler, 2015).

Impact of the duration of predation risk exposure on the bionomics of phloem-feeding insect was taken up. It was found that prey gets habituated to risk as the time progress. Prey behavioural response and its physiology could be tradeoff to long term risk exposure (Hamilton and Heithaus, 2001; Matassa and Trussell, 2014; Van Dievel *et al.*, 2016)

McCauley *et al.* (2011) have conducted the predation risk experiments of the naids of a dragonfly *Leucorrhinia intacta* (Hagen) by exposing to fish *Lepomis macrochirus* Rafinesque and an invertebrate predator, *Anax junius* (Drury). It was reported that the predation risk signals received in the form of cues increased the mortality in prey. Dragonfly naids showed higher mortality under the predation risk of both the predators. Nails mortality was reported to increase by 2.5 times in the presence of *A. junius* and more than four-fold in the presence of fish.

Hawlena *et al.* (2012) have described the predation risk effect of hunting spider *Pisuarina mira* (Walckenaer) on the physiology and biochemical constituent of grasshoppers, *Melanoplus femurrubrum* (De Geer). An increase in the body C:N ratio was noticed under predation risk. The indirect effect of predation risk might have wider impacts on the ecosystem in general and community in particular.

Predation risk might force prey to trade-off between safe but less nutritious hosts. Grasshopper, *M. femurrubrum* was seen to have moved to safe habitat with low nutritive requirements under predation risk of spider, *P. mira* than to un safe habitat (Schmitz, 1997). These predation risk led behavioural change in prey for seeking refuge in new niche areas might cause nutrient imbalance in them or might expose it to new host toxins (Hawlena *et al.*, 2012).

Ultraviolet (UV) radiation treated insect (*Helicoverpa armigera* Hubner) resulted high levels of antioxidant enzyme with the upregulation of all the genes coding for superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) (Wang *et al.*, 2012)

Interestingly, predator by products also proved as a source of chemical cues for prey to detect and respond. Field experiment was set to verify the effect of spider silk as a source of predator cues on the Japanese beetle, *P. japonica* and the Mexican bean beetle, *Epilachna varivestis* Mulsant. A significant reduction in plant damage due to reported pests in snap-bean, *Phaseolus vulgaris* L. was seen when spider the silk of *Tetragnatha elongata* Walckenaer was used as a predator cue compared to *Bombyx mori* L. silk (Rypstra and Buddle, 2013).

Janssens and Stoks (2013) have showed that under predation risk, damselfly (*E. cyathigerum*) naids witnessed higher lipid peroxidation, which was associated with the lower levels of superoxide dismutase (a major antioxidant enzyme in insects) and higher superoxide anion concentrations (a potent reactive oxygen species). The oxidative damage must be occurring due to non-supply of resources required for antioxidation mechanism and also due to enhanced metabolic rate, which suggest that the increased damage due to oxidation under predation risk could be widespread. This non-consumptive effect of predators on preys might be contributing to population dynamics of prey.

Keiser and Mondor (2013) have studied the transgenerational behavioural plasticity in the pea aphid, *A. pisum*. Pre-reproductive individuals when exposed to the alarm pheromone (E)- β -Farnesene (EBF), next-generation offspring altered their feeding site choices relative to the location of the maternal aphids. Offspring of EBF-treated aphids dispersed to neighbouring plant leaves in search of safer feeding site.

Ninkovic *et al.* (2013) have studied the predation risk effect of seven spotted ladybird beetle, *C. septempunctata* on the bird cherry-oat aphid, *R. padi* by exposing it to the predator's tracks. Ladybird's walking tracks left on the Petri plates induced aphid avoidance behavior which decreased with the time and number of beetles.

Presence of a generalised predator, *Tenodera sinensis* (Saussure) (Mantid) induced predation risk effect on honey bees and which got advanced with the predatory stage. Honey bees have shown high avoidance of predator (74%) in response to the olfactory cues

than visual cues (67%). Forage honey bee have reduced recruitment dance for a food source by 1.8-fold on just exposure to a live mantis without any attack (Bray and Nieh, 2014).

The predatory stink bug, *Podisus maculiventris* (Say) has altered the feeding behavior of its prey, Colorado potato beetle, *Leptinotarsa decemlineata* Say which has caused a significant reduction of feeding by beetle. Predation risk has reduced the feeding by beetles up to 29 per cent while, a reduction of 24 per cent was recorded on the leaves pre-exposed to the predator (Hermann and Thaler, 2014).

Import and release of exotic natural enemies under classical biological control programs is considered cost-effective strategy for invasive pests but the impact of predation risk effect from introduced predator is lacking on exotic pests. Hoki *et al.* (2014) have studied the effect of exotic predator, the sevenspotted lady beetle, *C. septempunctata* and native predator, nine-spotted lady beetle, *C. novemnotata* on the pea aphid, *A. pisum*. Introduced predator performed better for both the consumptive and non-consumptive effects compared to native predator. Whereas, the introduced predator has resulted in more dropping behaviour of aphid compared to native one.

Kersch-Becker and Thaler (2015) have studied the predation risk effect of *H. convergens* in combination with the plant resistance against potato aphid, *Macrosiphum euphorbiae* Thomas in tomato ecosystem. Both active predators and risk predators were not shown reduction in aphid population in high resistance plants, whereas on low resistance plants, predators reduced aphid density by 35 per cent and population growth by 86 per cent. However, dispersal of aphid was higher under predation risk on low resistance plants compared to high resistance plants.

Wineland *et al.* (2015) have the studied the predation risk effect of spiders on grass hoppers in detail. Predatory wolf spiders were used to only frightened the prey and not for subduing it. Grasshopper survival was significantly lower than control (without predator) in both the active and risk predation. Increased plant biomass was noticed under predation compared to control. Grasshoppers exhibited high vigilance, hiding behavior, and reduced

food intake under predation risk. The experiment proved that the non-consumptive predation risk effects could be lethal to prey.

Insects have a unique process of antioxidation governed by the array of enzymes acting against ROS formed during stress. Superoxide dismutases (SODs) are vital antioxidant enzymes converting superoxide into oxygen and hydrogen peroxide (Lomate *et al.*, 2015).

Bestion *et al.* (2015) have conducted the experiments in controlled environmental conditions using a multi-level food chain having top-predator cues (snakes), mesopredators (lizards), herbivores (crickets), and primary producers (plants). It was observed that the predation risk has the ability to affect both the herbivores and plants in a multi-level food chain.

Not only olfactory cues play an important role in conveying predation risk effect, but also visual cues alone could be helpful for the same as reported by Antiqueira and Romero (2016). It was observed that visual models of predatory crab spider alone effected visitation by pollinators leading to a 33 per cent reduction in fruit biomass and a 28 per cent in seed production.

Non-prey life stages of pests might also get affected by the predation risk of predator, hence, impacts across the life stages of prey is must for its further exploitation. Predation risk effect of predatory mite, *Neoseiulus cucumeris* (Oudemans) on a western flower thrips, *Frankliniella occidentalis* Pergande was attempted. The second instar thrips reduced feeding by 22 per cent when exposed to predator (Jandricic *et al.*, 2016).

Type and constituents of landscape determine the presence of different communities representing different trophic levels, and hence, maintain an equilibrium. More diverse habitats provide more diversity of natural enemies require to bring down prey populations. Better and deep understanding of non-consumptive effects could lead to manipulation of cues to exerts fear factor among insect pests in crop ecosystem. Predation risk effects could be better utilized by incorporating them in the form of predator cues and predator mimics in to integrated pest management and push-pull strategy. Predation risk induced

host avoidance behaviour could be employed as push component in push-pull strategy for pest management (Hermann and Landis, 2017).

Khudr *et al.* (2017) have studied the predation risk effect of lacewing, *Chrysoperla carnea* (Stephens) on different clones of green peach aphid *M. persicae*. Visual-olfactory cues from dead predator led to reduced aphid reproduction.

Spined soldier bug, *P. maculiventris* is a voracious predator of eggs and grubs of *L. decemlineata* but not adults. Hermann and Thaler (2018) have studied the predation risk effect of *P. maculiventris*, on *L. decemlineata* by assessing anti-predation behaviour viz., colonization, oviposition, feeding and their impact on host plant damage. Although, there was no difference in colonization of beetle in predation risk and control plots, the plant damage was reduced by 63.9 and oviposition by 37 per cent in predation risk plot.

Khudr *et al.* (2019) have studied the change in population of *M. persicae* under predation risk from live predator, dead predator, dummy predator scented and a dummy of *C. septempunctata* on host plant *Brassica oleracea* (L.). Predators have shown strong negative effects on aphid's reproductive aspects.

2.6 Orientation behaviour of *C. sexmaculata* towards prey and host plant extracts

Essential oil extracted from cotton (*Gossypium barbadense* L.) leaf exhibited the presence of δ -cadinene, caryophyllene oxide and α -copaene as major volatile components (Hedin *et al.*, 1972a). Similarly, aliphatic alcohols such as cis-3-hexen-1-ol, trans-2-hexen-1-ol, 1-penten-3-ol and 6-octen-4-ol and terpenoids viz., terpineol, bisabolol and bisabolene oxide were also reported from *G. hirsutum* (Hedin *et al.*, 1972b).

Similarly, aphid parasitoid, *Diaeretiella rapae* (M'Intosh) responded positively to the odors taken from aphid infested wheat leaves, cabbage leaves and aphids alone but not to the extracts taken from un-infested leaves of wheat or cabbage. However, it got attracted to the odours taken from un-infested leaves of *Brassica* sp. (Reed *et al.*, 1995).

Hamilton *et al.* (1999) have investigated the sensory system and olfactory ability of the convergent lady beetle, *Hippodamia convergens* Guerin-Meneville using an 8-arm air

flow olfactometer. It was reported that the beetles could perceive olfactory stimuli released by their prey or host plant or their combination. It was also reported that the olfactory receptors are located mainly on the tips of their antennae with chetiform sensilla at 11th antennal segments while trichoid sensilla on the terminal segments.

Similarly, olfactometer studies of *A. bipunctata* showed that the grub searching was more intensified when exposed to the odour from the aphids under predation than the odour of undisturbed aphids. The change in searching behavior might be due to presense of alarm pheromone, E- β -farnesene (Hemptinne *et al.*, 2000).

Schaller and Nentwing (2000) have assessed the orientation and choice arena of ladybird, *C. septempunctata* against 22 plants, aphid prey and conspecifics extracts in Y-tube olfactometer. Among different plants, two common weeds (*Cirsium arvense* L. and *Elytrigia repens* L.), chopped flower heads of *Berberis vulgaris* L., *Tripleurospermum inodorum* (L.), and the methanol extracts of *Beta vulgaris* (Bauhin) leaves were found to attract the ladybird beetle.

Vancanneyt *et al.* (2001) have studied the aphid performance on transgenic lines of potato which were modified to reduced the expression of Hydroperoxide lyases (HPLs) genes. HPLs acts on the linoleic and linolenic acids to form hexanal and 3-hexenal, respectively. Aphids feeding on the HPL-depleted plants (lower hexanal and 3-hexenal) nearly doubled their numbers compared to normal plants. It was inferred that hexanal, 3-hexenal and other HPL-derived products had a negative impact on aphid performance.

Han and Chen (2002) have reported that the volatile components from aphid infested tea shoots elicited higher EAG response, and stronger upwind flight and arresting behavior of natural enemies in the wind tunnel. More number of *C. septempunctata* got attracted than parasitoid, *Aphidius* sp., and the lacewing, *Chrysopa sinica* Tiedet. Most effective extract was found to have nine major compounds viz., E-2-hexenal, ocimene, Z-3-hexen-1-ol, butanoic acid-3-hexenyl ester, linalool, geraniol, indole, E-2-hexenoic acid and Z-3-hexenyl formate.

Cabbage root fly, *Delia radicum* L. infested plant (turnip) attracted the larval parasitoid, *Trybliographa rapae* Westwood, but did not to the volatiles emanating from uninfested plants, when presented as whole plant or roots, or leaves (Neveu *et al.*, 2002).

Francis *et al.* (2004) have evaluated the volatiles released from three aphid species (*M. persicae*, *A. pisum* and *B. brassicae*) and three host plant species (*V. faba*, *Brassica napus* L., and *Sinapis alba* L.) for its attraction to *C. septempunctata*. Volatiles taken from infested plants did not attract *C. septempunctata*. The results showed that both grub and adults attracted to *A. pisum* and *M. Persicae* but, not towards *Brevicornea brassicae* L. due to absence of alarm pheromone, (E)- β -Farnesene in the later species.

Nakashima *et al.* (2004) have studied the intraguild interactions between the seven spotted ladybird beetle, *C. septempunctata* and the aphid parasitoid, *A. ervi*. Ethanol extracts of *C. septempunctata* adults when subjected to GC-MS analysis showed the presence of n-tricosane ($C_{23}H_{48}$) and n-pentacosane ($C_{25}H_{52}$) as major constituents responsible for avoidance behaviour in *A. ervi*. Extract taken from grubs and footprint deposited by adult also showed the presence of same chemicals.

Ciarla *et al.* (2005) have studied the olfactory response of three ladybird beetles, *Eriopis connexa* (Germar), *Cycloneda sanguinea* (L.) and *H. axyridis* to extracts taken from *M. persicae* and the host plant, *Capsicum annuum* L. Infested plant has shown higher attraction to the odour source with significantly lower time. Higher attraction exerted by induced volatiles indicates their role as synomones.

Shepherd *et al.* (2005) have studied the olfactometer reaction of two predaceous histerid beetles, *Platysoma parallelum* (Say) and *Plegaderus transversus* (Say) against racemic mixtures of primary aggregation pheromones of their prey, pine bark beetles. Both the predator species got attracted to frontalin and ipsenol but not ipsdienol. Between these two attractants, frontalin was more attractive than ipsenol to *P. parallelum*. However, there was no significant difference between two components to *P. transversus*. It was inferred that the histerid beetle a predator of pine bark beetle employ a different mechanism for host habitat selection to avoid interspecific competition.

Tinzara *et al.* (2005) have studied the olfactory responses of two generalist predators, viz., *Dactylosternum abdominale* (Fab.) (beetle) and *Pheidole megacephala* Smith (ant) of banana weevil. Synomones from pseudostem and the synthetic chemical (Cosmolure+) attracted *D. abdominale*, while the predatory ant, *P. megacephala* was attracted only to the volatiles taken from fermented pseudostem. The attraction to volatiles extracted from damaged pseudostem, irrespective of weevil presence confirmed that both the predators used volatiles from fermented banana pseudostem as the major cue for searching prey.

Blande *et al.* (2007) have investigated the orientation behavior of parasitoid, *A. ervi* to the volatiles released from pea aphid, *A. pisum* and bean aphid, *A. fabae* reared on broad bean. It was noticed that parasitoid showed more attraction to volatiles derived from *A. pisum* than to *A. fabae*.

Qi *et al.* (2008) have evaluated the orientation response of ladybird beetle, *Popillia japonica* Newman against 27 volatile compounds from tea plants and their partial mixtures. Among different compounds tested such as linalool, E-2-hexen-1-ol and Z-3-hexen-1-ol and the mixture of 8 green leaf volatiles at varying dosages, 29 odours were detected by *P. japonica*. Some of the volatiles viz., benzaldehyde, methyl salicylate, linalool, hexanol, 1-penten-3-ol, 3-carene and benzyl alcohol led to significant attraction of the beetle. Beetle has shown attraction variability towards concentration gradient of respective compounds.

The response of Asian ladybird beetle, *H. axyridis* was studied using Y-tube olfactometer towards olfactory cues extracted from agricultural hedge row plants. Beetles preferred naturally infested buckthorn over artificially damaged or un-infested plants (Bahlai *et al.*, 2008).

Yu *et al.* (2008) have tested the seven synthetic Herbivore Induced Plant Volatiles (HIPVs) and a combination of nonanal+(Z)-3-hexen-1-ol for its ability to attract the natural enemies of insect pests in cotton field. Significantly higher attraction of *C. septempunctata* was observed towards (Z)-3-hexenyl acetate than to other HIPVs. Nonanal, (Z)-3-hexenyl acetate, and methyl salicylate (MeSA) shown attraction of *Erigonidium graminicolum* (Sundevall). The predatory bug, *Deraeocoris punctulatus* (Fallen) has

shown positive response to only octanal. Dimethyl octatriene, nonanal + (Z)-3-hexen-1-ol, and octanal were responsible for syrphid fly, *Paragus quadrifasciatus* Meigen attraction. The ladybird beetle, *P. japonica* and other natural enemies did not respond to any of the HIPVs tested.

Costello *et al.* (2008) have worked out the properties of four semiochemicals namely, α -pinene, 3-carene, ipsenol and ipsdienol against cerambycid wood borer viz., *Monochamus clamator* (LeConte), *Acanthocinus obliquus* (LeConte) and *Acmaeops proteus* (Kirby). Ipsenol and ipsdienol are pheromone of wood borer beetles whereas, α -pinene and 3-carene are produced by the pine trees. Experiment on trap catches using these semiochemicals resulted in attraction to ipsenol, ipsdienol and 3-carene but not to the α -pinene.

Volatile chemicals emitted during the interaction of plant and herbivore might attract either any one or both of herbivore and natural enemies. However, in nature plants get exposed to a variety of attackers, either simultaneously or sequentially, in shoots and roots. This might led to much more complex interactions which needs to be investigated in the context of HIPVs (Dicke *et al.*, 2009.).

Heuskin *et al.* (2009) have analyzed the chemical composition of essential oils extracted from *Matricaria chamomilla* L. and *Nepeta cataria* L. and their fractionization by column chromatography. Three main fractions with high degree of purity in E- β farnesene from the oil of *M. chamomilla*, one fraction enriched in (Z, E)-nepetalactone and β -caryophyllene were reported from the oil of *N. cataria*. These volatile fractions were subjected to further studies and found responsible to attract predators and parasitoids of aphids.

Seenivasagan *et al.* (2009) have studied the role of chemicals on tri-tropic interaction between plant, diamond back moth and parasitoid, *Cotesia plutellae* (Kurdjumov). Both the host plant and DBM larval extracts have played significant role in attraction of parasitoid. Plant extract was found responsible for long distance attraction while, larval body extracts were more promising for short distance attraction. Thus, the synomones released from host plant are found important in long distance attraction of

parasitoid whereas, the kairomones released from insect pest were responsible for short distance attraction.

Tapia *et al.* (2010) have conducted olfactometry studies for the two coccinellid beetles, *E. connexa*, *H. variegata* and a carabid beetle, *Trirammatus striatula* (Fabricius) against pea aphid, *A. pisum* infested plant and un-infested plant. *H. variegata* and *T. striatula* got attracted to infested plant extracts compared to un-infested plants. It indicates the importance of semiochemicals *i.e.*, Herbivore Induced Plant Volatiles (HIPVs) produced by infested plants towards the prey-searching behaviour of aphidophagous predators.

Methyl salicylic acid (MeSA) was used to test the attraction of different insect species comprising 27 natural enemies and 9 pests. Suitable traps were installed in strawberry plot with MeSA as lure to record the attraction of insect species. Positive response witnessed for *Chrysopidae* and *Orius tristicolor* (White) to MeSA based sticky traps (Lee, 2010).

Szendrei and Rodriguez-Saona (2010) have analyzed the published work on insect pest behaviour manipulation with plant volatiles. They reported a total of 374 insect-plant volatile interactions involving 50 species of insect pests. Volatiles effects are more prominent on chewing insects followed by wood borers and sap feeders. Females prefers plant volatiles more than the males. Similar level of response to plant volatiles was also observed in forest and agricultural habitats. More number of chemicals and method of volatile application showed a significant effect on attraction.

Rodriguez-Saona *et al.* (2011) have reviewed the published work on field responses of predaceous arthropods to MeSA. Out of 91 studies, 41 documented significant attraction response of different predator and parasitoid taxa to MeSA. MeSA-baited yellow sticky traps trapped higher number of adult ladybird beetles, hoverfly and green lacewings compared to un-baited traps in canberries. Mallinger *et al.* (2011) also reported the attraction of soybean aphid, *Aphis glycines* Matsumura to MeSA baited sticky traps. Abundance of soybean aphid was also reduced in the fields baited with MeSA. Gadino *et al.* (2012) evaluated the effect of synthetic MeSA lures (PredaLure) in vineyards and found

highly significant attraction of coccinellid beetles. Sticky cards baited with MeSA alone or volatile mixture also attracted ladybird beetle, *Stethorus punctum* (Casey) in vineyard fields (Maeda *et al.*, 2015). Similarly, soybean plant volatiles containing MeSA elicited significant electrophysiological response on *C. septempunctata*. However, MeSA baited traps did attract *C. septempunctata* but not *H. axyridis* (Zhu and Park, 2005).

Many studies proved that the volatiles released from both the prey as well as infested plant are more or less equally important in attraction of predators. Interestingly, the studies on ambulatory response of ladybird beetle *Laricobius nigrinus* Fender to odors of its prey *Adelges tsugae* (Annand) (hemlock woolly adelgid) and host plant resulted in attraction of predator to the volatiles from host trees only (Kimberly *et al.*, 2011).

Although the volatiles from infested-plant are known to attract predators and parasitoids, the olfactory learning behavior of predator might provide options to select genotypes for the pest. With the experiments on *C. septempunctata*, Glinwood *et al.* (2011) have reported that the coccinellid predator, *C. septempunctata* exhibited differential preferences to the odors from aphid-infested barley cultivars. After feeding on aphids for 24 h on a particular cultivar, the predator has shown the learned behavior of preference for that cultivar. Qualitative and quantitative differences in odors provided a potential basis for olfactory discrimination by the ladybird beetle.

Krakos *et al.* (2011) have studied the orientation of coccinellid beetle, *Curinus coeruleus* Mulsant to the nectar of Hawaiian plant, *Hibiscus brackenridge* (Roe). Extra floral nectar provides extra nourishment and energy to beetles to feed on pest. The sensory ability of the beetles was tested against visual and olfactory cues to detect the nectar in eight-armed air-flow olfactometer. The test results showed that the olfactory cues were mainly responsible for the attraction of beetles towards nectaries.

Adults of *C. septempunctata* preferred the odour of aphid-infested barley cultivar over un-infested plants of the same cultivar. There was no preference for cultivar when plants were un-infested. However, after feeding on aphids for 24 h on a particular cultivar, coccinellid preferred the odour of same cultivar (Glinwood *et al.*, 2011).

Essien *et al.* (2011) have assessed the un-infested cotton leaf extract for volatile chemical composition and reported different volatiles namely, (E)-2-hexenal (0.5%), tricyclene (29.6%), α -Thujene (0.2%) α -Pinene (12.8%), camphene (0.2%), sabinene (1.4%), β -Pinene (5.4%), myrcene (0.6%), α -Terpinene (11.1%), p -Cymene (2.1%), limonene (0.1%), (E)- β -Ocimene (0.3%), γ -Terpinene (0.3%), terpinolene (2.0%), bornyl acetate (18.6%), isodene (6.0%), β -Caryophyllene (0.2%), α -humulene (0.1%) and γ -Cadinene (1.1%).

Kaplan (2012) has evaluated the orientation response of different arthropod taxa to the herbivore-induced plant volatiles (HIPVs). Methyl salicylate (MeSA), cis-3-hexen-1-ol, and phenylethyl alcohol were used as individual compounds and equal part blends to observe the response of arthropods in maize and soybean fields. Out of 119 arthropod taxa, found attraction of four species in maize field and 16 in soybean. Only in very few cases HIPVs acted synergistically. Individual compounds attracted better than volatile the blend.

Ballhorn *et al.* (2013) have studied the influence of nitrogen-fixing rhizobia induced plant volatiles on indirect and direct defense of plant (Lima bean, *Phaseolus lunatus* L.) against Mexican bean beetle, *Epilachna varivestis* Mulsant. Results obtained through Y-tube olfactometry gave a simplified picture of insect decision making with display of concentration gradient dependent decision. Plant volatiles have affected the choice behavior of beetles as they preferred non-induced plants exhibiting a negative correlation with number of colonies of rhizobia.

Volatiles collected from *A. gossypii* infested plant showed significant attraction of coccinellid beetle, *H. variegata* when had high density of aphid (≥ 400 on *C. sativus* and ≥ 200 aphids/pot on *Cucurbita pepo* L.) but did not get attracted to volatiles taken when the aphid density was low (Li *et al.*, 2013).

Similarly, *C. sexmaculata* showed more attraction towards infested cowpea plants at high aphid (*A. craccivora*) population densities (150) thus, exhibited a positive correlation between number of aphids and attraction of *C. sexmaculata*. Alarm pheromone, E- β -Farnasene might be responsible for the higher attraction of predatory beetle (Jaba *et al.*, 2013).

Olfactometer experiment was conducted to assess the innate positive chemotaxis of two predators, *P. japonica* and *Orius sauteri* (Poppius) towards pollen of seven crop and banker plants. The results revealed that the maize and canola pollen were attractive to both the predators under laboratory as well as in field conditions. Whereas, among the banker plants, balm mint pollen highly attracted *P. japonica* while, alfalfa pollen attracted *O. sauteri* (Shu *et al.*, 2015).

Kemp and Cottrell (2015) have investigated the attraction of different species of coccinellid beetles to various colour traps with or without a lure. All the lures failed to attract beetles except limonene, which increased the beetle capture. Yellow traps consistently captured more number of lady beetles than red, orange, green, blue, purple, black, and white traps. Single-color yellow traps captured 95 per cent of the exotic species *i.e.*, *H. axyridis* and *C. septempunctata* of which former dominated with 74.1% of all ladybird beetle capture. Traps having combination of two colour (Yellow with other) could not capture more beetles than with only yellow traps.

Several natural enemies prefer MeSA as a cue to locate pest infested crop ecosystem. Different concentrations of MeSA were applied and studied against the cotton aphid, *A. gossypii* for its behaviour, population development, and attractiveness to natural enemies. Cucumber plants baited with 0.1 and 10 per cent MeSA attracted coccinellid, *Scymnus sodalis* (Weise) in similar proportion to aphid infested plants. The MeSA application has reduced the cotton aphid population significantly within 10 metre range of bait (Dong and Hwang, 2017).

Rondoni *et al.* (2017) have conducted Y-tube olfactometer studies on an invasive coccinellid predator, *H. axyridis* and the native species, *Oenopia conglobata* L. to assess the orientation behaviour towards odours of *V. faba* and *A. fabae*. Results revealed that both predators recognised the olfactory cues from plant and aphid, but invasive *H. axyridis* exhibited a complete response. This species has also showed the attraction to host plants previously infested by aphids, indicating the capacity to exploit synomones (HIPVs) induced by aphids.

Oliveira *et al.* (2017) have reported that the essential oils extracted from clove, *Syzygium aromaticum* (L.) and *Piper hispidinervum* Kunth exhibited the repellent action on adults of *Callosobruchus maculatus* (Fab.). The GC-MS analysis revealed the presense of 42 chemical compounds, among them safrole was the main component from *P. hispidinervum* whereas, eugenol was the major component in *S. aromaticum*.

Joo *et al.* (2017) have investigated the temporal dynamics of volatile composition released from tobacco *Nicotiana attenuata* Torr under the attack of *Manduca sexta* (L.) and *Tupiocoris notatus* (Distant). Presence of chemical compositions in the plant volatile released during the day and night separately was assessed. The synthetic blends resembling day and night released volatile were used to study the activity of predators, *Geocoris* spp. on *M. sexta* and *T. notatus*. Volatile blend with fast acting green leaf volatiles (GLVs) and slow released sesquiterpenes attracted the predator. The predator preferred day blend than the night released blend. Predation rate was found increased when the GLV composition of day blend was used whereas, the sesquiterpenes increased the predation when night blend was used.

Salamanca *et al.* (2017) have investigated the response of two predators viz., *H. convergens* and *Chrysoperla rufilabris* Burmeister to MeSA in the laboratory, greenhouse and field conditions. Strong Electro-Antennography (EAG) response was observed for females than males of both the predators. Wind tunnel studies in greenhouse also resulted the attraction and arrest of female to MeSA which further increased the predation. Mark-release-recapture experiments conducted in cranberry fields did not show significant attraction of predators to MeSA baited yellow sticky cards.

Orientation response of male and female transverse ladybird beetles, *C. transversalis* was tested against cowpea aphid, *A. craccivora* body extract and Herbivore Induced Plant Volatiles (HIPVs) using Y-tube olfactometer. Highest numbers of beetles were moved towards HIPVs followed by aphid body extract and least response was observed for un-infested leaf extract. Among the different concentrations, 10 per cent concentration was most effective followed by 15, 20 per cent, and least response was noticed at 5 per cent extract taken through both n-Hexane and DCM (Rakshith *et al.*, 2018).

3. MATERIALS AND METHODS

The present studies were carried out in Biological Control Laboratory (BCL), Division of Entomology, ICAR- Indian Agricultural Research Institute, New Delhi, during the year 2017-2020. The experimental location is situated at 28° 35' N latitudes and 77° 22' E longitudes and an altitude of 227 meters above mean sea level. The details of rearing materials, equipments, chemicals, the methodology adopted for carrying out various studies and statistical analysis used for interpretation of data are presented briefly in this chapter.

3.1 Materials

3.1.1 Glass/Plastic wares

1. Plastic pots (25 cm Ø x 30 cm height) for growing cotton plants which host aphid *A. gossypii* and its predator *C. sexmaculata*.
2. Screen cages made up of PVC sheets (30 x 40 x 60 cm size) with sliding door on one side for keeping potted cotton plants to avoid cross infestation.
3. Disposable plastic Petri plates of 10 cm diameter for rearing grubs of *C. sexmaculata* and experimentation on bionomics. Petri plates (10 cm Ø) were also used for rearing of cotton aphid, *A. gossypii* on mesta, *H. cannabinus* seedlings.
4. Plastic trays (30 x 45 cm size) for keeping the Petri plates containing test organisms.
5. Reagent bottles of various capacity for storing chemical solutions
6. Glass jars, beakers, conical flasks, pipettes etc., for preparation of reagents and carrying out reactions.
7. Microscope slides, watch glasses and glass Petri plates for keeping different

stages of *C. sexmaculata* under microscope to take morphometric measurements.

8. Volatile collection tubes for dynamic collection of plant and aphid volatiles.
9. HPLC grade reagent bottles (250 and 500 ml), plastic covers, trays, scissors etc., for the extraction of cotton leaf and aphid extracts.
10. GC-MS vials for storing concentrated extracts.
11. Elisa plates (96 wells) for spectrophotometric reading.
12. Test tubes to add reactants, keeping for incubation and for different reactions.
13. Pippettes and measuring cylinders of different sizes to measure reactants and reagents.

3.1.2 Equipments

1. Environmental rearing chamber (REMI) was used to conduct biological, FR, NR and other experiments by maintaining controlled conditions with respect to temperature, RH and photoperiod.
2. Separation and identification of compounds present in plant and aphid extracts was done by GC-MS (Gas Chromatography-Mass Spectroscopy): Shimadzu QP 2010 Ultra equipped with Rtx-5ms column measuring 30×0.25mm and NIST14 library.
3. Morphometric observations of different stages of *C. sexmaculata* were taken with the help of Nikon stereoscopic microscope (Nikon, 2010) with camera attachment (Nikon, 5MP) by using software “Vimage-2016”.
4. Y-tube olfactometer with two arms equipped with an air pump (KNF).
5. Extracts were concentrated using Rotary Evaporator (HS-3005N) with capacity of 500 ml round bottom flask placed on heating mantle (Electromantle™)

maintained at 40-45°C and 45-50°C for DCM and Hexane, respectively.

6. A container was designed with double cylinder system using Petri plates and iron mesh. Petri plates served as top and bottom of cylinder holding iron mesh as curved surface as fixed between two Petri plates. Inner small cylinder was made with Petri plates of size 10 cm diameter and kept in a bigger outer cylinder of size 18 cm diameter. This double cylinder container holds host seedlings along with aphids in the inner cylinder leaving empty inter cylinder space between the rims of two cylinders. These designed containers were used in the predation risk exposure treatments.
7. Plant volatile collection system (Mascot Enterprises VCS-15) for dynamic collection of volatiles released from cotton plants and aphids.
8. Elisa plate reader (Eon CS-234) was used to take spectrophotometric readings.
9. Thermostat centrifuge (Eppendorf 5424 R) was used for centrifugation of enzyme extracts of aphids.
10. Environmental growth chamber (REMI®) with 15W fluorescent tube lights was used to incubate reaction mixture for SOD analysis.
11. Ph meter for measuring pH of buffers.
12. -80°C deep freezer (Panasonic, MDF55 V, Ultra-deep freezer) was used to store the extracts for bioassay.
13. -20°C deep freezer was used to store the reagents and standard enzymes.

3.1.3 Experimental material

1. Cotton plants of variety, RCH-650 were grown in plastic pots keeping in plastic screen cages and used for rearing of aphid *A. gossypii* and predator *C. sexmaculata*.

2. Cotton plants were also grown in green house to collect leaf extracts from uninfested and aphid-infested plants.
3. The cotton aphid, *A. gossypii* was collected from infested cotton fields at ICAR-IARI farm and reared on potted cotton plants for studying biology, FP and foraging behaviour of predator, *C. sexmaculata*.
4. The cotton aphid, *A. gossypii* was also reared on mesta, *H. cannabinus* seedlings using absorbant cotton as substrate kept in a Petri plate. These aphids were used for the predation risk effect studies of predator *C. sexmaculata*.
5. The predator, *C. sexmaculata* adults were collected from different fields at ICAR-IARI, Pusa farm. It was later maintained on potted cotton plants established with cotton aphid in a screen cage. These adults were then used for studies on biology, FP, foraging behaviour, predation risk effect and orientation response.
6. The grubs of *C. sexmaculata* were reared and maintained in the Petri plates provided with cotton aphid on cotton leaf as substrate. These grubs were then used for studies on biology, FP and foraging behaviour.

3.1.4 Chemicals

3.1.4.1 For leaf and aphid extractions

1. HPLC grade dichloromethane (DCM)
2. HPLC grade n- hexane
3. Anhydrous sodium sulphate (Na_2SO_4) at 1g/10ml was used to remove the water from the extracts.
4. Activated charcoal (3g/ 100ml) was used to remove colour substances from the various extracts.

5. Acetone was used to clean the container (round bottom flask) of Rotary Evaporator during concentration of the extracts.

3.1.4.2 For superoxide dismutase (SOD) analysis

1. Nitrobluetetrazolium (NBT)
2. Methionine
3. EDTA
4. Riboflavin
5. Sodium phosphate and sodium ortho phosphate were used for preparation of phosphate buffers.

3.1.4.3 For catalase (CAT) analysis

1. Phosphate buffer 0.01 M (pH 7.8).
2. Hydrogen peroxide 0.2 M.
3. Potassium dichromate and glacial acetic acid (GAA) were used to prepare dichromate acetic acid (DAA).

3.1.4.4 For malondialdehyde (MDA) analysis

1. Malonedialdehyde (MDA)
2. Thiobarbituric acid (TBA)
3. Glacial acetic acid (GAA) used for preparation of MDA solution and TBA solution.

3.1.4.5 For protein estimation

1. Bradford reagent

3.1.4.6 For other experiments

1. Formalin solution (4 per cent) for disinfection of rearing containers, glass vials and other glass wares in order to prevent fungal or bacterial contamination.
2. Ethanol (70 %) for disinfection of hands and rearing trays.
3. Acetone (10 %) to remove the odour present in Y-tube olfactometer.
4. Hoagland's medium for preparation of plant nutrient solution.
5. Agar to prepare 2% agarose gel used to insert cut end of leaf petiole in to gel to maintain prolonged freshness of leaf when used as substrate for aphid in a Petri plate.
6. Double distilled water to prepare reagents for enzyme analysis.

3.1.5 Miscellaneous material

1. Plastic containers, camel hair brushes, muslin clothes and rubber bands to collect, transfer and secure the eggs, grubs, pupae and adults of *C. sexmaculata*.
2. Absorbant cotton to grow mesta seedlings in the Petri plates.
3. Whatman filter paper 1 for filtration of extracts.
4. Cotton plugs, tissue papers, blotting paper etc.
5. Needles, forceps, Camel hair brushes of various sizes for handling different stages of *A. gossypii* and *C. sexmaculata*.

3.2 Methodology

3.2.1 Programme of research work

Programme of research includes the following activities.

- i. Rearing of cotton aphid, *A. gossypii* and its predator, *C. sexmaculata* using cotton as host plant in the plastic pots.
- ii. Biology and recording of morphometrical observations of predator, *C. sexmaculata* on cotton aphid, *A. gossypii* as a prey.
- iii. Working out the feeding potential (FP) of different instars of grub, male and female of predator, *C. sexmaculata* on *A. gossypii* by providing enough prey continuously throughout the life of predator.
- iv. Analysing FR of different instars of grub, male and female of predator, *C. sexmaculata* to varying densities of prey, *A. gossypii*.
- v. Analysing NR and efficiency of conversion of ingested food (ECI) of female of *C. sexmaculata* to varying densities of prey, *A. gossypii*.
- vi. Elucidating the rate of natural intrinsic increase of prey, *A. gossypii* to use the data in simulating predator-prey population dynamics models.
- vii. Extraction and identification of volatile chemical compounds from un-infested cotton plant, aphid-infested cotton plant, only aphids, and aphid infested plant plus aphids.
- viii. Orientation behaviour of *C. sexmaculata* adults against different extracts of cotton plant and aphids by using Y-tube olfactometer.
- ix. Rearing of cotton aphid, *A. gossypii* on mesta, *H. cannabinus* for predation risk experimentation.
- x. Predation risk effect of predator, *C. sexmaculata* on its prey *A. gossypii* by assessing anti-oxidant enzymes activity and key biological parameters.

3.2.2 Growing of host plants

Cotton plants (*G. hirsutum*) of variety RCH-650 were grown in the plastic pots (Ø 20 cm). Pots were filled with fine loamy soil and coco peat in equal quantity and seeds were sown in July, 2018. These plants were maintained in screen cages (Plate 3.1) throughout the study period to avoid any cross infestation at controlled conditions of 25 ± 1 °C temperature, $65\pm 5\%$ of RH and 16 hrs of photo period in environmental chambers (REMI®).



a. Setup for rearing of *A. gossypii* and *C. sexmaculata*



b. Cotton plants kept in screen cages to avoid cross infestation

Plate 3.1: Screen cages used for rearing aphid and ladybird beetles

3.2.3 Rearing of *A. gossypii* and *C. sexmaculata*

Cotton aphid, *A. gossypii* was collected from the cotton plants from the fields of ICAR-Indian Agricultural Research Institute (IARI), Pusa, New Delhi. Parthenogenetic females were gently transferred with camel hair brush on to the potted cotton plants kept in the environmental chamber at controlled conditions of $25\pm1^{\circ}\text{C}$, $65\pm5\%$ RH and photo period of 16 hrs. This inoculation of field collected parthenogenetic females was carried out thrice at weekly interval. After 15-20 days of first inoculation, establishment of aphids was noticed with increase in numbers (Plate 3.2). Established aphid population on potted plants was maintained as stock culture throughout the study period. To avoid crowding of aphids, the grownup adults were frequently transferred to the fresh potted cotton plants.

Some of the potted cotton plants established with enough aphid populations were used for rearing of *C. sexmaculata*.

Adult beetles of six spotted zgzag ladybird beetle, *C. sexmaculata* were collected from cotton crop in the fields of ICAR-IARI, Pusa farm. Collected adult beetles were released on to potted cotton plants having established aphid population (in the screen cages). Due care was taken to avoid food shortage to the predatory beetles by adjusting beetle number as per the availability of aphid on plants. Ladybird beetles laid eggs mostly on the leaves of cotton plant. These eggs were regularly collected along with the leaf portion and kept for hatching in a Petri plate by providing aphid to newly hatched grubs.

These grownup first instar grubs were transferred to other Petri plates and reared individually till the pupation to avoid cannibalism (Hesler *et al.*, 2012). Prior to transfer of grubs, 5 ml of agarose-based Hoagland's media was poured in a Petri plate by keeping it in slant position and allowed to solidify. Then, freshly collected cotton leaf was cut at petiole and the cut end was inserted in to agarose-based Hoagland's media by keeping the top surface of leaf down in a Petri plate (Plate 3.3). Aphids were then transferred gently on to the leaf with camel hair brush and allowed to settle. Healthy grub of predator was then released in to Petri plate. Fresh aphids were added daily whereas, the cotton leaf was changed once in 3-4 days. Grubs were reared in the Petri plates till the pupation. Pupae were

kept separately for adult emergence and the emerged adults were transferred on to the cotton plants maintained in the screen cages having *A. gossypii* colonies. Eggs laid by the second-generation beetles were used for the study of biology and morphometry (Plate 3.4).



a. *A. gossypii* transfer to cotton plant



b. Parthenogenetic reproduction



c. Established aphid colony on cotton



d. Population buildup on young leaves



e. Established *A. gossypii* on cotton with different stages adult mortps

Plate 3.2 Rearing of *A. gossypii* from the field collected adults



Plate 3.3: Aphids are provided on cut leaf kept in a Petri plate. Leaf petiole was inserted in to agar-based Hoagland's media to maintain freshness



a. I instar grub feeding on aphid



b. IV instar grub and pupa



c. Adults

Plate 3.4: Stages of *C. sexmaculata* on cotton

3.2.4 Study of biology and morphometrics of predator

The study on biology of *C. sexmaculata* was conducted in the Biological Control Laboratory, Division of Entomology, ICAR-IARI at controlled conditions of $25 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH and photo period of 14 hr. Eggs laid by adult beetles were kept in the petri plate to observe incubation period and hatching percent. Growth and development were observed by providing aphid on cotton leaf substrate kept in a petri plate (100 mm Ø). Freshly collected cotton leaf was spread upside down in the petri plate, mixed population of second and third instar nymphs of aphid were gently transferred on to the leaf and allowed to settle. Newly hatched grub of *C. sexmaculata* was transferred on to the leaf (Dixon and Agarwala, 1999). Aagarose based Hoagland's media containing essential plant nutrients was used to keep the prolonged freshness of leaf.

In each petri plate 5 ml of Hoagland's media (1.5 %) was poured at one side by keeping in slant position and kept for solidifying. Cut end of petiole of cotton leaf was inserted in to Hoagland's media before transferring the aphids. Total 60 plates were maintained as replicates. Aphids were replaced every day with fresh ones where as cotton leaf for every three days.

The grubs were observed for every 3 hr for moulting, pre-pupation, pupation, adult emergence and mortality. For I instar 50, II instar 100, III and IV instar grub 150 aphids were provided as prey/ day.

The same procedure was followed for adult beetles. One day old adult beetles were transferred individually in to a petri plate containing 150 aphids. After premating period, pairing was done and allowed for 24 hr, and then the pair was separated and maintained individually till their mortality. Different biological parameters viz., fecundity, incubation period, hatching per cent, duration of each stage, sex ratio, oviposition period, longevity and mortality were recorded. During each stage, observed under stereo-binocular microscope for colour pattern. Nikon microscope (SMZ 800N) having camera (USB 500) with software, "VIMAGE 2016" was used to record morphometrics and bionomics parameters of the predator.

3.2.5 Foraging behavior of *C. sexmaculata* on *A. gossypii*

To understand the overall foraging behavior of *C. sexmaculata* and its influence on population dynamics of aphids, the studies on FP and life table (FP) of different stages of *C. sexmaculata* throughout their life period, then studies on FR and NR to draw the the TR of predator were under taken. Efficiency of conversion of ingested food (ECI) and intrinsic rate of increase in prey, *A. gossypii* were also studied to draw the population dynamics of predator and prey for simulation of models in future.

3.2.5.1 Predatory Potential (PP) of different stages of *C. sexmaculata* on *A. gossypii*

These studies were conducted at controlled conditions of $25 \pm 1^{\circ}\text{C}$, $60 \pm 5\%$ RH and a photo period of 14 hour. In the Petri plate (10 cm Θ), about 5 ml of agarose-based Hoagland's media containing essential plant nutrients was poured at one side by keeping Petri plate in slant position for solidifying. Freshly collected cotton leaf was spread upside down in the Petri plate by inserting cut end of petiole in to Hoagland's media to maintain prolonged freshness of leaf. Mixed population containing second and third instar of aphid was gently transferred with a camel hair brush on to the cotton leaf and allowed to settle. Newly hatched grubs of *C. sexmaculata* were collected from the stock culture and released individually on to aphids. Total 30 plates were maintained as replicates, each containing a single predator and 160 aphids, whereas two Petri plates were kept without predator as a control to observe natural mortality of prey. After every 24 hours, number of remaining aphids was noted and replaced with fresh aphids to maintain density of 160. Grubs were observed daily for moulting, pre-pupation, pupation, adult emergence and mortality. Number of preys consumed was worked out by deduction of remaining aphid count and control mortality from the total number of aphids.

Prey consumed = No. of prey provided – (No. of prey remained + No. of control mortality)

The same procedure was followed for adult male and female beetles. One day old adult beetles were transferred individually in to a Petri plate containing 160 aphids and daily count of remaining aphids was recorded.

3.2.5.2 Functional response studies

Various densities of aphid viz., 5, 10, 20, 40, 80, 120 and 160 were provided on cotton leaf substrate in a Petri plate. Aphids were gently transferred from stock culture on to the cotton leaf substrate placing with even distribution and allowed for three hours to settle. After settling, the leaf substrates with unevenly distributed aphids were discarded to ensure proper density of aphids. Mixed population containing second and third instar of aphid was used and discarded adults to avoid offspring production during the experiment. A single randomly selected individual of grub (I to IV instar), adult male and female was released on to each density of prey (Plate 3.5). First instar grub was released on to the prey immediately after hatching, whereas second instar was pre-starved for 3 hours, third instar for 6 hours, fourth instar for 12 hours and adult for 24 hours to normalise their appetite among the individuals. For adults, fifteen days old mated adults were used to represent an active reproductive adult. After releasing the predator, it was allowed to feed for 24 hours. After 24 hours, predator was removed and remaining aphids were counted. Ten replicates were maintained for each density of prey and one was kept without predator as a control to observe natural mortality of prey. Prey consumption was worked out by taken in to account of control mortality of aphids (Omkar and Kumar, 2012).

The polynomial logistic regression of the proportion of aphid killed in relation to the initial numbers was carried out for an insight about the type of functional response (Trexler and Travis, 1993; Juliano, 2001).

$$\frac{N_e}{N_0} = \frac{\exp(P_0 + P_1 N_0 + P_2 N_0^2)}{1 + \exp(P_0 + P_1 N_0 + P_2 N_0^2)}$$

Where, N_e is the number of preys eaten, N_0 is initial prey density, and P_0 , P_1 and P_2 are the intercept, linear and quadratic coefficients (maximum likelihood estimates), respectively. A significant negative value of P_1 on polynomial logistic regression indicates the Type-II FR. Otherwise a positive value of P_1 with a significant negative value of P_2 confirms the Type-III FR (Juliano, 2001). As the consumed prey is not replaced during the foraging period, Roger's random attack equation (Rogers-II FR equation) of type-II FR was used to fit the curve and estimate the FR parameters (Royama, 1971; Rogers, 1972).

Roger's random attack equation:

$$N_e = N_0[1 - \exp(a'(T_h N_e - T_t))]$$

Where,

N_e is the number of preys eaten, N_0 is initial prey density, a' is coefficient of attack, T_h and T_t is handling time and total time, respectively.



a. Functional response of *C. sexmaculata* adult



b. Functional response study of *C. sexmaculata* grub

Plate 3.5: Functional response of *C. sexmaculata* against *A. gossypii* kept on cotton leaf in Petri plate

3.2.5.3 Numerical response studies

NR was determined by using the method of Veeravel and Baskaran (1997). Fifteen days old active egg laying adult female beetles were starved for 24 hours prior to experimentation. These pre-starved female beetles were released individually on to different densities of aphid viz., 5, 10, 20, 40, 80, 120 and 160 on cotton leaf substrate kept in a Petri plate. Each Petri plate carried one density of prey and a female beetle. Five replicates for each prey density were kept and one plate without beetle as a control to record natural mortality of prey. After 24 hours, the beetles were removed and the data on number of aphid predated and eggs laid by beetle was noted. Fecundity curve was drawn with number of eggs laid as a function of initial prey density and resulted regression equation was used to work out the NR equation. The numerical response (NR) as a net reproductive turnout was worked out with the obtained fecundity curve equation by taking in to consideration the sex ratio and fraction of oviposition period to the total life period of female. The contribution of ingested food towards egg biomass at different prey densities was worked out using the equation given below. It is also referred to as efficiency of conversion of ingested food (ECI).

$$ECI = \frac{\text{Number of eggs laid}}{\text{Number of prey consumed}} \times 100$$

3.2.5.4 Rate of natural increase of prey, *A. gossypii*

Fecundity life table of cotton aphid, *A. gossypii* was constructed to know the natural increase. Newly formed adult females were transferred individually on to a cotton leaf kept in a Petri plate. Daily observations on mortality and fecundity were recorded for a total of 30 individual females until their death. Finite rate of increase (λ) per day was calculated from the constructed table.

3.2.5.5 Total response of *C. sexmaculata* and population change in *A. gossypii*

The TR of predator was taken as a product of FR and NR. All the parameters viz., finite rate of increase of prey (λ), FR, NR and TR of predator were assessed as a function of

single factor i.e., initial prey density (N_0) by keeping the duration of one day as total time (T_t). The change in aphid population was projected graphically by simulating the respective equations for the different instars of grub, male and female adults of *C. sexmaculata*.

3.2.6 Predation risk effect of *C. sexmaculata* on the cotton aphid, *A. gossypii*.

Although these experiments were planned to carryout with the aphids grown on cotton plant, faced difficulties while handling such as lower number of aphids per unit space, and difficulty in imposing predation risk due to larger size of plant. It was also tried on cut leaf of cotton where observed the disturbance to aphids while transferring and also a possibility of nutritional stress as using detached leaf. Hence, pre-experimental trials were carried out to find more feasible options involving less disturbance to aphids, accommodating a greater number of aphids per unit space, easy to handle while treatment exposure and easy to count.

Rearing of aphids on mesta, *H. cannabinus* was found more suitable for the present predation risk experiments. So, the cotton aphids, *A. gossypii* were exposed to the predation risk along with the host seedlings grown in a Petri plate.

3.2.6.1 Rearing technique for cotton aphid, *A. gossypii* on mesta, *H. cannabinus*

Mesta, *H. cannabinus* was selected for rearing of *A. gossypii* based on screening of different host plants in which had shown certain advantages such as very feasible to grow in Petri plate, small size of seed (29.91g/1000 seed), high germination per cent with viability of more than one year, very low cost, and longer life of seedling with a limited height when grown in a Petri plate. Initially it was observed that, cotton aphids settled nicely and completed development easily on seedlings. Considering this, an experiment was laid out to compare development and population increase at four different temperatures viz., 18, 21, 24 and 27 °C.

Petri plates (10 cm ø) were used as container to rear the aphids. In a Petri plate about 7-8 g of absorbent cotton was placed uniformly and about 100 ml of purified water (RO-Kent Water Purifier's) was poured. On this wet cotton, thirty seeds of *H. cannabinus*

were placed with uniform spacing and gently pressed. These Petri plates were arranged vertically one on another and kept for germination. After two days, when sprouting was observed, the Petri plates were kept open and allowed to grow for a day. Thinning was done to maintain 25 seedlings in each Petri plate uniformly. Germination and initial growth of seedling was obtained at 27 ± 1 °C and $65 \pm 5\%$ RH. These 3 days old seedlings were used for aphid rearing experiment.

From the stock culture of *A. gossypii* reared on potted cotton plants, parthenogenetic females were collected and transferred on to the *H. cannabinus* seedlings grown in Petri plate. Every day, 15-20 ml of purified water was poured in each Petri plate to maintain moisture. Aphid rearing was carried out at four different temperatures viz., 18, 21, 24 and 27 °C in the environmental chamber, Remi®-Walk-in-Stability Chamber (Plate 3.6) at 16:8 h of photo period and $65 \pm 5\%$ RH.

In order to know the total developmental period of nymph from the birth of first instar to become an adult through all the four instars, a single parthenogenetic adult was released on to each seedling (25 females for 25 seedlings in each Petri plate) and allowed to lay the nymphs for 24 hrs only and removed thereafter. The laid nymphs were observed daily for recording adults if any to know the developmental period. The developmental periods were also observed till the completion of three generations. Whereas for the developmental periods of each instar, count of each instar and adults were recorded at 24 h interval and removed the adults to avoid overlapping population. This was continued till every individual had developed in to an adult.

For population increase, a single parthenogenetic aphid per plant (25 females for 25 seedlings in each Petri plate) was released, allowed for reproduction and buildup of population. Regular count of nymphs, adults and winged morphs was recorded from the continued population separately on each plant for 30 days with an interval of 3 days. For each temperature three Petri plates (each containing 25 seedlings) were maintained as replicates. Along with the population count of aphids, plant height was also recorded to get idea about plant growth. After the completion of rearing experiments, aphid population was

maintained throughout the year by transferring them on to new seedlings after every 30 days and reared at 24 °C and 65±5% RH.



a. Aphid stock culture maintained on potted cotton plants



b. Aphid rearing on mesta seedlings at controlled conditions

Plate 3.6: Remi[®]-Walk-in-Stability Environmental Chamber used for aphid rearing

After one year, the aphids reared on *H. cannabinus* were transferred on to cotton plants for the establishment studies. Aphid establishment was tested on field plant, potted plant and also on excised leaves of cotton by transferring aphids @ of 100 adults/leaf.

3.2.6.2 Predation risk exposure and analysis of risk effect in *A. gossypii*

Conducted a preliminary study to know the effect of risk exposure duration on oxidative stress in *A. gossypii* through assessing activity levels of superoxide dismutase (SOD). By fixing 24 hours as treatment duration, assessed the predation risk levels under active predation and risk predation comparing to the un-exposed control. Enzyme analysis was done for the stress bio-markers taken SOD, CAT, MDA and GST. The ultimate effect of predation risk was assessed by observing the biological parameters.

3.2.6.2.1 Treatments

A container was designed with double cylinder system using Petri plates and iron mesh (Plate 3.7). Petri plates served as top and bottom of cylinder holding iron mesh as curved surface as fixed between two Petri plates. Inner small cylinder was made with Petri plates of size 10 cm diameter and kept in a bigger outer cylinder of size 18 cm diameter. This double cylinder container holds host seedlings along with aphids in the inner cylinder leaving empty inter cylinder space between the rims of two cylinders. For active predation, adult beetles of *C. sexmaculata* were released directly on to the aphids in the inner cylinder where beetles can consume aphids. For predation risk affect (Risk predation) beetles were released in to the inner cylinder space where beetles could not consume the aphid as it were separated by the iron mesh allowing only the olfactory cues to get in (Plate 3.8). Container having aphids without release of beetles was used as un-treated control.



a. Iron mesh used as a barrier to avoid entry of beetle



b. Closed container with Petri plates



c. Iron mesh container covered with a plastic jar

Plate 3.7: Container made with Petri plate and iron mesh to impose predation risk effect



a. Mesta Seedlings grown in a Petri plate



b. Aphids reared on mesta seedlings



c. Beetles released inside the container
(Active Predation)



d. Beetles released outside the container
(Risk Predation)

Plate 3.8: Imposing predation risk effect of *C. sexmaculata* on *A. gossypii*

3.2.6.2.2 Influence of exposure duration on predation risk effect and sustainability

To assess the oxidative stress under varying durations of predation risk exposure, a mixed population of nymphs and adults of *A. gossypii* was exposed to the predation risk (Non-consumptive) by releasing *C. sexmaculata* beetles in to inter cylinder space @ 100 per container and allowed for different durations viz., 1, 6, 12 and 24 hours. From the exposed populations, aphids were collected at 0, 1, 6, 12 and 24 hours after removal of predator and analyzed for SOD activity. Predation risk effect was assessed by comparing SOD levels with the control (without predator release) at varying time periods.

Duration of predator allowed for risk predation on aphid			Time of aphid sample collected after removal of predator for enzyme analysis				
			0 h	1 hr	6 hrs	12 hrs	24 hrs
		1 h					
	6 hours						
	12 hours						
24 hours							

3.2.6.2.3 Oxidative stress and change in biological parameters under active and risk predation

Based on the expression of SOD levels in the above experiment, the treatment (predator release) duration was fixed as 24 hours. A mixed population of nymphs and adults of *A. gossypii* grown on mesta seedlings was used for the treatment. Oxidative stress levels and biological parameters were assessed and compared among the following treatments.

1. Active predation (Consumptive+Non-consumptive): Predators released on to aphids and allowed to prey.
2. Risk predation (Non-consumptive): Predators released but not allowed to prey
3. No predation (Control): Predator was not released.

Immediately after 24 h of treatment, aphid was collected @ 100 mg per treatment and stored at -20 °C for the enzyme analysis. Simultaneously, 75 adult aphids from each

treatment were transferred on to the new seedlings of host plant to observe the biological parameters.

The collected aphid of 100 mg from each treatment was cold homogenized in 1 ml of 50 mM phosphate buffer (pH 7.0) and centrifuged at 3000 rpm for 15 min at 4 °C. The supernatant was used for the enzyme assay analyzed for the activity of SOD, CAT and Malonedialdehyde (MDA) to assess the oxidative stress levels.

3.2.6.2.4 Superoxide Dismutase (SOD) activity

Superoxide dismutase was quantified on the basis of its ability to inhibit photochemical reduction of nitrobluetetrazolium (NBT) following the method given by Beauchamp and Fridovich (1971). Reaction mixture of 3 ml was prepared by adding 0.2 ml of each methionine (200 mM), NBT (1.125 mM), EDTA (1.5 mM), riboflavin (75 µM), 25 µL of enzyme extract and remaining volume was made with 50 mM phosphate buffer (pH 7.8). After adding riboflavin, incubated under 15W fluorescent lamp for 10 minutes, taken in to dark for stopping the reaction and absorbance was read at 560 nm. One unit of SOD activity defined as the amount of enzyme required to inhibit 50% of NBT reduction under assay conditions. The values were given as SOD units/mg protein.

3.2.6.2.5 Catalase (CAT) activity

Reaction mixture was prepared with 1 mL of 0.01 M phosphate buffer (pH 7.8), 475 µL of double distilled water, 25 µL of enzyme extract and 0.5 mL of 0.2 M hydrogen peroxide. Exactly after 1 minute, 2 mL of dichromate acetic acid (DAA) was added and heated the tubes for 15 min in a boiling water bath. After cooling, absorbance was measured at 570 nm (Sinha, 1972). A standard curve was prepared with the range of 500 to 850 µL of H₂O₂ without containing enzyme extract. Activity of CAT was given as µM of H₂O₂decomposed per minute per mg protein.

3.2.6.2.6 Malondialdehyde (MDA) content

From the standard MDA solution (1 μ M) prepared a series of solutions ranging from 0.01 nM to 1000 nM were prepared by diluting with Glacial Acetic Acid (GAA). Thio Barbituric Acid (TBA) of 4 mM was prepared using GAA. One ml of each MDA and TBA was taken in 10 ml test tube and heated in a water bath at 95 $^{\circ}$ C for 60 minutes. After cooling, absorbance was read at 532 nm. Standard curve was prepared with serial concentrations. For samples, 50 μ L of extract was added to the GAA (950 μ L) then added 1 ml of TBA. Sample values were drawn from the standard curve. Units are expressed as nM/mg protein (Ohkawa et al., 1979). The protein content in each sample was determined using Bradford (1976) method and used for calibration of enzyme activity.

3.2.6.2.7 Effect of treatments on aphid biology

After 24-hour exposure to the treatments, parthenogenic adults were transferred on to fresh seedlings of host plant @ one aphid per seedling. Transferred adults were allowed for 24 hours to lay the nymphs, then transferred to other fresh seedlings and maintained till their death. These adults were observed for their survival, longevity, reproductive period and fecundity. Whereas, the laid nymphs were observed for their developmental period, mortality and adult morphs.

3.2.7 Orientation response of *C. sexmaculata* towards the prey and host plant extracts.

Extraction was done from aphid infested cotton leaves, only aphids, infested leaves along with aphids and leaves from an uninfested plant for the comparison. Two solvents, n-Hexane and Dichloromethane (DCM) were used for the extraction separately. Concentrated extracts were used for the identification of chemical compounds through GC-MS analysis and for the study of orientation behavior of *C. sexmaculata* in Y-tube olfactometry.

3.2.7.1 Extraction and identification of volatile chemical compounds

The following abbreviations were used for the extracted material.

UL (Uninfested Leaves extract): Leaves were collected from 45 days old, healthy and infestation-free cotton plants.

WOA (With Out Aphid extract): Leaves were collected from the aphid infested cotton plant and used for the extraction after removing the aphids with a clean brush. Prior to collection of leaves, potted cotton plants maintained in the screen cages were inoculated with aphids at 30 days after sowing and leaves were collected 15 days after inoculation (45 days old plants).

WA (With Aphid extract): Infested leaves with aphids extracted.

AO (Aphid Only): Aphids, *A. gossypii* were collected from infested cotton plants with the help of clean camel hair brush and extracted with the solvents.

Extracts collected from un-infested plant at 2, 4 and 10 h after spraying of 0.1% Methyl Salicylic acid (MeSA) to know the relativity of compounds. Volatiles also collected directly through Vacuum plant volatile collection chamber for the comparision.

For each treatment, fifty grams of extraction material (leaves with or without aphids) was collected, chopped into small pieces using scissors and immersed overnight in 150ml of hexane and dichloromethane (DCM) separately. Whereas, for AO (Aphid Only) 10 g of aphids were used for the extraction and immersed overnight in 30 ml of solvents. Then the extract was filtered using Whatman No.1 filter paper by passing through 15gm of anhydrous sodium sulphate (Na_2SO_4) to remove water from extracts. Then the extracts were decolourised by passing through the activated charcoal and concentrated under *vacuo* at 40-45 °C and 45-50 °C for hexane and DCM, respectively by using rotary evaporimeter (Plate 3.9). The extracts were concentrated to final volume of 1ml and collected in a separate GC-MS vials and stored in deep freezer at -80⁰C. These concentrated extracts were used for GC-MS and olfactometer studies (Seenivasagan *et al.*, 2009; Rakshith *et al.*, 2018). Similarly, the volatiles collected through Vacuum plant volatile collection system were also analysed by GCMS for the identification of compounds (Plate 3.10).



a. Leaf collection



d. Filtration of extract



b. Cutting of leaf in to small pieces



e. Concentration through Rotary evaporator



c. Soaking in DCM/n-Hexane



f. Identification of volatiles through GCMS

Plate 3.9: Extraction and identification of volatile chemicals from cotton



Plate 3.10: Volatile collection from cotton plant through Vacuum Plant Volatile Collection System (Mascot Enterprises, VCS-15)

3.2.7.2 Separation and characterisation of extracted compounds

Separation and identification of chemical components in different extracts were done by using Gas Chromatography-Mass Spectrometry (GC-MS) (Shimadzu QP 2010 Ultra) equipped with Rtx-5ms column measuring 30×0.25 mm and NIST14 library (Plate 3.9). Helium gas with a flow rate of 1ml/min was used as a carrier. Each extract used for GC-MS analysis was passed through 0.2μ nylon syringe filter. Then, 1 μ l volume of each sample (extract) was injected with the help of auto sampler. The injector and ion source temperatures were maintained at 230°C and 200°C , respectively. The compounds were identified by comparison of mass spectral fragmentation pattern with those stored in MS library (Seenivasagan *et al.*, 2009; Rakshith *et al.*, 2018).

Oven temperature	Raising rate	Hold time
Initially 40°C	-	1min
$40 - 60^{\circ}\text{C}$	3°C	10 min
$60 - 80^{\circ}\text{C}$	2°C	10 min
$80-120^{\circ}\text{C}$	1°C	2 min
$120-300^{\circ}\text{C}$	5°C	-

3.2.7.3 Y-tube olfactometer bio-assay

Extracts from 50 g of leaves (150 ml of n-hexane or DCM) and 10 g of aphids (30 ml of solvent) were concentrated to 1 ml which were considered as 100 per cent stock solutions. Pre-experimental trials were conducted with the different concentrations of stock solutions to standardise the test concentrations to be used in the actual experiments. Based on the results obtained in the pre-experimental trials, four concentrations viz., 1, 5, 10 and 15 per cent were fixed as the test concentrations for each treatment. Ten minutes duration was taken as the time to be observed (Plate 3.11).

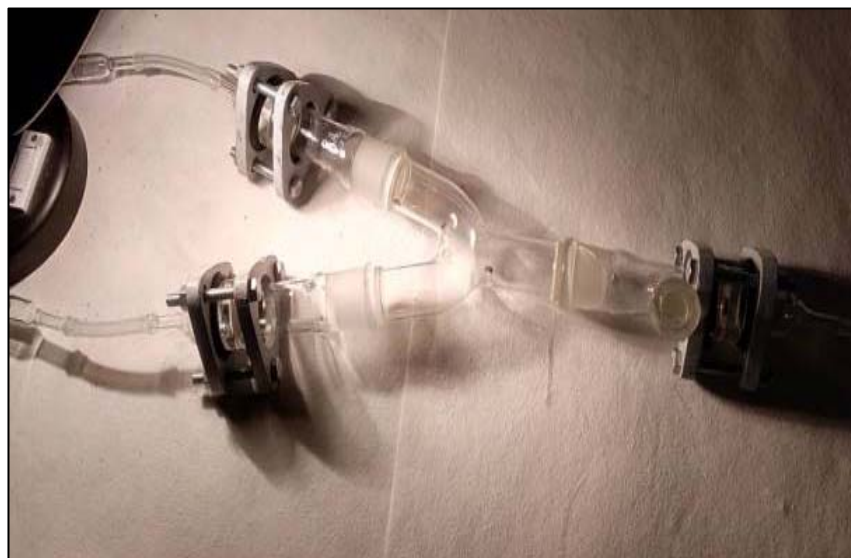


Plate 3.11: Y-tube olfactometry for orientation response studies

The dosage responses of *C. sexmaculata* males and females to various extracts were assessed using Y-tube olfactometer (Arm length 13 cm, main stem length 13cm and internal diameter 2 cm). The experimental room was maintained at 27 ± 1 °C and $65 \pm 5\%$ RH with a 40W fluorescent lamp as the source of light. Average air flow in each arm was 0.5ml/min.

For each concentration, 50 μ l of solution was applied on a filter paper strip (Whatman No.1, 2.5x0.5 cm size) and kept 30 seconds to evaporate then, it was placed

inside the olfactometer odour arm. Another arm was used as control with the respective solvent as an odour source.

A group of ten pre-starved (for 8 hrs) adult beetles of *C. sexmaculata* were released at the end of main stem of olfactometer. Studies were conducted for male and female beetles separately. For each sex, a total of 80 beetles in 8 batches were observed. Test solution was applied freshly for every batch. The olfactory response of each batch was recorded for 10 min. continuously.

The number of beetles moved towards the odour source and the time taken by an individual beetle to reach the odour source or control (solvent) was noted to record the orientation behaviour of *C. sexmaculata*. The Olfactometer was cleaned with acetone and allowed to dry in between each replication. The direction of odour arm was also changed after each replication (Seenivasagan *et al.*, 2009; Rakshith *et al.*, 2018).

3.2.8 Statistical analysis

Biological parameters and morphometric measurements of *C. sexmaculata* are expressed in mean $\pm$ SE using MS Excel of MS Office 2010.

Feeding potential throughout the life of predator was analysed through 'CONSUME-MSChart' (Chi 2012a) to display age-specific consumption (k_x), age-stage-specific consumption (C_{xj}) and the net consumption rate (C_0). Data on duration, mortality and number of eggs laid was subjected to the construction of age-stage and two-sex life tables (Chi and Liu 1985; Chi 1988) using TWSEX-MSChart (Chi 2012b).

The type of FR is assessed by polynomial logistic regression analysis of the proportion of prey consumed in relation to initial prey density (Juliano, 2001). Though Holling's Disc equation is known for fitting type-II FR (it assumes prey density is constant), we preferred Roger's equation as it assumes non-replacement of prey as followed in the study. With the best fitted line, estimated the coefficient of attack (a') and handling time (T_h). Statistical analysis is done using R software (R-Core Team, 2013). Logistic regression analysis is done using 'glm' function to determine the type of FR and confirmed

with ‘frair_test’ function using FRAIR package (Pritchard *et al.*, 2017). The same package is used for estimation of FR parameters and pair-wise comparison of different stages of predator for the parameters.

Olfactometric studies on dual choice tests is done by using Y-tube olfactometer for orientation response parameters. Responses of predator to different extracts at various concentrations is worked out with per cent means and their significant difference is analyzed through two sample t-test. Responses of male and female to different extracts at different concentrations are compared using GLM univariate analysis (SPSS) and Tukey’s homogeneity test.

The test for the significant differences among various biological parameters, enzyme activities and others is done using one-way analysis of variance (ANOVA) and grouping is done by Tukey’s HSD test using ‘agricolae’ package of R software.

4. RESULTS

The experimental results on different aspects of *C. sexmaculata* which includes biology, predation behavior (Feeding potential and functional response), numerical response, total response, orientation behaviour and, the natural increase of prey, rearing technique on mesta seedlings and predator induced predation risk effect on, *A. gossypii* is furnished in the following order.

- 4.1 Biology and morphometry of *C. sexmaculata* on cotton aphid, *A. gossypii*
- 4.2 Foraging behaviour of *C. sexmaculata* on *A. gossypii*
- 4.3 Rearing technique for cotton aphid, *A. gossypii* on mesta, *H. cannabinus*
- 4.4 Predation risk effect of *C. sexmaculata* on *A. gossypii*
- 4.5 Orientation behaviour of *C. sexmaculata* towards prey and host plant extracts

4.1 Biology and morphometry of *C. sexmaculata* on cotton aphid, *A. gossypii*

Studies were conducted at the controlled conditions of $25 \pm 1^{\circ}\text{C}$, $65 \pm 5\%$ RH and photo period of 16 hrs. A detailed account of biology with developmental periods and reproduction is given in Table 4.1. Morphometrical measurements for different stages is given in Table 4.2 along with images (Plate 4.1).

4.1.1 Eggs

Eggs were laid mostly on cotton leaf sometimes on the inner surface of petriplate in clusters of 6 to 24 eggs. Freshly laid eggs were cigar shaped, yellow in colour with smooth chorion and without any reticulations. With advancement of age, eggs changed to pale yellow then turned greyish and became completely black just before hatching. Length of eggs varied from 0.88 to 1.18 mm with a mean of 1.06 ± 0.06 mm and the breadth ranged from 0.34 to 0.45 mm with a mean of 0.38 ± 0.02 mm.

Table 4.1. Biology of *C. sexmaculata* reared on *A. gossypii*.

S.No.	Particulars	Period (Days)			Number observed
		Minimum	Maximum	Mean±SE	
1	Incubation	3.0	3.6	3.2±0.1	60
2	First instar grub	1.625	1.875	1.729±0.018	60
3	Second instar grub	1.000	1.375	1.161±0.022	56
4	Third instar grub	1.125	1.625	1.323±0.022	55
5	Fourth instar grub	1.750	2.250	2.000±0.027	54
6	Total grub period	6.000	6.375	6.214±0.030	54
7	Pre-pupal	1.000	1.875	1.318±0.050	54
8	Pupal	3.250	3.875	3.585±0.043	54
9	Adult-Male	19.0	86.0	65.1±7.3	24
10	Adult-Female	34.0	87.0	67.0±4.7	26
11	Pre-oviposition	5.0	8.0	6.0±0.4	26
12	Oviposition	20.0	49.0	42.0±3.3	26
13	Post-oviposition	9.0	36.0	19.0±3.1	26
14	Total life cycle of Male	97.25	30.12	75.56±8.76	24
15	Total life cycle of Female	98.0	45.0	78.06±5.96	26
16	Survival of grub (%)	-	-	90.0	60
17	Survival of pupa (%)	-	-	92.6	54
18	Adult emergence (%)	-	-	83.33	60
19	Sex ratio (Male: Female)	-	-	0.92:1	54
20	Fecundity (No. of eggs/female)	241	1377	756.5±128.9	26
21	Hatching percentage	69.2	100.0	94.9±4.7	100 clusters

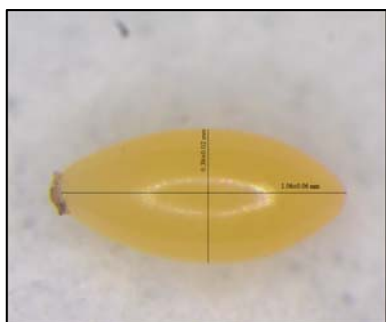
Table 4.2. Morphometrics of different stages of *C. sexmaculata* reared on *A. gossypii*.

Stage	Length (mm)±SE	Breadth (mm)±SE	Head width (mm)±SE	Number observed
Egg	1.06±0.06	0.38±0.02	-	30
Grub				
1 st instar	1.49±0.05	0.42±0.01	0.23±0.01	30
2 nd instar	4.13±0.09	0.68±0.03	0.45±0.03	30
3 rd instar	5.74±0.15	0.83±0.02	0.55±0.02	30
4 th instar	7.37±0.12	1.36±0.02	0.65±0.02	30
Pupa	3.83±0.13	2.32±0.1	-	30
Male	4.23±0.09	3.75±0.09	-	20
Female	5.35±0.25	4.06±0.15	-	20

Incubation period showed consistency and ranged from 3 to 3.6 days with an average of 3.2 ± 0.1 days. Almost all eggs laid in clusters hatched simultaneously without any lagging period, particularly, those in small clusters of 6 to 12 eggs. Hatching percentage varied from 69.2 to 100 percent with a mean of 89.9 ± 4.7 .

4.1.2 Grub

The grubs of *C. sexmaculata* completed development through four instars with three moults, then went for pupation with considerable pre-pupation period. Freshly hatched grubs were dark grey in colour with shining dark head capsule and legs. Length and breadth of grub ranged from 1.33 to 1.64 mm and 0.38 to 0.47 mm, respectively. Average length and breadth were 1.49 ± 0.05 and 0.42 ± 0.01 mm, respectively. Average width of head capsule of I instar grub was 0.23 ± 0.01 mm. Second instar grubs were greyish black in colour with black head capsule and legs, and white coloured patches on first and fourth abdominal segments. Length and breadth of II instar grub ranged from 3.94 to 4.42 mm and 0.58 to 0.75 mm, respectively.



Egg: Longitudinal view



Eggs: Radial view



Grub: I instar



Grub: II instar



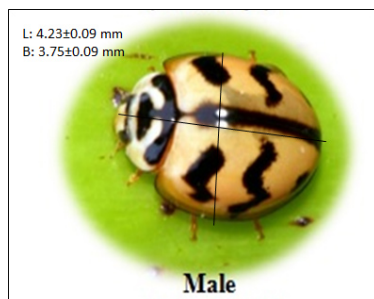
Grub: III instar



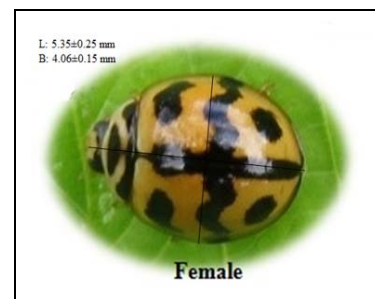
Grub: IV instar



Pupa



Male



Female

Plate 4.1: Morphometrics of different stages of *C. sexmaculata*

Average length and breadth was 4.13 ± 0.09 and 0.68 ± 0.03 mm, respectively. Average width of head capsule was 0.45 ± 0.03 mm. Third instar grub was glistening black in colour with the development of white spots on mid-dorsal line of meso thorax, meta thorax and first abdominal segments. Length and breadth of III instar grub ranged from 5.26 to 6.13 mm and 0.76 to 0.88 mm, respectively. Average length and breadth were 5.74 ± 0.15 and 0.83 ± 0.02 mm, respectively. Average width of head capsule was 0.55 ± 0.02 mm. Fourth instar grubs were yellowish black in colour with black colour legs. Length and breadth of IV instar grub ranged from 6.94 to 7.63 mm and 1.28 to 1.43 mm, respectively. Average length and breadth were 7.37 ± 0.12 and 1.36 ± 0.02 mm, respectively. Average width of head capsule was 0.68 ± 0.02 mm.

The duration of I, II, III and IV instar grub ranged from 1.625 to 1.875, 1 to 1.375, 1.125 to 1.625 and 1.75 to 2.25 days, respectively with a mean duration of 1.729 ± 0.018 , 1.161 ± 0.022 , 1.323 ± 0.072 and 2 ± 0.027 days, respectively. The total grub period ranged from 6 to 6.375 days with mean developmental period of 6.214 ± 0.03 days. Pre-pupation period was 1.318 ± 0.05 days. Faster development of grub might be due to good quality prey maintained on cotton leaf with agar-based Hoagland's media for prolonging freshness of leaves.

4.1.3 Pupa

Freshly formed pupae were whitish yellow then turned pale orange yellow. Pupae had symmetrically arranged black spots on each segment. Mean length and breadth of pupae was 3.83 ± 0.13 and 2.32 ± 0.1 mm., respectively. Pupation period ranged from 3.25 to 3.875 days with mean of 3.585 ± 0.043 days.

4.1.4 Adults

Newly emerged adults were yellowish, which later turned shining yellow with black spots. Later, adult sported zigzag markings on elytra. Length and breadth of male ranged from 3.98 to 4.47 and 3.56 to 4.02 mm with a mean of 4.23 ± 0.09 and 3.75 ± 0.09 mm, respectively. Whereas, in case of female, it ranged from 4.46 to 5.84 and 3.57 to 4.46 mm, with a mean of 5.35 ± 0.25 and 4.06 ± 0.15 mm, respectively. After adult emergence, three

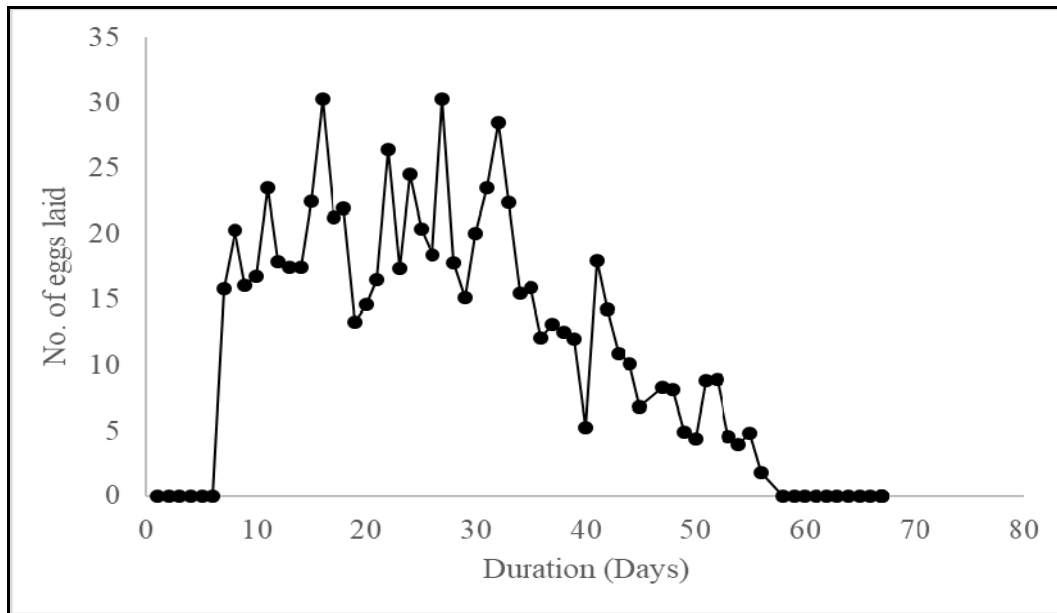
days of pre-mating period was observed whereas, pairing was noticed from fourth day onwards. Total longevity of male and female ranged from 19 to 86 and 34 to 87 days, respectively. However, mean duration of longevity was noticed as 65.1 ± 7.3 and 67 ± 4.7 days, respectively for male and female. Despite of shorter duration of grub stage, longevity of adults was found on higher side. In a total adult life span of 67 days for female, 6 ± 0.4 days were devoted for pre-oviposition, 42 ± 3.3 days for oviposition and 19 ± 3.1 days for post oviposition period.

4.1.5 Fecundity

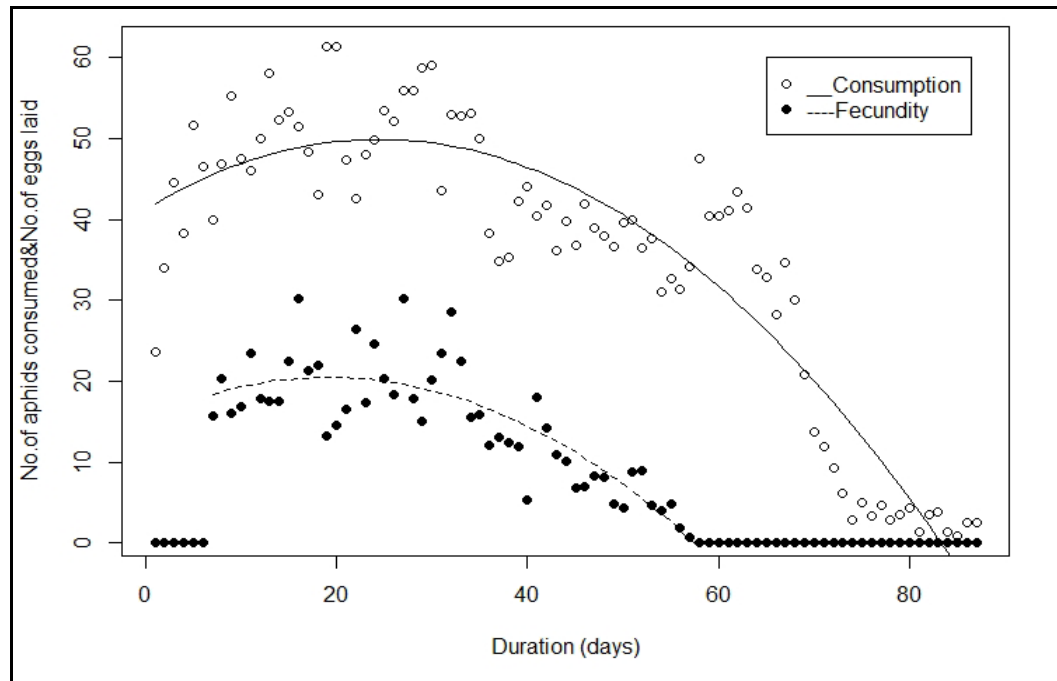
During the mean oviposition period of 42 days, fecundity has been found to increase for first 30 days and there after decreased gradually (Figure 4.1). The highest per day fecundity for individual was 72 whereas, the average fecundity was found to be 18.01 ± 0.88 eggs/day/female. The total fecundity varied between 241 and 1377 with pooled mean of 756.5 ± 128.96 eggs/female. Higher longevity and oviposition period contributed to enhanced fecundity (756.5).

4.1.6 Total life cycle

Life cycle of *C. sexmaculata* is depicted in the Plate 4.2. Total life cycle was completed with a mean of 78.06 ± 5.96 days in female and was found higher than male (75.56 ± 8.76). Survival percentage of egg, grub and pupa were 94.9, 90.0 and 92.6, respectively.



a. Fecundity during the life period of female



b. Fecundity and consumption during the life period of female

Figure 4.1: Fecundity of *C. sexmaculata* reared on *A. gossypii*

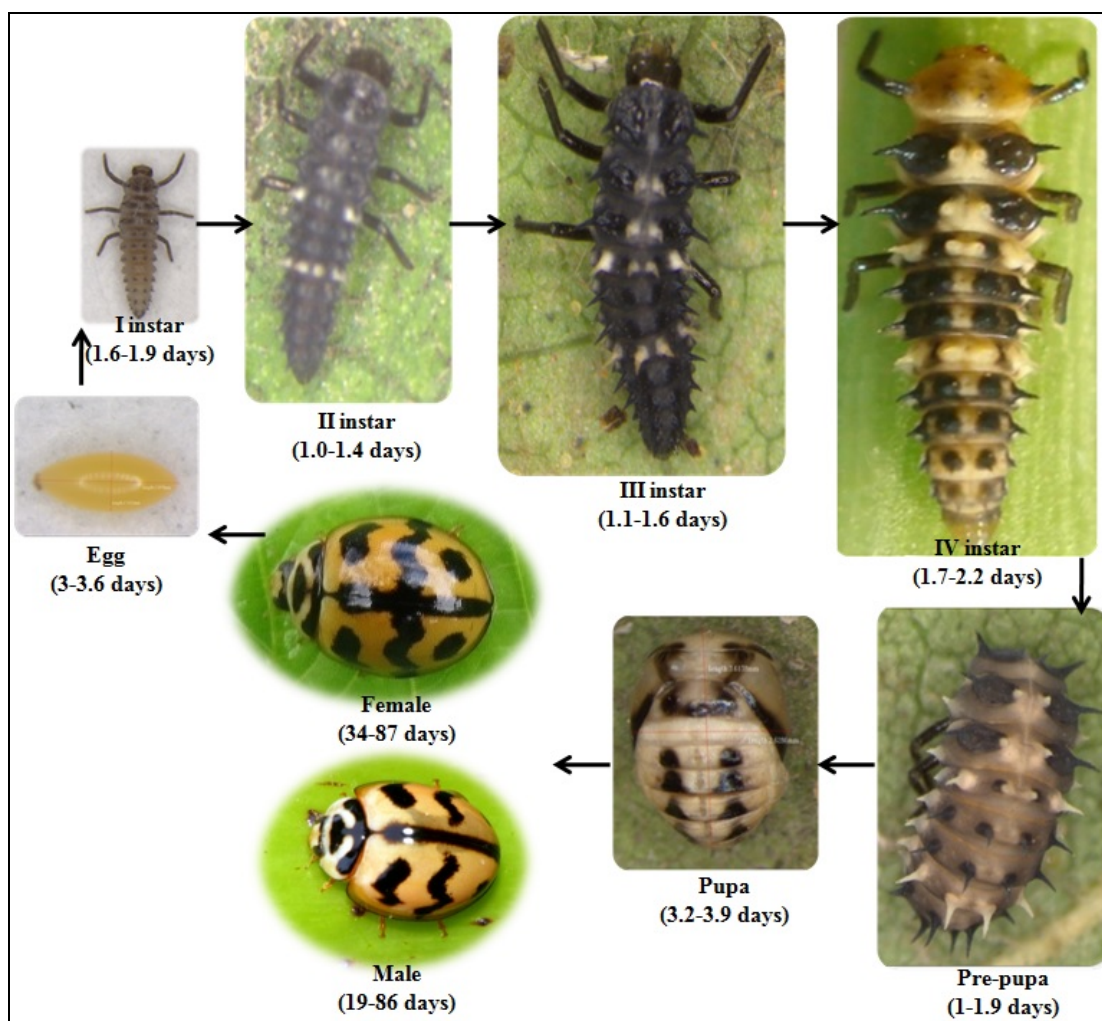


Plate 4.2: Life cycle of *C. sexmaculata* on *A. gossypii*

4.1.7 Life table

Under life table studies, age-specific survival rate (l_x) ranged from 0.03 to 0.91. The age-specific fecundity (m_x), age-specific maternity ($l_x m_x$) and age-stage-specific fecundity (f_x) of *C. sexmaculata* preyed on *A. gossypii* were maximum at 27 days age with the values of 14.63, 13.3 and 27.44, respectively (Figure 4.2). Life table studies showed intrinsic rate of increase (r) of 0.2156, net reproductive rate of 383.6, mean fecundity of 790.19 and the mean generation time of 27.29 days.

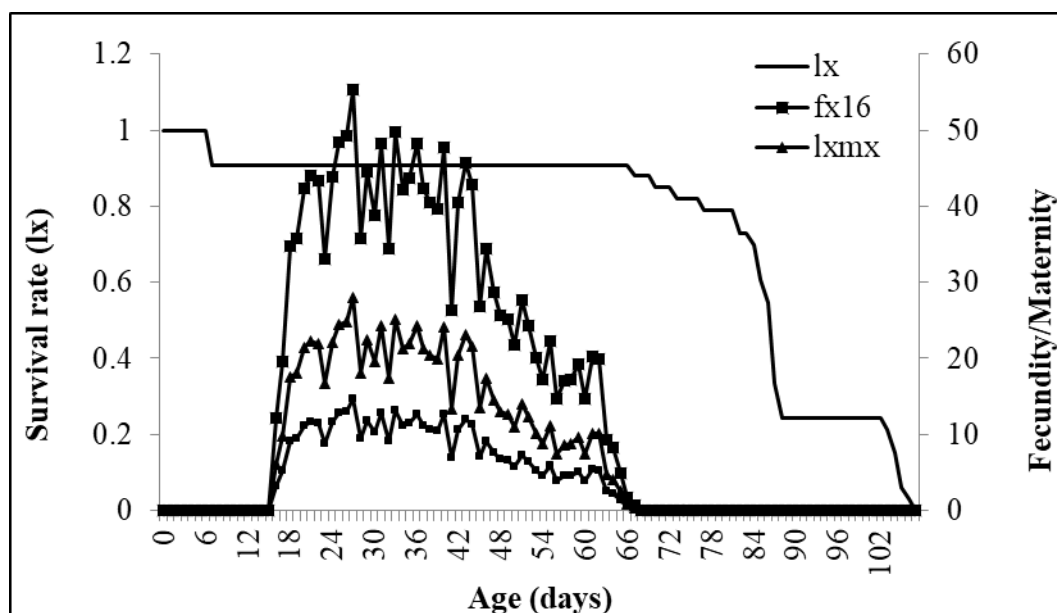


Figure 4.2: Age-specific survival rate (l_x), age-specific fecundity (m_x), age-specific maternity ($l_x m_x$) and age-stage-specific fecundity (f_x) of *C. sexmaculata* on *A. gossypii*.

4.2 Foraging behaviour of *C. sexmaculata* on *A. gossypii*

4.2.1 Feeding potential of different stages of *C. sexmaculata* on *A. gossypii*

The grub of *C. sexmaculata* developed faster with a mean duration of 6.66 days and displayed increasing consumption with the advancement of age and stage. Grub stages showed significant difference for predation rate with each other by displaying increase in consumption with advancement of stage and showed maximum consumption at fourth instar grub (101.43 ± 3.04). All the four instars of grub differed significantly with each other for daily consumption as well as total consumption.

Instar wise total consumption was recorded as 16.24 ± 1.26 , 20.3 ± 1.89 , 37.13 ± 2.10 and 101.43 ± 3.04 aphids per each individual of I, II, III and IV instar grub, respectively. Similarly, the mean daily consumption by the I, II, III and IV instar grub was recorded as 9.74 ± 0.74 , 18.61 ± 1.31 , 26.04 ± 2.33 and 36.66 ± 2.02 aphids, respectively.

Adult beetles exhibited high consumption rate over grub because of long duration of adult life with a significant more consumption of aphids by female compared to male.

Mean daily consumption analysed for all the stages of grub, male and female have shown significant superiority of female with the consumption of 43.13 ± 0.54 aphids/day followed by fourth instar grub (36.66 ± 2.02), male (36.62 ± 1.01), third instar (26.04 ± 2.33), second instar (18.61 ± 1.31) and the first instar grub (9.74 ± 0.74). The total consumption of the cohort of size 33 was 96842.5 aphids with net predation rate of 2934.62 aphids/predator. Female beetle has consumed a total of 3389.84 ± 70.26 aphids while the male consumed 3036.93 ± 109.37 aphids (Table 4.3).

Table 4.3: Predation rate of *C. sexmaculata* on *A. gossypii*

Predator stage		Predation rate	
		No. of aphids consumed by predator stage	No. of aphids consumed per day
Grub	First instar	$16.24 \pm 1.26a$	$9.74 \pm 0.74a$
	Second instar	$20.3 \pm 1.89b$	$18.61 \pm 1.31b$
	Third instar	$37.13 \pm 2.10c$	$26.04 \pm 2.33c$
	Fourth instar	$101.43 \pm 3.04d$	$36.66 \pm 2.02d$
Pre-adult	1 st to 4 th instar	173.69 ± 3.71	-
Adult	Male	$2856.36 \pm 3.74a$	$36.62 \pm 1.01d$
	Female	$3216.16 \pm 4.00b$	$43.13 \pm 0.54e$
Total	Male	3036.93 ± 109.37	-
lifespan	Female	3389.84 ± 70.26	-
Total consumption of the cohort (N=33)		96842.5	-
Net predation rate (C_o)		2934.62	-
Tukey's HSD		$P < 0.01$	$P < 0.001$

Predation rate (Mean $\pm$ SE) analysed with least square means and separated using Tukey's HSD test. Different letters next to the results denotes significant differences.

When sex and stage differentiation was taken in to consideration and analysed using CONSUME-MSChart, the age-specific consumption rate (k_x) and age-specific net consumption rate (q_x) and cumulative net consumption rate (C_y) of *C. sexmaculata* on *A.*

gossypii has been depicted in Figure 4.3. The maximum value for k_x and q_x was obtained at the age of 33 days with net predation rate (C_o) of 2934.62aphids/predator.

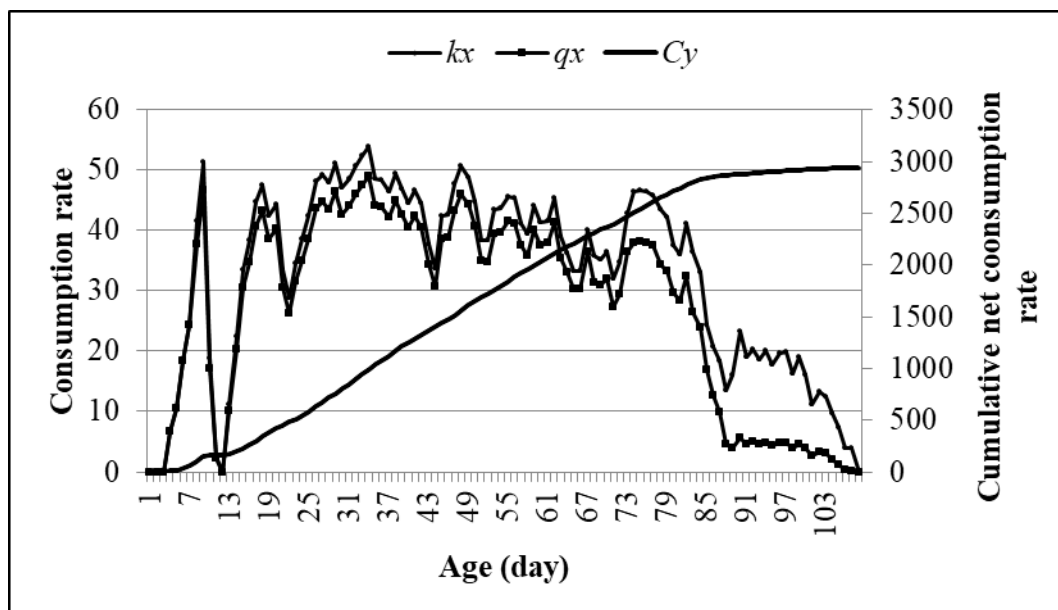


Figure 4.3: Age-specific consumption rate (k_x), age-specific net consumption rate (q_x) and cumulative net consumption rate (C_y) of *C. sexmaculata* on *A. gossypii*.

4.2.2 Functional responses of different stages of *C. sexmaculata* against *A. gossypii*

Polynomial logistic regression analysis of proportion of prey consumed (N_e/N_0) to the initial prey density (N_0) is resulted in significant negative linear parameters ($P_1 < 0$) and confirmed the type-II response for all the predatory stages (Table 4.4). The line fitted with Roger's model exhibited a characteristic polynomial decelerating type-II curve for all predatory stages (Figure 4.4). The curves displayed the increase in predation with the increase in prey density until reaching the satiation for all the stages of predator. Polynomial logistic regression curves also confirmed the type-II response by showing declining in proportion of predation with the increasing prey density (Figure 4.5). Female beetle has shown the highest coefficient of attack (a') followed by male, fourth and other instar grubs.

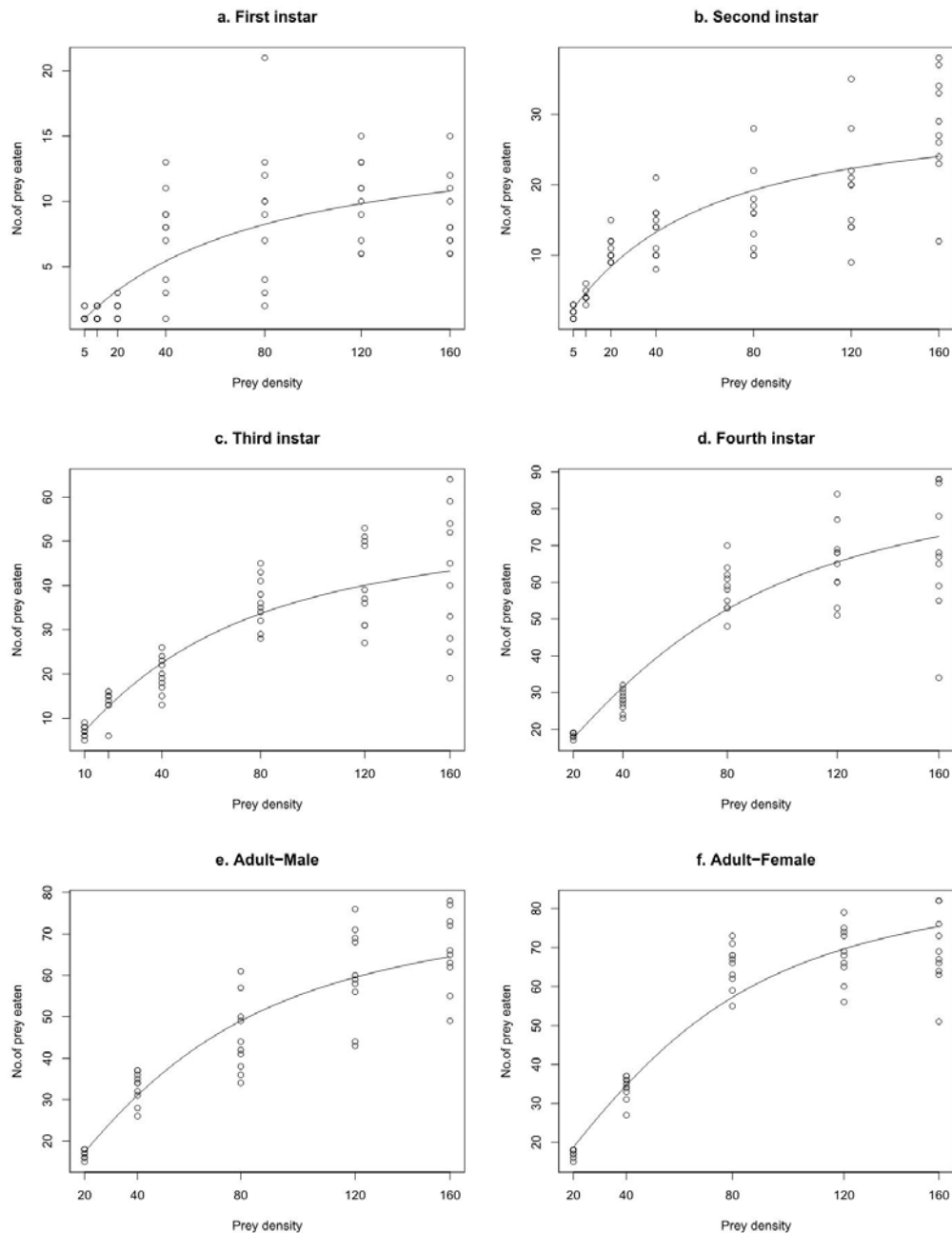
However, the fourth instar grub recorded lowest handling time (0.01041 day) followed by female, male and other instar grubs. Coefficient of attacks (a') displayed by the I, II, III and IV instar grub, male and female were 0.23456, 0.74714, 1.40934, 2.60469, 2.54052 and 3.45257, respectively. Among all the stages, fourth instar grub has showed high efficiency with maximum predation of 96.1 aphids per day followed by female (94.6), male (82.6), third instar (57.3), second instar (31.3) and first instar grub (15.6). The handling times (T_h) recorded for the I, II, III and IV instar grub, male and female were 0.06404 days, 0.03193, 0.01745, 0.01041, 0.01211 and 0.01 days, respectively (Table 4.5).

Pair-wise comparison of different stages of predator for the FR parameters viz., coefficient of attack (a') and handling time (T_h) revealed the significant difference among each other, except for fourth instar grub which was found non-significant with male for attack rate (a'), and female for handling time (T_h) (Table 4.6).

Table 4.4: Maximum likelihood estimates from polynomial logistic regression analysis of the proportion of prey eaten (N_e/N_0) by different stages of *C. sexmaculata* against initial number of aphids offered (N_0).

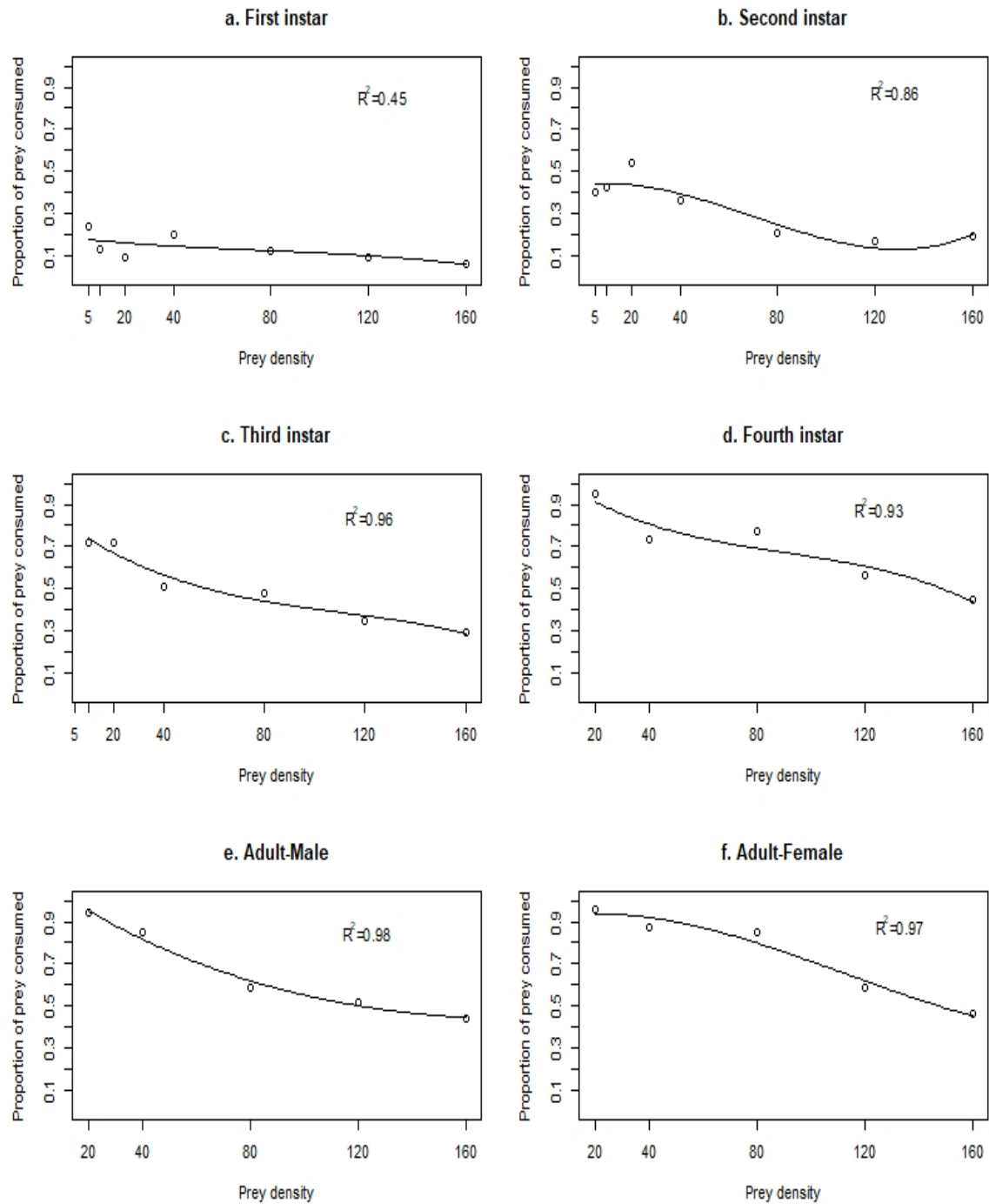
Growth stage	Parameter	Estimate	S.E.	Z value	P value
I instar grub	Intercept (P_0)	-1.4027	0.1141	-12.29	< 2e-16 ***
	Linear (P_1)	-0.0085	0.0011	-7.59	3.1e-14 ***
II instar grub	Intercept (P_0)	-0.2999	0.0854	-3.51	0.000447 ***
	Linear (P_1)	-0.0092	0.0008	-11.50	< 2e-16 ***
III instar grub	Intercept (P_0)	0.7597	0.0862	8.81	< 2e-16 ***
	Linear (P_1)	-0.0116	0.0008	-14.93	< 2e-16 ***
IV instar grub	Intercept (P_0)	2.0785	0.1069	19.44	< 2e-16 ***
	Linear (P_1)	-0.0150	0.0009	-17.30	< 2e-16 ***
Adult (Male)	Intercept (P_0)	1.6805	0.1004	16.74	< 2e-16 ***
	Linear (P_1)	-0.0133	0.0008	-15.95	< 2e-16 ***
Adult (Female)	Intercept (P_0)	2.8464	0.1252	22.73	< 2e-16 ***
	Linear (P_1)	-0.0201	0.0010	-20.18	< 2e-16 ***

*Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$
Significant negative value of linear parameter (P_1) confirmed the Type-II FR for all the stages of *C. sexmaculata* on *A. gossypii*.



Fitted lines exhibiting decelerating curves as a result of reduction in proportion of prey consumption with increasing prey density which indicates Type-II FR for all the four instars of grub (a, b, c, d), male (e) and female (f). Here, slope of curve indicates coefficient of attack (a') whereas, height of peak indicates lower handling time.

Figure 4.4: Functional response of *C. sexmaculata* against *A. gossypii* through Rogers-II functional response model



Gradual reduction in proportion of prey consumption with increasing prey density confirms type-II functional response.

Figure 4.5: Polynomial logistic regression of proportion of prey consumed to the initial prey density.

Table 4.5: Estimates of coefficient of attack (a') and handling time (T_h) for different stages of *C. sexmaculata* on *A. gossypii* ($T_t=1$ day) analysed through Rogers-II functional response equation.

Predator stage	Coefficient of attack (a')	95% CI		Handling time (T_h) (Days)	95% CI		Maximum predation per day (T_t/T_h) (Aphids/day)
		Lower	Upper		Lower	Upper	
First instar grub	0.23456	0.181	0.323	0.06404	0.048	0.081	15.6
Second instar grub	0.74714	0.596	0.940	0.03193	0.025	0.040	31.3
Third instar grub	1.40934	1.130	1.754	0.01745	0.013	0.021	57.3
Fourth instar grub	2.60469	2.138	3.230	0.01041	0.009	0.013	96.1
Adult (Male)	2.54052	2.081	3.144	0.01211	0.011	0.014	82.6
Adult (Female)	3.45257	2.845	4.215	0.01057	0.010	0.012	94.6

Table 4.6: Pair-wise differences between FR parameters of different stages estimated through FR coefficient test using delta parameter.

Stages of comparison	$D_{a'}$ (SE)	D_{Th} (SE)
I and II instar grub	-0.51260 (0.07904)***	0.03213 (0.00868)***
I and III instar grub	-1.17520 (0.11141)***	0.04659 (0.00843)***
I and IV instar grub	-2.36779 (0.16546)***	0.05358 (0.00838)***
I instar grub-Male	-2.30545 (0.18636)***	0.05193 (0.00839)***
I instar grub-Female	-3.21959 (0.20889)***	0.05353 (0.00838)***
II and III instar grub	-0.66360 (0.12896)***	0.01447 (0.00247)***
II and IV instar grub	-1.85684 (0.17786)***	0.02150 (0.00232)***
II instar grub-Male	-1.79322 (0.19727)***	0.01981 (0.00234)***
II instar grub-Female	-2.70605 (0.21858)***	0.02135 (0.00231)***
III and IV instar grub	-1.19344 (0.19442)***	0.00705 (0.00105)***
III instar grub-Male	-1.13111 (0.21244)***	0.00535 (0.00109)***
III instar grub-Female	-2.04381 (0.23233)***	0.00689 (0.00102)***
IV instar grub-Male	0.06348 (0.24512)NS	-0.00170 (0.00068)*
IV instar grub-Female	-0.84968 (0.26266)**	-0.00016 (0.00055)NS
Male-Female	-0.91269 (0.27613)***	0.00154 (0.00063)*

*Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$, NS-Non-significant. $D_{a'}$ and D_{Th} are estimates of the differences between stages for the parameters a' and T_h , respectively.

4.2.3 Numerical response of *C. sexmaculata* against *A. gossypii*

Prey density dependent fecundity and Efficiency of Conversion of Ingested (ECI) food of *C. sexmaculata* has been given with the corresponding number of preys consumed in Table 4.7. Egg laying by female has started at prey density of 20 with a mean fecundity of 8.4 ± 1.2 and increased with the prey density to reach the maximum of 21.8 ± 2.2 eggs at a prey density of 160. Fecundity has shown a strong correlation ($R^2=0.969$) with the prey density by exhibiting logarithmic curvilinear regression line (Figure 4.6). ECI was highest ($47.6 \pm 8.1\%$) at prey density of 20 which then decreased ($33.9 \pm 3.1\%$) with an increase in prey density by displaying a linear regression relationship (Figure 4.7).

The mean number of eggs laid per day was recorded as 0, 0, 8.4 ± 1.2 , 13.2 ± 1.4 , 19.4 ± 2.9 , 21.4 ± 0.7 and 21.8 ± 2.2 at the prey density of 5, 10, 20, 40, 80, 120 and 160 aphids, respectively.

Fecundity regression curve as a function of prey density (N_0) resulted the following equation.

$$\text{No. of eggs laid/female} = 7.173 \log_e N_0 - 13.42$$

Oviposition period (32 days), total period of female (67 days) and female to adult ratio (0.533) obtained in the present study were used to draw the NR equation.

$$NR = (7.173 \log_e N_0 - 13.42) 32/67 \times 0.533$$

Where, N_0 is the initial prey density.

Table 4.7: Oviposition and Efficiency of Conversion of Ingested (ECI) food of *C. sexmaculata* on *A. gossypii*

Prey density	No. of aphids consumed	No. of eggs laid	ECI
5	4.8±0.2	0	0
10	9.8±0.2	0	0
20	18±0.7	8.4±1.2	47.6±8.1
40	31.6±1.4	13.2±1.4	41.8±4.0
80	54±1.8	19.4±2.9	35.8±5.1
120	61.6±2.2	21.4±0.7	34.8±1.3
160	64.2±2.7	21.8±2.2	33.9±3.1

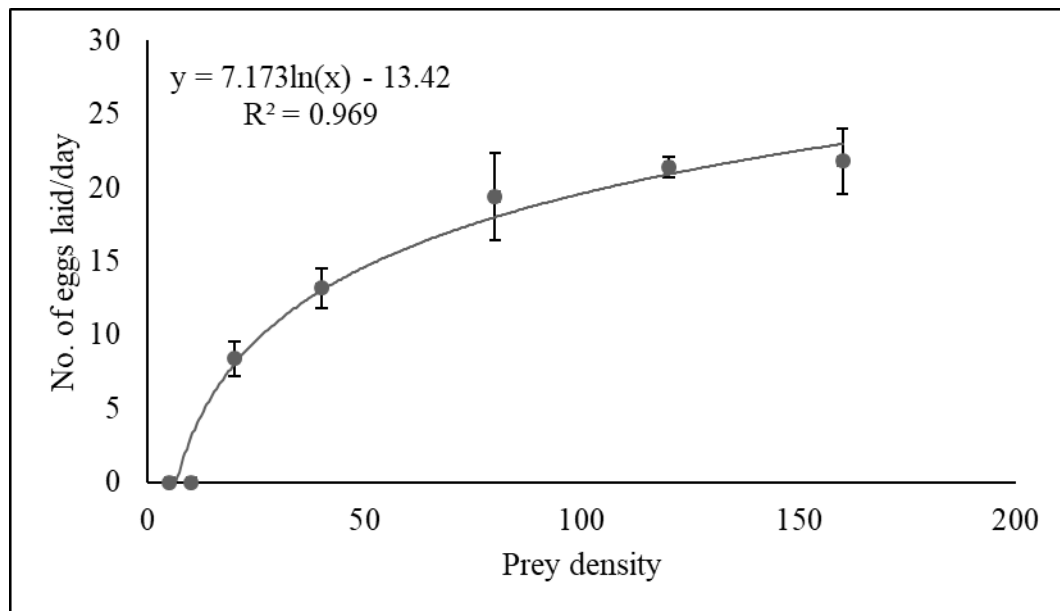


Figure 4.6: Fecundity of *C. sexmaculata* in response to the initial prey density of *A. gossypii*

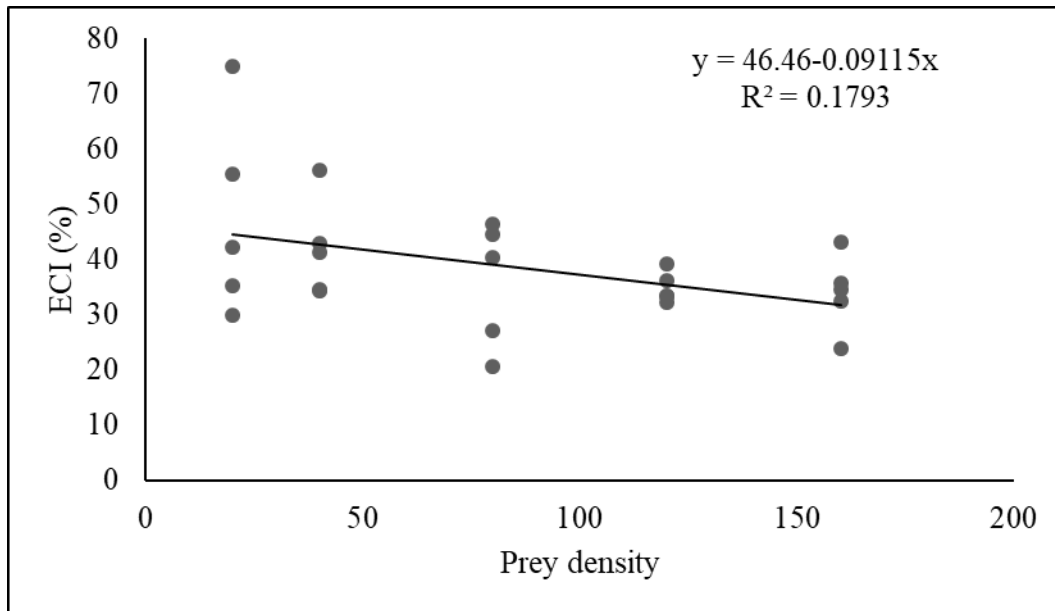


Figure 4.7: Efficiency of conversion of ingested food (ECI) of *C. sexmaculata* on *A. gossypii*

4.2.4 Natural increase of prey, *A. gossypii*

In the absence of predator, fecundity life table study of parthenogenetic aphid population has resulted in the finite rate of increase (λ) of 2.162 per day (Table 4.8).

4.2.5 Total response of predator and population change in prey

The TR of predator was attained by multiplying NR equation with the respective FR equations for the different stages. Increase in prey population at a finite rate of increase ($\lambda=2.162$) was taken as a prey recruitment factor while depletion at a rate of TR of predator. The difference of TR to the natural increase of prey was considered as a net population to be resulted for the next day.

Prey recruitment= $N_0 \lambda$; Depletion of prey=TR; Net population= $N_0 \lambda$ -TR; Time=1 day.

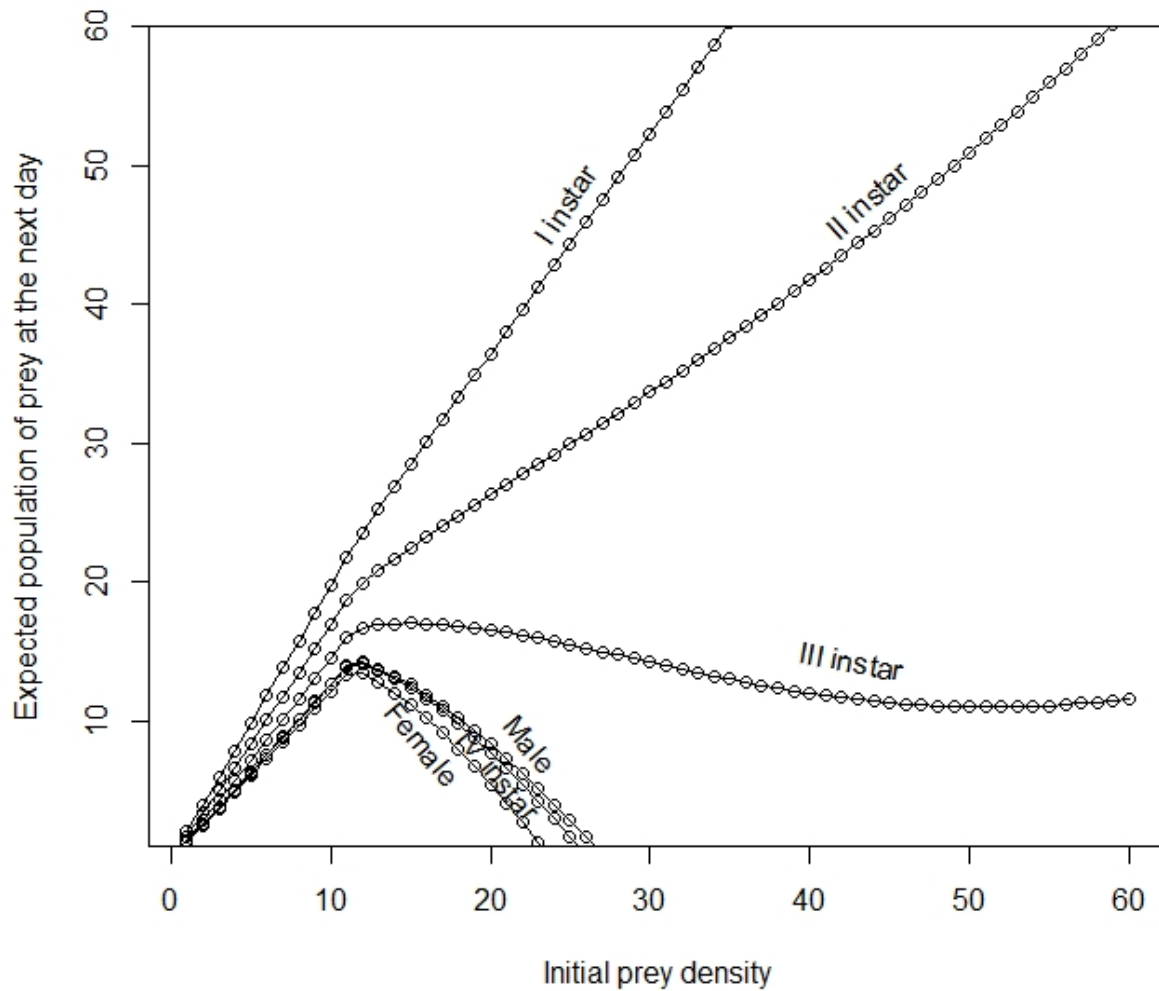
Population change of prey was projected by simulating respective equations, as taken initial prey density (N_0) on x-axis and the expected net population at the next day on

y-axis. The simulated curves for different stages of predator exhibited prey elimination by female, male and fourth instar grub of predator while other instars could not. Among all the stages, female was the most effective, as it showed rapid prey elimination by keeping it below 25, while male and fourth instar grub could kept it below 30 (Figure 4.8).

Table 4.8: Fecundity life table of *A. gossypii* in the absence of predator

Age in days (X)	lx		fx	lxfx	X.lxfx
	Number	Probability			
0	100	1.00	0.00	0.00	0.00
1	98	0.98	2.47	2.42	2.42
2	95	0.95	4.15	3.94	7.89
3	91	0.91	5.16	4.70	14.09
4	85	0.85	3.96	3.37	13.46
5	85	0.85	3.15	2.68	13.39
6	82	0.82	2.14	1.75	10.53
7	80	0.80	1.77	1.42	9.91
8	73	0.73	1.46	1.07	8.53
9	65	0.65	0.91	0.59	5.32
10	54	0.54	0.55	0.30	2.97
11	36	0.36	0.30	0.11	1.19
12	12	0.12	0.35	0.04	0.50
13	10	0.10	0.03	0.00	0.04
14	8	0.08	0.00	0.00	0.00
15	0.00	0.00	0.00	0.00	0.00
		Σ	26.40	22.38	90.24

	Formula	Value
Gross Reproductive Rate (GRR)	Σfx	26.40
Net reproductive rate (R_0)	$\Sigma lxfx$	22.38
Approximate generation time (T_c) in days	$\Sigma X.lxfx / \Sigma lxfx$	4.03
Intrinsic rate of natural increase (rm)	$\log_e R_0 / T_c$	0.771
Finite rate of increase (λ)	e^{rm}	2.162



A continuous vector taken on X-axis as initial prey density (N_0), simulated for expected net population to be remained for the next day as a result of difference between increase of prey at finite rate of increase ($N_0\lambda$) and removal of prey at a rate of TR (FR X NR). NR value ≤ 1 is considered as 1. Respective TR equations for different instars of grub, male and female were used for simulation of curves for respective stages. Curves for first and second instar showing continuous increase of aphid whereas, fourth instar, male and female has brought down the aphid population to zero by keeping it below 30.

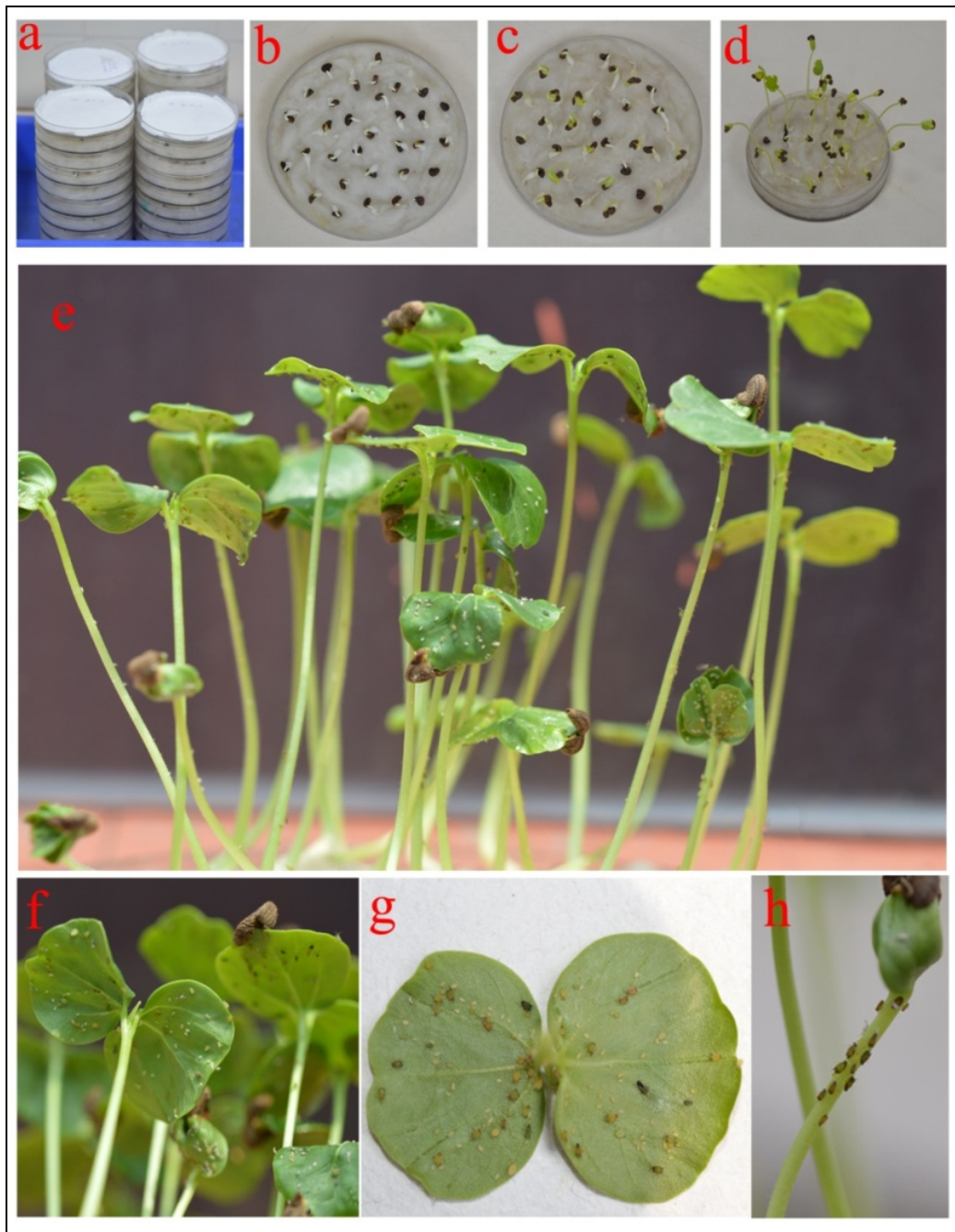
Figure 4.8: Population change of *A. gossypii* according to the total response of different stages of predator, *C. sexmaculata*.

4.3 Rearing technique for cotton aphid, *A. gossypii* on mesta, *H. cannabinus*

Among the different host species tested in the preliminary studies, mesta was found most suitable for rearing cotton aphid, *A. gossypii* in a larger number. Seedlings grown in a Petri plate supported aphid rearing in good numbers for about 25-30 days. Details of observations on rearing technique and results of different biological parameters along with population growth of aphid at various temperatures are furnished.

4.3.1 Rearing method

Aphid rearing setup on host seedlings raised in Petri plates is depicted in plate 2. Seeds of mesta, *H. cannabinus* grew well on absorbent cotton with germination per cent more than 90. Just in three days after sowing the seedlings with fully opened cotyledonary leaves were ready to host the aphids. Each Petri plate (10 cm Ø) could accommodate a stand of 25 seedlings (Plate 4.3 a-d). These seedlings supported the population buildup of *A. gossypii* up to 33 days after sowing. At all the four temperatures viz., 18, 21, 24 and 27 °C the seedlings were healthy without variations in size of cotyledonary leaves. Normal leaves started coming just after 25 days of sowing but not fully developed till the completion of 30 days. The seedlings grew with increase in height up to 12-15 days after sowing and attained maximum height as 8.5, 10, 10 and 13cm at 18, 21, 24 and 27 °C, respectively. Initially, aphids colonized on cotyledonary leaves, mostly confining to underside. However, the adults later moved to upper side of leaf and towards stem. At 30-35 days of sowing the seedlings started turning yellow, little early in seedlings raised at 27 °C. Aphids completed three generations within 30 days of sowing at all the temperatures, except 18 °C, where it took 35 days (Plate 4.3 e-h).



Rearing stages and colonization of *A. gossypii* on seedlings of *H. cannabinus*. Seed kept for germination: (a-d) 0, 1, 2 and 3 days after keeping for germination (e) Aphid establishment 18 days after release (f) Aphid on both sides of leaf (g) Different stages of aphid on underside of leaf (h) Aphid on stem.

Plate 4.3: Rearing technique for *A. gossypii* on mesta seedlings

4.3.2 Developmental period

Variation in the developmental periods with respect to the temperature and generation was observed. The fastest development was recorded at 27 °C with a mean duration of 4.22 ± 0.69 days over three generations. Developmental period increased with reduction in temperature with a significant difference between all the treatments. The first generation took significantly higher duration for all the temperatures, except for 27 °C, for which a significant difference was not observed for developmental period among the generations (Table 4.9).

Developmental periods were also observed for individual instars (I to IV) of cotton aphid. The first instar recorded the highest developmental period, while fourth instar the least. However, the duration of first instar got reduced with increasing temperature and differed significantly with other treatments. For the fourth instar a significant difference was observed for all the temperatures, except at 18 and 21 °C (Table 4.10).

4.3.3 Population growth

Population of different morphs of aphids viz., nymphs, wingless adults and winged adults at four different temperatures was seen as follows.

4.3.3.1 Nymphs

Nymphal population increased geometrically from first day to 12 days of releasing, then increased exponentially up to 21-24 days and there after a faster decline was observed. The highest number of 568.9 ± 24.8 nymphs per seedling was recorded at 24 °C. Population growth of nymphs was maintained more than 400 consecutively from 18 to 21 days after release at 24 °C. There was a rapid decline in population growth of nymphs towards reaching 30 days of releasing for all the temperatures and maintained more than 100 nymphs per seedling (except 27 °C) which was substantially higher to transfer on to new seedlings for continuous rearing (Table 4.11 & Figure 4.9).

Table 4.9: Developmental period for each generation of *A. gossypii* at different temperatures

Generation of aphid	Developmental period in days (Mean±SE)			
	18 °C	21 °C	24 °C	27 °C
I gen.	11.12±0.96a	7.13±0.81a	6.69±0.39a	4.25±0.70a
II gen.	9.21±1.08b	6.71±1.06b	4.97±0.62b	4.31±0.74a
III gen.	10.58±0.80c	6.75±0.97b	5.49±0.57c	4.10±0.61a
<i>P</i> value	<2e-16	0.00295	<2e-16	0.0718
HSD (0.05)	0.314201	0.308544	0.175544	0.224474
Mean	10.29±1.25a	6.86±0.97b	5.69±0.89c	4.22±0.69d

Tukeys HSD, $P < 0.05$, d.f. (2, 312). Values with the same letter are not significantly different for generations (*i.e.*, between the rows). Means over 3 generations significantly differed with each other for temperatures with *P* value of 2.49e-05 and Tukeys HSD value of 0.999 (*i.e.*, between the columns).

Table 4.10 Developmental period of each instar of *A. gossypii* at different temperatures

Stage of aphid	Developmental period in days (Mean±SE)				<i>P</i> value	HSD (0.05)
	18 °C	21 °C	24 °C	27 °C		
First instar	3.89±0.75a	1.94±0.50b	1.70±0.40c	1.30±0.40d	<2e-16	0.1902
Second instar	2.21±0.90a	1.64±0.54b	1.04±0.19c	1.06±0.43c	<2e-16	0.2061
Third instar	1.79±0.53a	1.71±0.60a	1.15±0.36b	1.10±0.43b	<2e-16	0.1752
Fourth instar	1.46±0.50a	1.43±0.50a	1.07±0.30b	0.79±0.41c	<2e-16	0.1546

Tukeys HSD, $P < 0.05$, d.f. (3, 414). Values with the same letter are not significantly different for temperatures (*i.e.*, between columns).

Table 4.11 Population growth of different morphs of *A. gossypii* reared at different temperatures (mean±SE)

Days After Release (DAR)	Aphid Morph	Temperature				F cal	P value
		18 °C	21 °C	24 °C	27 °C		
3	N	5.5±0.2	5.2±0.5	5.2±0.3	4.6±0.1	0.724	0.0608NS
6	N	14.1±0.4	14.2±0.2	12.7±0.5	8.6± 0.7	0.741	0.557NS
	WLA	Nil	Nil	1.2±0.4b	2.0±0.2a	9.72	0.0356*
9	N	18.1±0.7b	24.9±1.2a	28.7±1.2a	19.2±1.6b	17.88	0.0007***
	WLA	Nil	Nil	5.4±0.5b	7.6±0.7a	8.522	0.0433*
12	N	19.3±2.7b	15.9±0.6b	50.1±2.4a	45.7±5.7a	38.64	4.2e-05***
	WLA	2.4±0.3c	4.4±0.8c	27.7±3.3a	18.0±1.3b	74.3	3.5e-06***
15	N	29.3±5.0c	61.2±2.9c	153.0±1.9b	229.1±30.7a	66.0	5.5e-06***
	WLA	10.3±1.1c	15.6±1.8bc	26.1±3.6a	22.2±3.4ab	19.5	0.0005***
18	N	104.4±6.5c	247.9±18.7b	412.8±17.7a	312.0±11.0b	75.88	3.2e-06***
	WLA	12.5±0.6c	21.3±2.9b	26.6±0.8a	19.9±2.1b	22.74	0.0003***
	WA	Nil	Nil	Nil	6.3 ±1.4		
21	N	293.7±32.9b	444.1±12.1a	516.6±39.4a	221.4±14.3b	47.89	1.9e-05***
	WLA	18.6±2.8b	23.9±4.5ab	30.0±1.8a	19.0±2.5b	7.479	0.0104*
	WA	11.4±1.5bc	16.3±1.6b	8.5±1.5c	24.7±1.2a	28.98	0.0001***
24	N	391.2±13.9b	406.7±42.4b	568.9±24.8a	133.4±7.9c	121.6	5.2e-07***
	WLA	19.2±3.0b	27.3±0.9a	17.7±3.8bc	11.3±1.0c	20.85	0.0004***
	WA	13.3±1.1bc	17.9±1.4b	9.9±2.0c	27.7±1.5a	31.92	8.4e-05***
27	N	366.8±12.7a	258.5±20.5b	234.1±19.0b	51.4±9.2c	126.6	4.4e-07***
	WLA	12.9±1.0b	20.5±1.6a	16.7±1.9b	6.6±0.8c	39.31	3.9e-05***
	WA	15.8±2.4bc	20.3±1.9b	12.7±1.1c	29.2±1.1a	42.87	2.8e-05***
30	N	157.8±10.5a	139.6±4.8a	137.9±8.2a	25.8±7.0b	52.16	1.3e-05 ***
	WLA	6.3±0.6b	14.7±1.5a	10.5±1.6ab	6.5±0.6b	13.47	0.0017**
	WA	17.8±2.6c	22.5±2.1b	14.7±1.6c	33.3±1.8a	63.21	6.5e-06***

N-Nymphs, WA-Winged Adults and WLA-Wingless Adults. Wingless aphids started appearing from 6 DAR. Winged adults started appearing from 18 DAR.

Values with same letters are not significantly different between the columns. *Significant at $P<0.05$, ** Significant at $P<0.01$, *** Significant at $P<0.001$, NS-Non-significant.

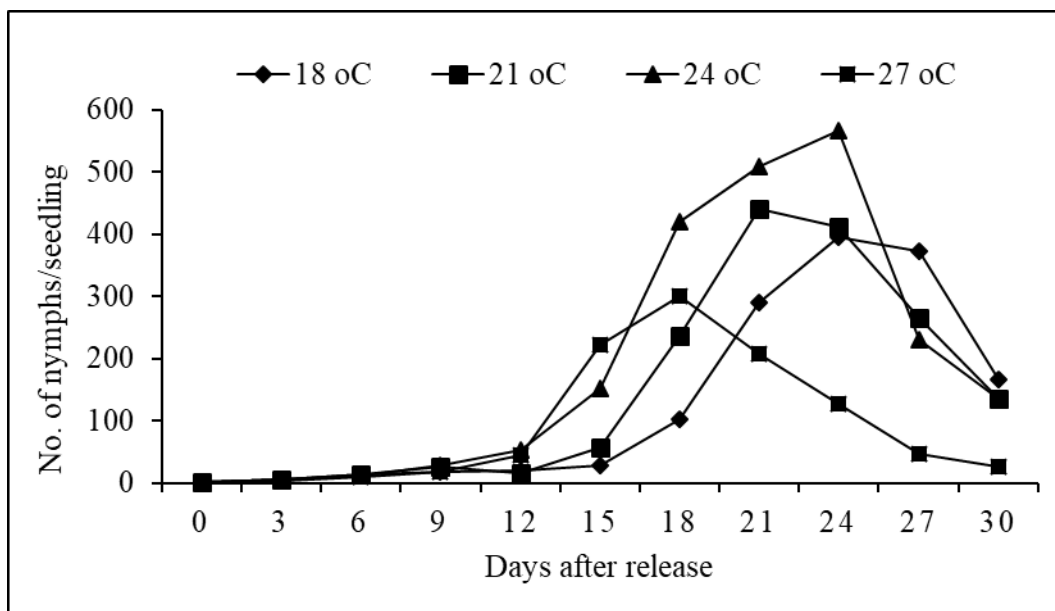


Figure 4.9: Population of nymphs of *A. gossypii* reared at different temperatures on mesta seedlings.

4.3.3.2 Wingless adults

As nymphs developed and progressed in to adults, the wingless adult population has started appearing from sixth day of releasing at 24 and 27 °C whereas, it was observed from 12th day after releasing at 18 and 21 °C. The adult population was noticed highest at 24 °C (30 ± 1.8) followed by 21 (27.3 ± 0.9), 27 (22.2 ± 3.4) and 18 °C (19.2 ± 3.0) at 21, 24, 15 and 24 days after release, respectively. It was noticed consistently higher for ten consecutive days from 12th to 21st day after release at 24 and 27 °C with more than 25 and 20 adults per seedling, respectively. At 21 °C, it was observed more than 20 adults per seedling between 18 to 27th day after release. At 18 °C, the wingless adult population was found lowest with delayed occurrence compared to other temperatures (Figure 4.10).

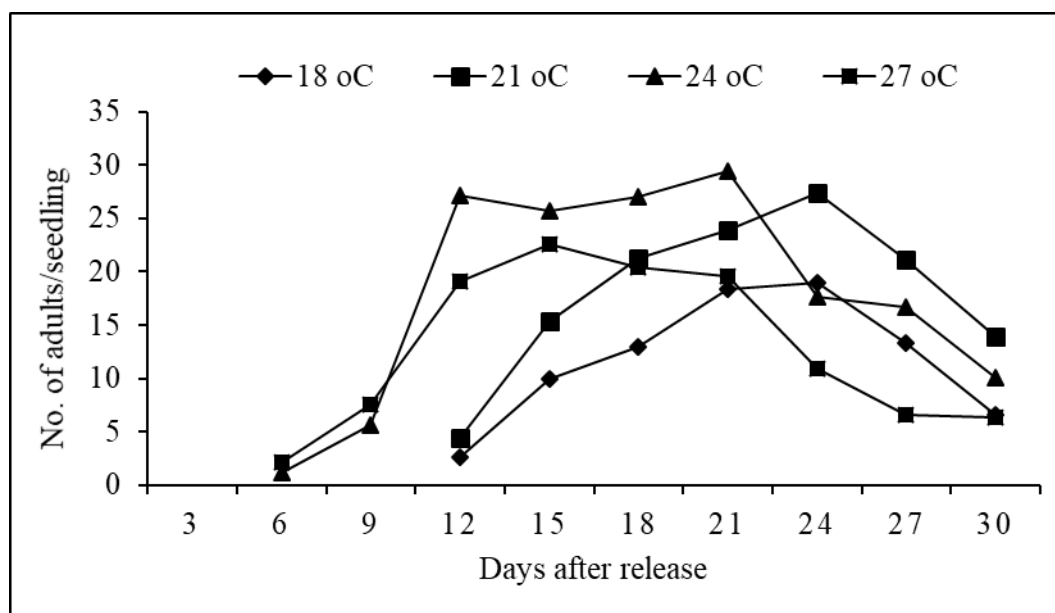


Figure 4.10: Population of wingless adults of *A. gossypii* reared at different temperatures on mesta seedlings.

4.3.3.3 Winged adults

Winged adults were observed early in case of 27 °C where, they started appearing from 18th day after release whereas, for other temperatures observed from 21 days after release. The number of winged aphids gradually increased from the first appearance till the end of experiment (up to 30 days after initial release) at all the temperatures. The cumulative total of winged aphids at 30 days after release was noticed highest at 27 °C (33.3 ± 1.8 /seedling), which is significantly better with other temperatures. Among the other temperatures, the cumulative total was highest at 21 °C (22.5 ± 2.1) followed by 18 (17.8 ± 2.6) and 24 °C (14.7 ± 1.6) however, there is no significant difference among them (Figure 4.11).

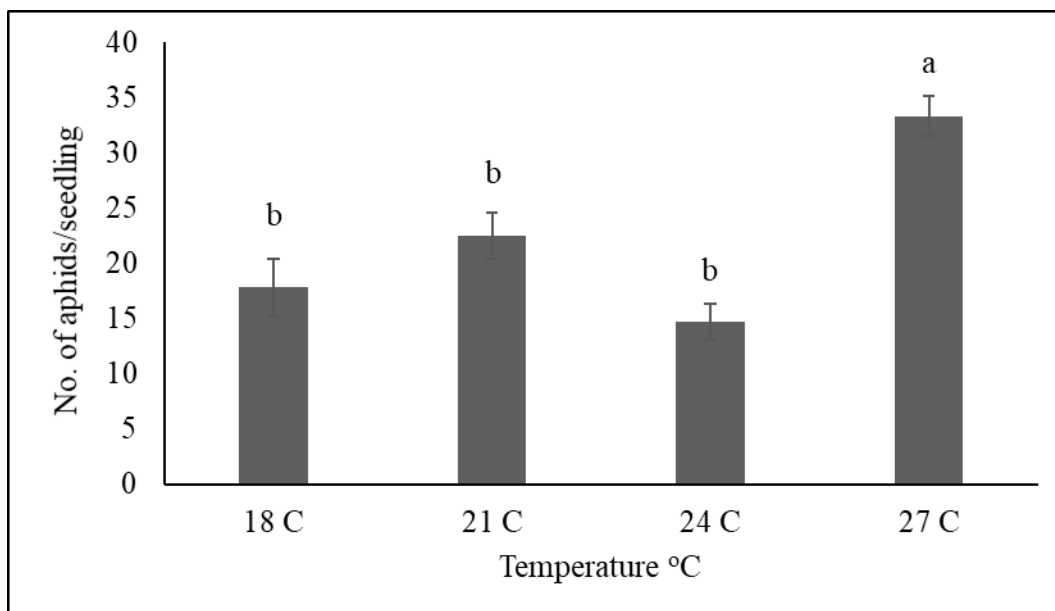


Figure 4.11: Population of winged adults of *A. gossypii* reared at different temperatures on mesta seedlings (30 days after release).

4.3.3.4 Continuous rearing and establishment on cotton

Cotton aphid, *A. gossypii* was reared continuously for a year on the host, *H. cannabinus* seedlings in the Petri plate. During the continuous rearing, it was clearly observed that a Petri plate could accommodate 25 seedlings which produced not less than 10,000 nymphs within a short period of 18 days. Similarly, the same seedlings (25) produced more than 600 parthenogenetic adults from 12th day to 24th day after release. After one year, parthenogenetic females were transferred and established on cotton. These transferred aphids showed survivability of 62.8 ± 2.1 , 74.6 ± 3.4 and 93.2 ± 1.0 per cent on field plant, potted plant and excised leaves of cotton, respectively. These aphids were continuously reared for more than three generations with proper development and population increase, thus confirmed the establishment on cotton. Aphid population gradually increased from 6th day to 30 days after release on both the field and potted plant. However, excised leaf has not supported population buildup as leaf dried just in 5 days. After 30 days of release, aphid population has increased to 893.4 ± 9.5 per leaf on field plant as recorded 760.2 ± 46.4 on potted plant (Table 4.12).

Table 4.12: Survival and establishment of *A. gossypii* on cotton when transferred from Mesta, *H. cannabinus*

Host plant used	No. of aphids released (Adults/leaf)	Survival % at 3 days after release	Aphid population (Nymphs+Adults) per leaf at days after release (Mean±SE)				
			6	12	18	24	30
Field plant	100	62.8±2.1	90±6.1	141.6±12.5	237.8±8.2	397.0±37.0	893.4±9.5
Potted plant	100	74.6±3.4	113±11.3	149.4±8.0	332.4±12.2	457.4±19.2	760.2±46.4
Excised leaf*	100	93.2±1.0	-	-	-	-	-

*Excised leaves got dried on 5th day after release.

4.4 Predation risk effect of *C. sexmaculata* on *A. gossypii*

Prior to assess predation risk exposure treatments, risk effect and its sustainance was assessed by analysing SOD activity levels on exposure to different durations viz., 1, 6, 12 and 24 hours. Exposure for 24 hours has shown high SOD activity sustainably for 24 hours after exposure. Hence, the treatment duration was taken as 24 hours. Different oxidative stress enzymes viz., SOD, CAT and MDA activity was analysed in aphids exposed to direct predation, risk predation and no-predation (control). Treated populations of aphid were also studied for biological parameters.

4.4.1 Influence of exposure duration on predation risk effect and sustainability

Enzyme activity of SOD in the cotton aphids when exposed to varying durations of risk predation imposed by six spotted beetle is given with grouping (Table 4.13). Among the different treatments of predator exposure duration, 24-hour risk predation has shown maximum SOD activity (6.38 ± 0.36) immediately after removal of predator (0-hour post exposure period) and maintained stable and consistent status throughout the post exposure period up to 24 hours (6.60 ± 0.32). Except, the 1-hour exposure, all the other treatments exhibited significantly higher SOD values throughout the post exposure of 24 hours compared to control. However, 1-hour exposure treatment did show higher SOD activity but not significantly better than control. Even though, the one-hour exposure showed SOD activity on par with the control at 0-hour post exposure period, it gradually reach to maximum at 6 hours after exposure (8.39 ± 0.32) and again declined significantly but higher than the control at 24 hours of post exposure period. Among all the treatments, 24-hour exposure displayed stability (linear $R^2 = 0.75$) whereas, 1-hour treatment displayed more dynamism (linear $R^2 = 0.076$) (Figure 4.12). Based on the high SOD activity and its stability throughout the post exposure period after removal of predator, 24-hour predation exposure was taken as treatment duration for conducting oxidative stress experiment under active predation and risk predation.

Table 4.13: Superoxide Dismutase (SOD) activity in the cotton aphid, *A. gossypii* under predation risk of *C. sexmaculata*.

Treatment (Duration of exposure)	SOD units/mg protein $\pm$ SE in cotton aphid at post exposure period (After removal of predator)				
	0 hours	1 hour	6 hours	12 hours	24 hours
Control (0-hour)	3.60 $\pm$ 0.63 ^b	4.16 $\pm$ 0.32 ^b	3.67 $\pm$ 0.55 ^c	4.23 $\pm$ 0.75 ^c	4.19 $\pm$ 0.33 ^c
1-hour	3.97 $\pm$ 1.37 ^b	5.71 $\pm$ 0.93 ^{ab}	8.39 $\pm$ 0.32 ^a	7.61 $\pm$ 1.09 ^a	5.53 $\pm$ 0.39 ^b
6-hour	5.16 $\pm$ 0.80 ^{ab}	6.08 $\pm$ 0.59 ^a	6.79 $\pm$ 0.45 ^b	6.79 $\pm$ 0.17 ^{ab}	6.08 $\pm$ 0.40 ^a
12-hour	5.75 $\pm$ 0.73 ^{ab}	6.38 $\pm$ 0.86 ^{ab}	6.64 $\pm$ 0.40 ^b	5.7 $\pm$ 0.56 ^{ab}	6.01 $\pm$ 0.23 ^{ab}
24-hour	6.38 $\pm$ 0.36 ^a	6.31 $\pm$ 0.72 ^a	6.49 $\pm$ 0.28 ^b	6.23 $\pm$ 0.39 ^{bc}	6.60 $\pm$ 0.32 ^{ab}
P value	0.0113	0.0196	1.24e-06	0.00116	6.31e-05
HSD	2.27	1.94	1.10	1.79	0.91

Values with the same letter for the respective columns are not significantly different.

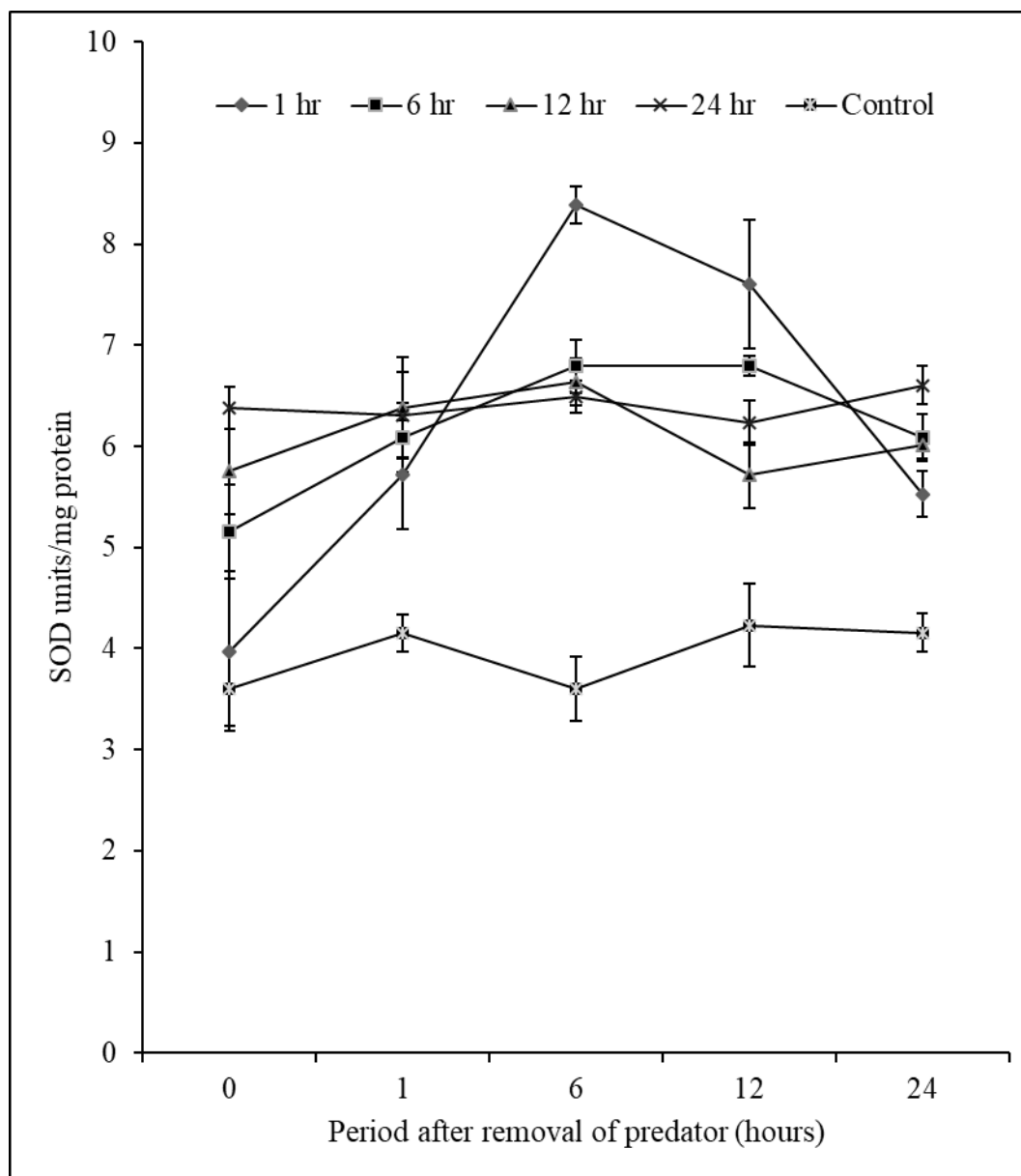


Figure 4.12: Superoxide Dismutase (SOD) activity levels it sustenance over 24 hrs of duration in *A. gossypii* under predation risk of *C. sexmaculata*.

4.4.2 Oxidative stress under active and risk predation

Superoxide dismutase (SOD) activity was observed significantly higher for both the active predation and risk predation over control. It clearly indicated oxidative stress under active predation and the same amount of effect was also induced through the olfactory cues as expressed in risk predation without significant difference between active and risk predation. Whereas, catalase (CAT) showed higher levels of activity compared to the control and there is also a significant higher expression under active predation compared to the risk predation. Malonedialdehyde (MDA) content showed significantly higher levels as in case of SOD. An increased MDA level clearly indicates the increased lipid oxidation in the aphids under predation.

Activity levels of SOD were recorded under active predation and risk predation as 9.75 ± 0.603 and 9.30 ± 0.406 units per mg protein and both have shown significant difference with the control (5.39 ± 0.13) with the minimum significant difference of 1.07. Whereas, MDA content was obtained 3.41 ± 0.312 , 2.73 ± 0.786 and 1.40 ± 0.0398 nM per mg protein under active predation, risk predation and no-predation, respectively. MDA content has shown higher under predation with a significant difference between each other.

Similarly, for catalase (CAT) also showed significant difference with each other and recorded 78.12 ± 5.266 , 62.62 ± 5.222 and 26.48 ± 5.999 H_2O_2 decomposed per min. per mg protein under active predation, risk predation and no-predation, respectively (Table 4.14 & Figure 4.13).

Table 4.14: Activity of antioxidant enzymes (SOD and CAT) and MDA content in the cotton aphid, *A. gossypii* under predation risk of *C. sexmaculata*.

Treatment	Superoxide Dismutase (SOD) [SOD units/mg protein]	Catalase (CAT) [H₂O₂ decomposed μM/min/mg protein]	Malonedialdehyde (MDA) [nM/mg protein]
Active predation	9.75±0.60 ^a	78.12±5.26 ^a	3.41±0.3 ^a
Risk predation	9.30±0.4 ^a	62.62±5.22 ^b	2.73±0.39 ^b
No predation (Control)	5.39±0.13 ^b	26.48±5.99 ^c	1.40±0.04 ^c
P value	2.87e-05	7.1e-05	0.0065
HSD	1.07	13.79	1.22

Values with the same letter for the respective columns are not significantly different.

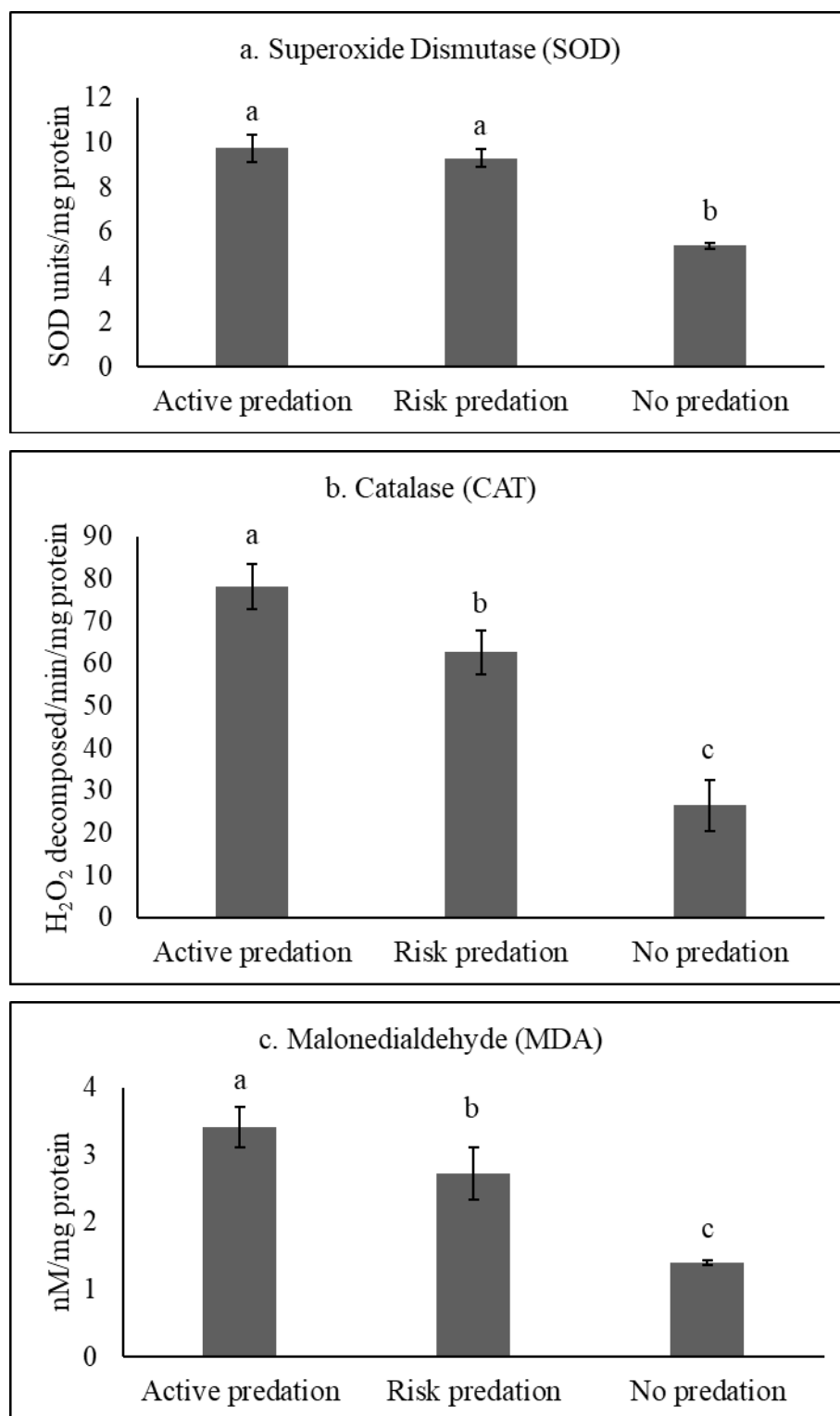


Figure 4.13: Expression of oxidative stress markers (SOD, CAT and MDA) in cotton aphid, *A. gossypii* under predation risk of *C. sexmaculata*.

4.4.3 Predation risk effect on aphid biology

An account of different biological parameters of adult aphids and their offsprings under active and risk predation is given with statistical comparison and grouping (Table 4.15). Predation risk did not affect the longevity of exposed aphid adults, as there is no significant difference between the treatments and control. Reduction in reproduction performance of exposed aphids with the reduced reproductive period and the number of nymphs produced per female during the total life span was noticed. Both the active predation and risk predation exhibited similar trend in inducing lower reproduction compared to the control, without showing significant difference between them. However, there was no significant difference between the treatments for the number of nymphs produced in first 24 hours of exposure period.

Female longevity was recorded as 17.03 ± 4.191 , 16.63 ± 3.253 and 18.31 ± 3.382 days under active predation, risk predation and no-predation, respectively. Number of nymphs produced per female was least under risk predation (30.00 ± 4.402) followed by active predation (32.35 ± 4.666) and control (41.62 ± 5.602).

Although, there was no influence on mortality and developmental period of nymphs laid by the females exposed to predator, it clearly paved the way for production of winged morphs. There was more than three-fold increase in the number of winged adults in both active and risk risk predation compared to no-predation. No significant difference was observed for winged morphs between the active and risk predation. Per cent winged morph produced to the total adults was highest under active predation (21.53 ± 1.05) followed by risk predation (18.28 ± 4.87) and control (6.00 ± 2.99). The minimum significant difference for winged morphs production was 8.396.

Among the different biological parameters viz., the reproductive period of females, number of nymphs produced per female and the per cent winged morphs to the total adults showed differences with no-predation parameters (Figure 4.14).

Table 4.15: Biological parameters of the cotton aphid, *A. gossypii* under predation risk of *C. sexmaculata*.

Biological parameters	Treatments			P value	HSD
	Active predation	Risk predation	No predation (Control)		
Female longevity (days)	17.03±4.19	16.63±3.25	18.31±3.38	NS	
Reproductive period (days)	13.48±1.61 ^b	13.20±1.56 ^b	14.93±1.19 ^a	3.14e-05	0.963
Number of nymphs produced per female	32.35±4.65 ^b	30.00±4.40 ^b	41.62±5.60 ^a	2.21e-14	4.692
Number of nymphs produced/day/female in first 24 hours after exposure period	3.12±1.15	2.99±1.05	2.81±0.95	NS	-
Developmental period of nymph (days)	4.62±0.45	4.63±0.48	4.61±0.46	NS	-
Mortality of nymphs (%)	6.74±1.16	5.70±1.91	4.91±1.037	NS	-
Winged morphs to the total adults (%)	21.53±1.05 ^a	18.28±4.86 ^a	6.00±2.99 ^b	0.0029	8.391

Values with the same letter for the respective rows are not significantly different.

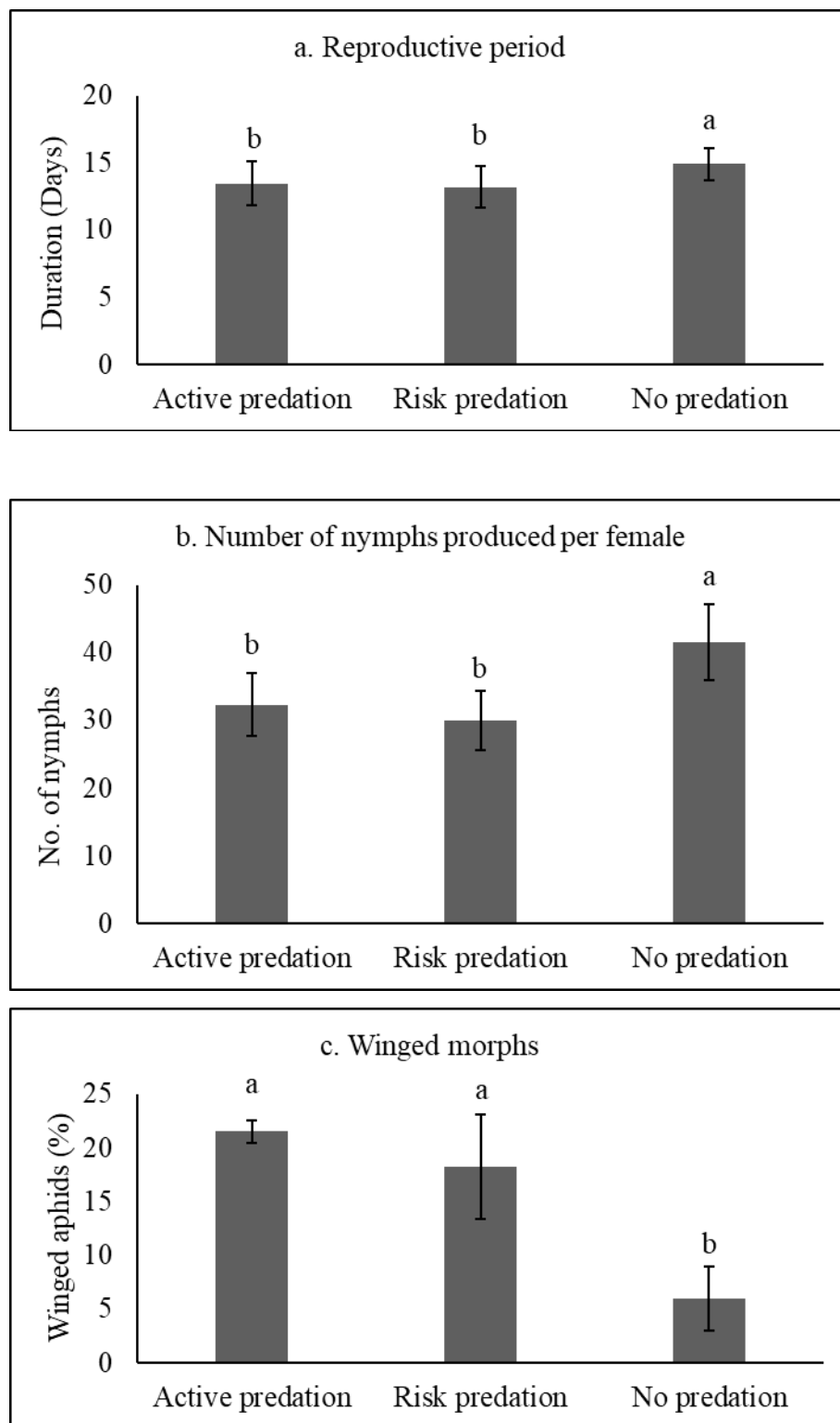


Figure 4.14: Biological parameters differentially expressed in cotton aphid, *A. gossypii* under predation risk of *C. sexmaculata*.

4.5 Orientation behaviour of *C. sexmaculata* towards prey and host plant extracts

Orientation response of *C. sexmaculata* males and females was studied towards different odour sources viz., un-infested leaf extract (UL), infested leaf without aphid (WOA), infested leaf with aphids (WA) and aphids only (AO). All these four odour sources were extracted separately with DCM and n-Hexane. These eight extracts were tested at four different concentrations (1%, 5%, 10% and 15%) for beetles orientation response studies. Adult beetle response to the odour source and the time taken to reach the odour source was assessed against four concentrations of each extract.

4.5.1 Orientation response of male

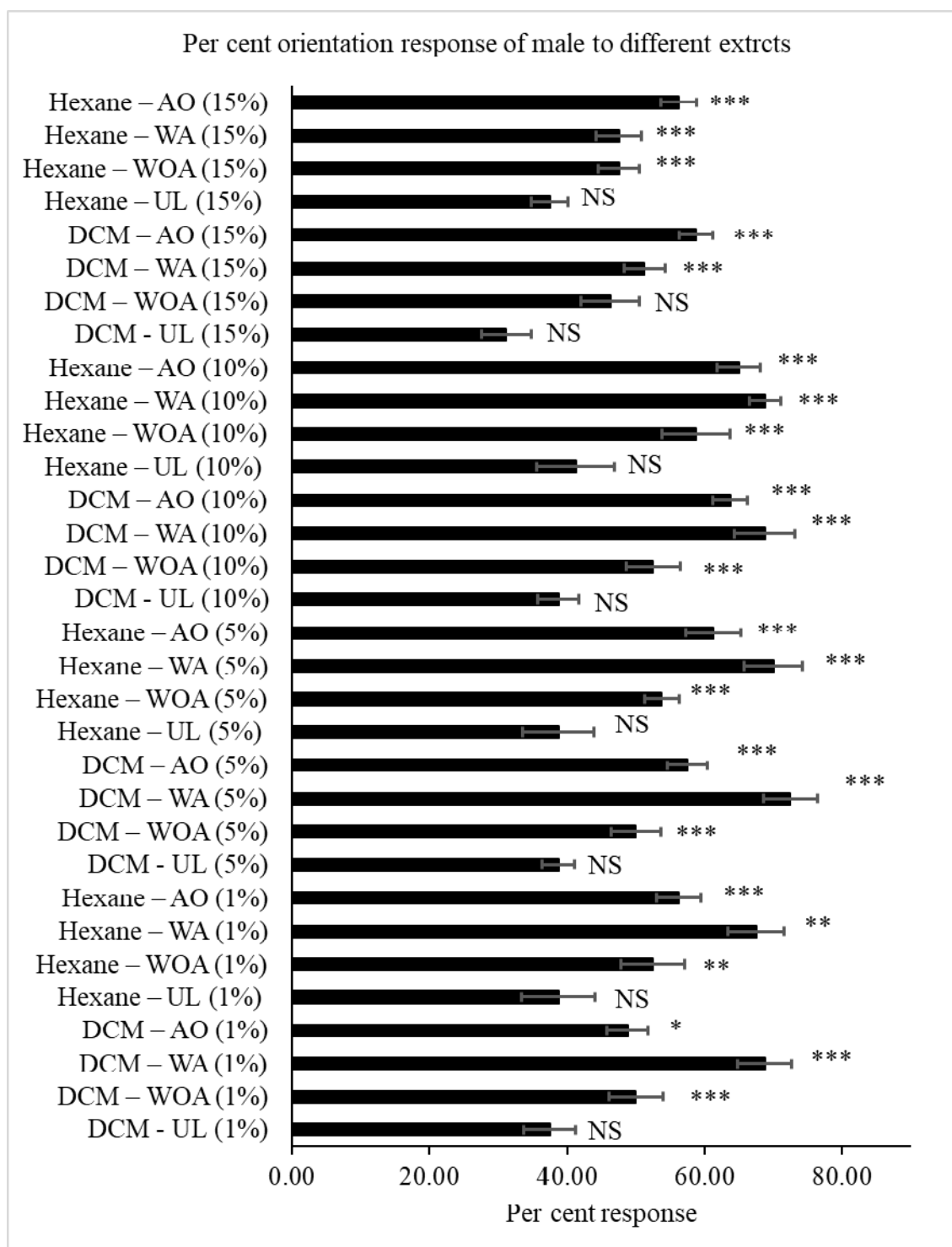
Orientation response of *C. sexmaculata* male to the different odours is shown with mean per cent attraction (mean $\pm$ SE) and it's significance difference with the control (Table 4.16 & Figure 4.15). A detail account of per cent orientation of male beetle towards the different sources such as odour, control and no-response is given by comparing variances among the different extracts for its significance (Table 4.17).

Only three extracts of 1 per cent concentration namely, infested leaf with aphids extracted with DCM (DCM-WA), infested leaf with aphids extracted with n-Hexane (Hexane-WA) and aphids only extracted with n-Hexane (Hexane-AO) have shown significant higher attraction of male beetles compared to the control (Figure 4.16). Among the different extracts, WA extracts of both DCM and n-Hexane have shown higher attraction of male beetles compared to other extracts. Among the remaining extracts, AO extracts of n-Hexane has shown significant more attraction whereas, the remaining extracts did not differ significantly with the control. The order of higher to lower response was DCM-WA (68.8 $\pm$ 6.39%) $\square$ Hexane-WA (67.5 $\pm$ 3.66%) > Hexane-AO (56.3 $\pm$ 2.63%) $\square$ Hexane-WOA (52.5 $\pm$ 5.59%) $\square$ DCM-WOA (50.0 $\pm$ 5.35%) $\square$ DCM-AO (48.8 $\pm$ 5.15%) > Hexane-UL (40.0 $\pm$ 4.63%) and $\square$ DCM-UL (37.5 $\pm$ 3.66%).

Table 4.16: Orientation response of *C. sexmaculata* male to different extracts

Concen Tration	Extractant	Extract	Orientation response of male (%)±SE		T cal	P value	
			Odour	Control			
1 %	DCM	UL	37.5±3.66	38.8±5.15	0.198	0.846	NS
		WOA	50.0±5.35	38.8±4.41	1.624	0.127	NS
		WA	68.8±6.39	22.5±5.90	5.317	0.000	***
		AO	48.8±5.15	40.0±4.63	1.263	0.227	NS
	n-Hexane	UL	40.0±4.63	38.8±5.15	0.180	0.859	NS
		WOA	52.5±5.59	37.5±4.53	2.084	0.056	NS
		WA	67.5±3.66	20.0±3.27	9.674	0.000	***
		AO	56.3±2.63	36.3±3.24	4.793	0.000	***
5 %	DCM	UL	36.8±3.18	37.5±5.26	0.189	0.852	NS
		WOA	50.0±4.63	38.8±4.41	1.760	0.100	NS
		WA	72.5±3.66	18.8±2.95	11.434	0.000	***
		AO	57.5±2.50	36.3±3.24	5.194	0.000	***
	n-Hexane	UL	38.8±3.98	37.5±3.66	0.231	0.821	NS
		WOA	53.8±4.98	35.0±4.23	2.872	0.012	*
		WA	70.0±4.23	20.0±2.67	10.000	0.000	***
		AO	61.3±2.95	30.0±2.67	7.850	0.000	***
10 %	DCM	UL	38.8±2.95	40.0±5.35	0.205	0.841	NS
		WOA	52.5±4.12	38.8±4.41	2.280	0.039	*
		WA	68.8±3.98	17.5±2.50	10.902	0.000	***
		AO	63.8±4.20	26.3±4.98	5.758	0.000	***
	n-Hexane	UL	41.3±4.41	37.5±3.66	0.655	0.523	NS
		WOA	58.8±2.27	30.0±2.67	8.205	0.000	***
		WA	68.8±2.95	16.3±2.63	13.282	0.000	***
		AO	65.0±3.27	26.3±3.24	8.415	0.000	***
15 %	DCM	UL	31.3±2.95	32.5±4.53	0.231	0.821	NS
		WOA	46.3±3.24	38.8±4.41	1.371	0.192	NS
		WA	51.3±2.95	18.8±2.95	7.789	0.000	***
		AO	58.8±3.98	36.3±4.60	3.696	0.002	**
	n-Hexane	UL	38.8±2.50	37.5±3.66	0.290	0.776	NS
		WOA	47.5±3.13	37.5±4.53	1.815	0.091	NS
		WA	47.5±2.50	20.0±3.78	6.068	0.000	***
		AO	56.3±2.63	38.8±2.27	5.041	0.000	***

*Significant at $P<0.05$, ** Significant at $P<0.01$, *** Significant at $P<0.001$, NS-Non-significant.



*Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$, NS-Non-significant.

Figure 4.15: Orientation response of *C. sexmaculata* male to different extracts

Table 4.17: Orientation response of *C. sexmaculata* male towards different extracts with variances among the extracts with in the same concentration

Extractant	Extract	Orientation response of male (%)±SE towards different extracts at the respective concentration											
		1 %			5 %			10 %			15 %		
		Odour	Control	No response	Odour	Control	No response	Odour	Control	No response	Odour	Control	No response
DCM	UL	37.5±3.7b	38.8±5.2	23.8±6.3	36.8±3.2d	37.5±5.3	23.8±5.3	38.8±2.9c	40.0±5.3	21.3±5.5	31.3±2.9	32.5±4.5	36.3±5.9
	WOA	50.0±5.4ab	38.8±4.4	11.3±3.5	50.0±4.6cd	38.8±4.4	11.3±4.8	52.5±4.1ab	38.8±4.4	8.8±3.9	46.3±3.2ab	38.8±4.4	15.0±5.7
	WA	68.8±6.4a	22.5±5.9	8.8±2.3	72.5±3.7a	18.8±3.0	8.8±3.0	68.8±3.9a	17.5±2.5	13.8±2.6	51.3±2.9a	18.8±2.9	30.0±5.0
	AO	48.8±5.2ab	40.0±4.6	11.3±4.4	57.5±2.5abc	36.3±3.2	6.3±3.2	63.8±4.2ab	26.3±4.9	10.0±3.3	58.8±3.9a	36.3±4.6	5.0±2.7
n-Hexane	UL	40.0±4.6b	38.8±5.1	21.3±5.1	38.8±4.0d	37.5±3.7	23.8±3.2	41.3±4.4c	37.5±3.6	21.3±3.5	38.8±2.5bc	37.5±3.6	25.0±4.6
	WOA	52.5±5.6ab	37.5±4.5	10.0±3.8	53.8±5.0bcd	35.0±4.2	11.3±3.0	58.8±2.3ab	30.0±2.7	11.3±2.3	47.5±3.1ab	37.5±4.5	15.0±4.6
	WA	67.5±3.7a	20.0±3.3	12.5±3.7	70.0±4.2ab	20.0±2.7	10.0±2.7	68.8±2.9a	16.3±2.6	15.0±4.2	47.5±2.5ab	20.0±3.8	32.5±4.1
	AO	56.3±2.6ab	36.3±3.2	7.5±4.1	61.3±3.0abc	30.0±2.7	8.8±4.0	65.0±3.3ab	26.3±3.2	8.8±2.9	56.3±2.6a	38.8±2.3	5.0±2.7
d.f. 7		d.f. 7			d.f. 7			d.f. 7			d.f. 7		
MSS: 182.4		MSS: 124.1			MSS: 103.1			MSS: 73.0			MSS: 73.0		
F cal. 5.67		F cal. 10.41			F cal. 10.97			F cal. 9.10			F cal. 9.10		
P value:		P value:			P value:			P value:			P value:		
5.57E-05		2.73E-08			1.25E-08			1.88E-07			1.88E-07		
MSD: 21.26		MSD: 17.5			MSD: 16.0			MSD: 13.4			MSD: 13.4		

Values with the same letter for the respective column are not significantly different.

UL: Un-infested Leaf, WOA: infested leaf WithOut Aphid, WA: infested leaf With Aphid, AO: Aphid Only.

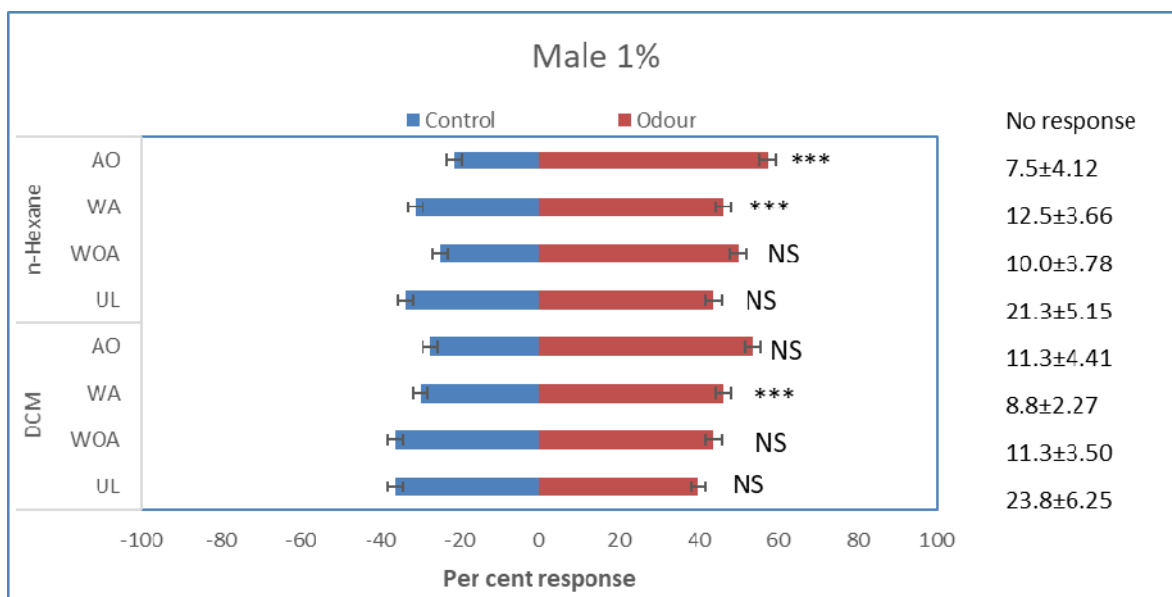


Figure 4.16: Per cent orientation of *C. sexmaculata* male to different extracts at 1% concentration

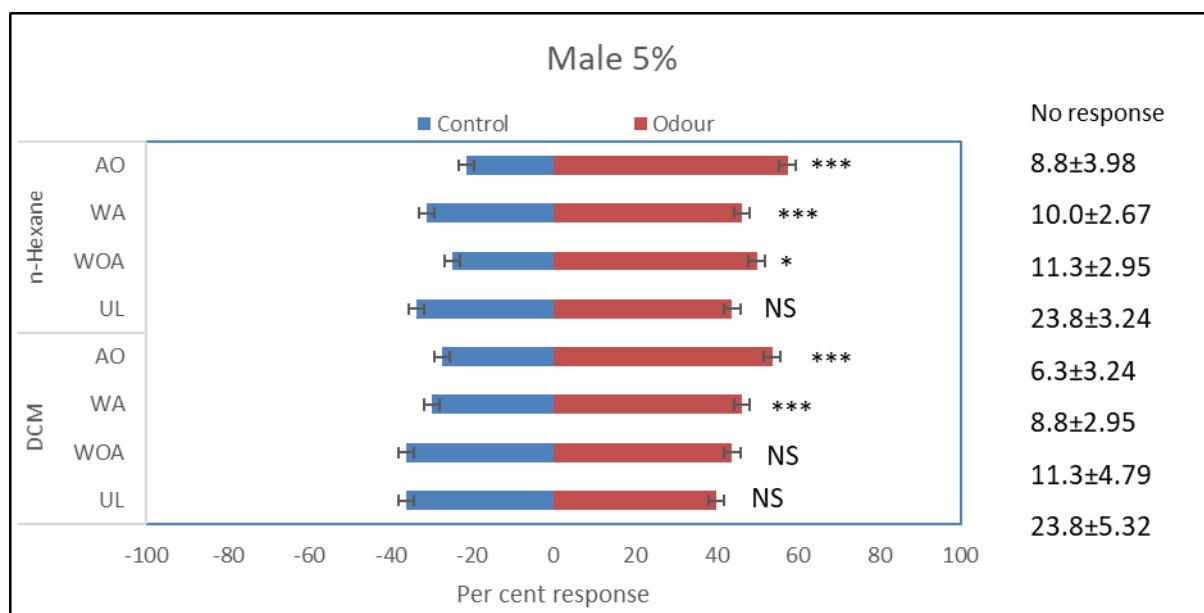
Similar trend with an increase in per cent attraction was observed when the concentration of extracts was increased to 5 per cent. Except, un-infested leaf (UL) and infested leaf without aphid (WOA) extracted with DCM and UL extracted with n-Hexane, all the other extracts resulted in significant higher attraction of male beetles compared to control (Figure 4.17).

WA extract of both DCM and n-Hexane was the most attractive followed by AO to the male beetles. However, no significant difference was observed for the remaining extracts like DCM-UL, DCM-WOA and Hexane-UL compared to the control. The decreasing order of different extracts (5%) for the per cent attraction of male beetles of *C. sexmaculata* was DCM-WA (72.5 ± 3.66) > Hexane-WA (70.0 ± 4.23) > Hexane-AO (61.3 ± 2.95) > DCM-AO (57.5 ± 2.50) > Hexane-WOA (53.8 ± 4.98) > DCM-WOA (50.0 ± 4.63) and > Hexane-UL (38.8 ± 3.98) > DCM-UL (36.8 ± 3.18).

The concentration of 10 per cent of all the extracts except, UL have displayed significant difference for the per cent attraction of beetles compared to the control (Figure

4.18). The decreasing order of different extracts (5%) for the per cent attraction of male beetles of *C. sexmaculata* was Hexane-WA (68.8 ± 2.95) □ DCM-WA (68.8 ± 3.98) > Hexane-AO (65.0 ± 3.27) □ DCM-AO (63.8 ± 4.20) □ Hexane-WOA (58.8 ± 2.27) □ DCM-WOA (52.5 ± 4.12) > Hexane-UL (41.3 ± 4.41) and □ DCM-UL (38.8 ± 2.95).

Whereas, at 15 per cent concentration, attraction got decreased substantially compared to other concentrations. Although, the per cent attraction got reduced, a significant difference with the control in case of DCM-WA, DCM-AO, Hexane-WA and Hexane-AO was noticed (Figure 4.19). The decreasing order of different extracts for the per cent attraction of beetles was DCM-AO (58.8 ± 3.98) □ Hexane-AO (56.3 ± 2.63) □ DCM-WA (51.3 ± 2.95) > Hexane-WA (47.5 ± 2.50) □ Hexane-WOA (47.5 ± 3.13) □ DCM-WOA (46.3 ± 3.24) > Hexane-UL (38.8 ± 2.50) and > DCM-UL (31.3 ± 2.95).



*Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$, NS-Non-significant.

Figure 4.17: Per cent orientation of *C. sexmaculata* male to different extracts at 5% concentration

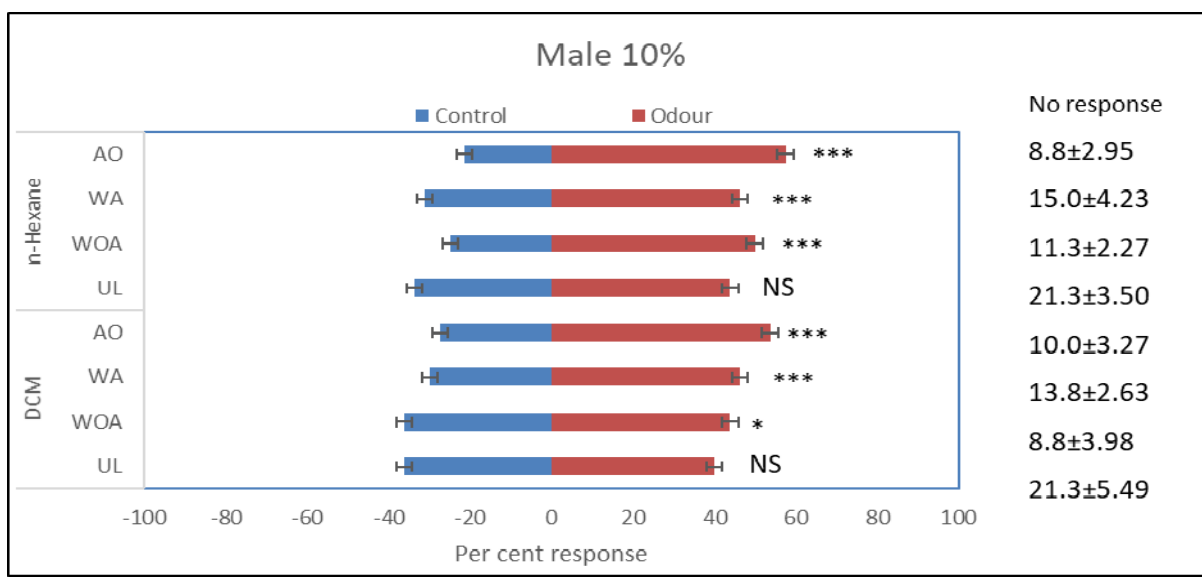
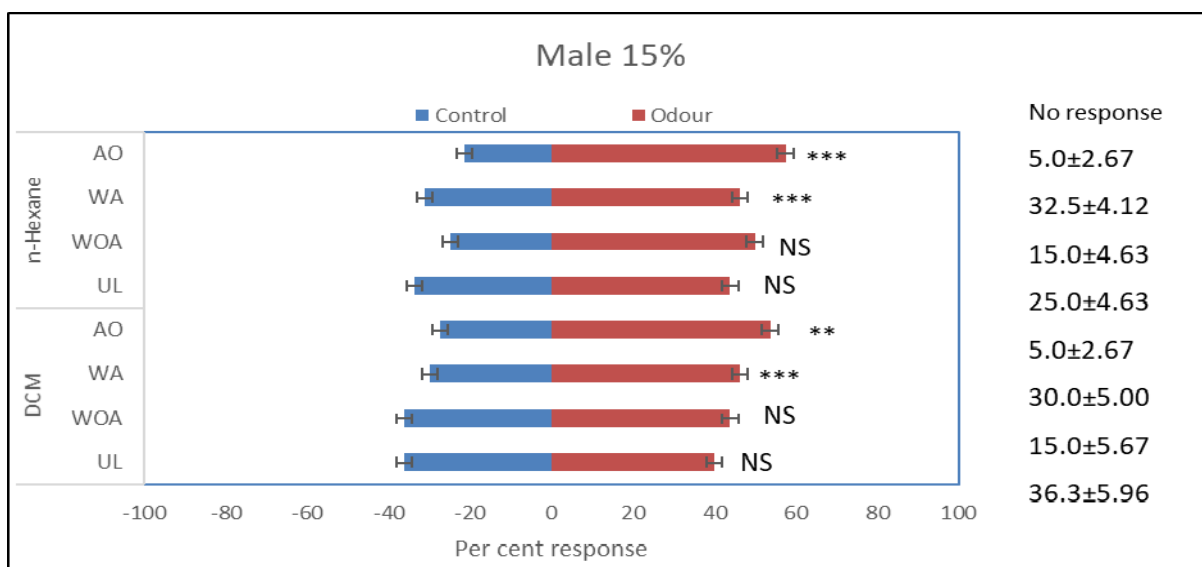


Figure 4.18: Per cent orientation of *C. sexmaculata* male to different extracts at 10% concentration



*Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$, NS- Non-significant.

Figure 4.19: Per cent orientation of *C. sexmaculata* male to different extracts at 15% concentration

Per cent response of attraction towards different extracts in the order of highest to lowest was DCM-WA (5%), Hexane – WA (5%), DCM-WA (1%), DCM-WA (10%), Hexane-WA (10%), Hexane-WA (1%), Hexane-AO (10%), DCM-AO (10%), Hexane-AO (5%), Hexane-WOA (10%), DCM-AO (15%), DCM-AO (5%), Hexane-AO (1%), Hexane-AO (15%), Hexane-WOA (5%), Hexane-WOA (1%), DCM-WOA (10%), DCM-WA (15%), DCM-WOA (1%), DCM-WOA (5%), DCM-AO (1%), Hexane-WOA (15%), Hexane-WA (15%), DCM-WOA (15%), Hexane-UL (10%), Hexane-UL (1%), DCM-UL (5%), Hexane-UL (5%), DCM-UL (10%), DCM-UL (1%), Hexane-UL (15%) and DCM-UL (15%) with the attraction of 72.5 ± 3.66 , 70.0 ± 4.23 , 68.8 ± 6.39 , 68.8 ± 3.98 , 68.8 ± 2.95 , 67.5 ± 3.66 , 65.0 ± 3.27 , 63.8 ± 4.20 , 61.3 ± 2.95 , 58.8 ± 2.27 , 58.8 ± 3.98 , 57.5 ± 2.50 , 56.3 ± 2.63 , 56.3 ± 2.63 , 53.8 ± 4.98 , 52.5 ± 5.59 , 52.5 ± 4.12 , 51.3 ± 2.95 , 50.0 ± 5.35 , 50.0 ± 4.63 , 48.8 ± 5.15 , 47.5 ± 3.13 , 47.5 ± 2.50 , 46.3 ± 3.24 , 41.3 ± 4.41 , 40.0 ± 4.63 , 38.8 ± 3.98 , 38.8 ± 3.98 , 38.8 ± 2.95 , 37.5 ± 3.66 , 37.5 ± 2.50 and 31.3 ± 2.95 per cent, respectively.

When the orientation response of male beetle was compared to the concentration gradient of different extracts, infested leaf with aphid (WA) has exhibited significant difference for both the extractants (DCM and n-Hexane). Attraction of beetle to DCM-WA and Hexane-WA was highest and consistent among the concentrations of 1, 5 and 10 per cent and was also found on par with each other. Whereas, the per cent attraction got significantly reduced at higher concentration of 15%.

The DCM extract of infested leaf with aphid (DCM-WA) responded with 68.8 ± 6.39 , 72.5 ± 3.66 , 68.8 ± 3.98 and 51.3 ± 2.95 per cent attraction of male beetles at the concentration of 1, 5, 10 and 15 per cent, respectively. Similarly, with n-Hexane it was recorded 67.5 ± 3.66 , 70.0 ± 4.23 , 68.8 ± 2.95 and 47.5 ± 2.50 per cent, respectively (Table 4.18).

Table 4.18: Comparative response of *C. sexmaculata* male to the different concentrations of each extract

Extractant	Extract	Orientation response of male (%±SE) to the extracts at the respective concentration				F cal	P value
		1%	5%	10%	15%		
DCM	UL	37.5±3.66	36.8±3.18	38.8±2.95	31.3±2.95	0.959	0.426NS
	WOA	50.0±5.35	50.0±4.63	52.5±4.12	46.3±3.24	0.343	0.794NS
	WA	68.8±6.39a	72.5±3.66a	68.8±3.98a	51.3±2.95b	8.451	0.000372***
	AO	48.8±5.15	57.5±2.50	63.8±4.20	58.8±3.98	2.349	0.0939NS
n-Hexane	UL	40.0±4.63	38.8±3.98	41.3±4.41	38.8±2.50	0.165	0.919NS
	WOA	52.5±5.59	53.8±4.98	58.8±2.27	47.5±3.13	1.203	0.327NS
	WA	67.5±3.66a	70.0±4.23a	68.8±2.95a	47.5±2.50b	9.863	0.000132***
	AO	56.3±2.63	61.3±2.95	65.0±3.27	56.3±2.63	2.177	0.113NS

*Significant at $P<0.05$, ** Significant at $P<0.01$, *** Significant at $P<0.001$, NS-Non-significant. Values with the same letter for the respective rows are not significantly different.

4.5.2 Orientation response of female

In case of female, all the extracts except DCM-UL, Hexane-UL and DCM-WOA of 15% concentration attracted a more number of *C. sexmaculata* female by exhibiting significant difference with the control (Table 4.19 & Figure 4.20). Similarly, as observed in case of male, female beetle also showed varying degree of orientation response to different sources like odour, control and no-response, and which is given by comparing variances among the different extracts for its significance (Table 4.20).

At one per cent concentration, DCM-WA attracted highest beetles (58.8 ± 4.79) followed by DCM-WOA (52.5 ± 1.64), Hexane-AO (52.5 ± 2.50), Hexane-WA (52.5 ± 4.53), Hexane WOA (48.8 ± 2.95), DCM-AO (48.8 ± 2.95), DCM-UL (40.0 ± 2.67) and Hexane-UL (38.8 ± 2.95). However, the last three extracts have not shown a significant difference with the control. All the extracts which have shown a significant attraction, stood on par with each other, except DCM-WA which was significantly superior over the others (Figure 4.21).

At 5 per cent concentration, all the extracts except DCM-UL and Hexane-UL have shown increasing trend of attraction of female beetles with significant difference over the control (Figure 4.22). Among all the extracts, DCM-WA was the superior with 83.8 ± 3.24 per cent attraction of female beetles over other extracts. Among the other extracts, the decreasing order of per cent attraction was Hexane-WA (72.5 ± 2.50) $\square$ DCM-AO (70.0 ± 2.67) $>$ Hexane-AO (66.3 ± 3.24) $>$ Hexane-WOA (56.3 ± 2.63) $>$ DCM-WOA (51.3 ± 3.98) $\square$ Hexane-UL (41.3 ± 2.27) and $>$ DCM-UL (37.5 ± 3.66). However, un-infested leaf extracts (DCM-UL and Hexane-UL) have not shown significant difference with the control.

Table 4.19: Orientation response of *C. sexmaculata* female to different extracts

Concentration	Extractant	Extract	Orientation response of female (%)±SE		T cal	P value
			Odour	Control		
1 %	DCM	UL	40.0±2.67	35.0±4.23	1.030	0.334 NS
		WOA	52.5±1.64	31.3±3.50	5.495	0.000***
		WA	58.8±4.79	18.8±2.27	7.542	0.000***
		AO	48.8±2.15	37.5±3.66	2.393	0.031*
	n-Hexane	UL	38.8±2.35	35.0±4.23	0.728	0.479NS
		WOA	47.8±2.95	35.0±2.67	3.454	0.004**
		WA	52.5±4.53	27.5±4.91	3.742	0.002**
		AO	52.5±2.50	33.8±2.63	5.167	0.000***
5 %	DCM	UL	37.5±3.66	36.3±4.98	0.202	0.843NS
		WOA	51.3±3.98	30.0±2.67	4.432	0.001***
		WA	83.8±3.24	13.8±2.63	16.776	0.000***
		AO	70.0±2.67	16.3±2.63	14.333	0.000***
	n-Hexane	UL	41.3±2.27	42.5±3.66	0.290	0.776NS
		WOA	56.3±2.63	30.0±3.27	5.158	0.000***
		WA	72.5±2.50	17.5±2.50	15.556	0.000***
		AO	66.3±3.24	18.8±2.95	10.841	0.000***
10 %	DCM	UL	36.8±2.35	33.8±4.20	0.974	0.346NS
		WOA	53.8±3.24	30.0±2.67	5.656	0.000***
		WA	66.3±4.98	15.0±1.89	9.626	0.000***
		AO	55.0±3.27	22.5±3.13	7.172	0.000***
	n-Hexane	UL	38.8±2.95	36.3±3.75	0.524	0.609NS
		WOA	53.8±1.83	30.0±1.89	9.029	0.000***
		WA	63.8±4.60	21.3±2.95	7.771	0.000***
		AO	61.3±3.98	23.8±2.63	7.859	0.000***
15 %	DCM	UL	40.0±3.78	36.3±3.24	0.753	0.464NS
		WOA	41.8±3.20	36.3±4.20	1.263	0.227NS
		WA	46.3±3.24	30.0±1.89	4.333	0.001**
		AO	53.8±2.63	27.5±2.50	7.233	0.000***
	n-Hexane	UL	43.8±4.20	33.8±3.24	1.886	0.080NS
		WOA	50.0±3.78	25.0±1.89	5.916	0.000***
		WA	46.3±1.83	31.3±2.27	5.150	0.000***
		AO	57.5±1.64	21.3±2.27	12.969	0.000***

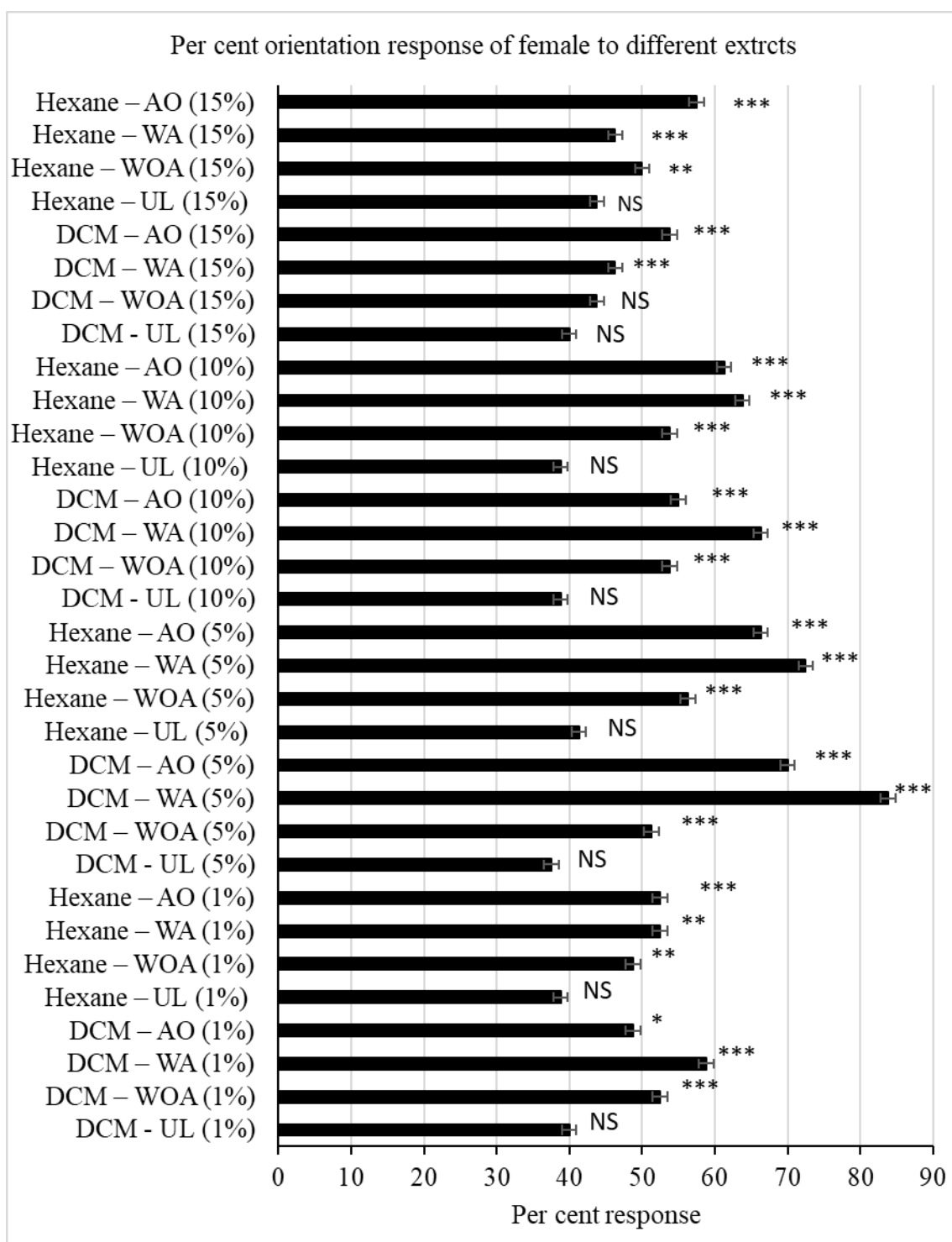
*Significant at $P<0.05$, ** Significant at $P<0.01$, *** Significant at $P<0.001$, NS-Non-significant.

Table 4.20: Orientation response of *C. sexmaculata* female towards different extracts with variances among the extracts with in the same concentration

Extractant	Extract	Orientation response of female (%)±SE towards different extracts at the respective concentration											
		1 %			5 %			10 %			15 %		
		Odour	Control	No response	Odour	Control	No response	Odour	Control	No response	Odour	Control	No response
DCM	UL	40.0±2.7b	35.0±4.2	25.0±4.6	37.5±3.7e	36.3±4.9	26.3±5.3	36.8±2.3b	33.8±4.2	27.5±5.2	40.0±3.8b	36.3±3.2	23.8±6.2
	WOA	52.5±1.6ab	31.3±3.5	16.3±3.7	51.3±3.9de	30.0±2.	18.8±3.5	53.8±3.2ab	30.0±2.6	16.3±3.2	41.8±3.2ab	36.3±4.2	20.0±4.6
	WA	58.8±4.8a	18.8±2.2	22.5±5.6	83.8±3.2a	13.8±2.6	2.5±1.6	66.3±4.9a	15.0±1.8	18.8±4.4	46.3±3.2ab	30.0±1.9	23.8±3.7
	AO	48.8±2.1ab	37.5±3.6	13.8±3.2	70.0±2.7ab	16.3±2.6	13.8±3.7	55.0±3.3a	22.5±3.1	22.5±3.1	53.8±2.6ab	27.5±2.5	18.8±2.3
n-Hexane	UL	38.8±2.3b	35.0±4.2	26.3±4.9	41.3±2.3de	42.5±3.6	16.3±3.7	38.8±2.9b	36.3±3.7	25.0±4.6	43.8±4.2ab	33.8±3.2	22.5±4.5
	WOA	47.8±2.9ab	35.0±2.6	16.3±4.2	56.3±2.6cd	30.0±3.2	16.3±4.2	53.8±1.8ab	30.0±1.8	16.3±2.6	50.0±3.8ab	25.0±1.9	25.0±3.3
	WA	52.5±4.5ab	27.5±4.9	20.0±2.7	72.5±2.5ab	17.5±2.5	10.0±2.7	63.8±4.6a	21.3±2.9	15.0±2.6	46.3±1.8ab	31.3±2.3	22.5±3.1
	AO	52.5±2.5ab	33.8±2.6	13.8±1.8	66.3±3.2bc	18.8±2.9	15.0±2.7	61.3±3.9a	23.8±2.6	15.0±1.8	57.5±1.6a	21.3±2.3	21.3±2.9
		d.f. 7			d.f. 7			d.f. 7			d.f. 7		
		MSS: 85.7			MSS: 79.2			MSS: 103.8			MSS: 87.3		
		F cal. 4.24			F cal. 26.41			F cal. 8.41			F cal. 3.05		
		P value:			P value:			P value:			P value:		
		0.0008			1.4E-15			5.43E-07			0.0086		
		MSD: 14.57			MSD: 14.0			MSD: 16.03			MSD: 14.7		

Values with the same letter for the respective rows are not significantly different.

UL: Un-infested Leaf, WOA: infested leaf WithOut Aphid, WA: infested leaf With Aphid, AO: Aphid Only



*Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$,
NS-Non-significant.

Figure 4.20: Orientation response of *C. sexmaculata* female to different extracts

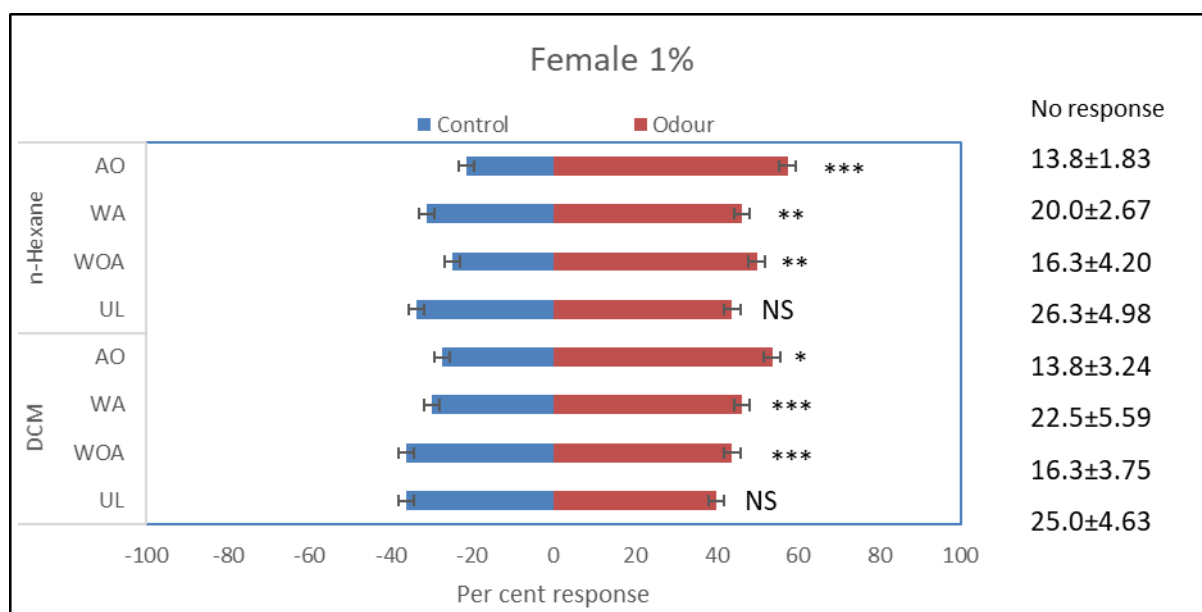
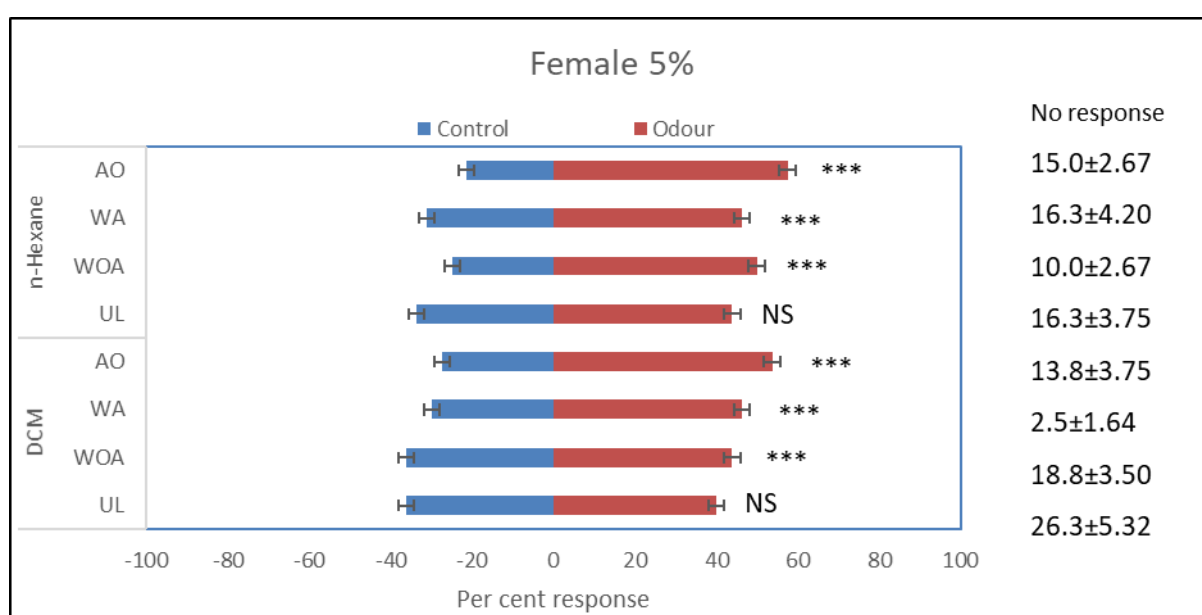


Figure 4.21: Per cent orientation of *C. sexmaculata* female to different extracts at 1% concentration



*Significant at $P<0.05$, ** Significant at $P<0.01$, *** Significant at $P<0.001$, NS-Non-significant.

Figure 4.22: Per cent orientation of *C. sexmaculata* female to different extracts at 5% concentration

The extracts concentration of 10 per cent also witnessed the similar trend of significant attraction over control for all the extracts except DCM-UL and Hexane-UL (Figure 4.23). Although, there was a similar trend in significant differences with the control, reduction in attraction was observed for most of the extracts. Among all the extracts, four extracts viz., DCM-WA, DCM-AO, Hexane-WA and Hexane-AO have shown the significant higher attraction but found on par within the group. The decreasing order of extracts for the per cent attraction of female beetle was DCM-WA (66.3 ± 4.98), Hexane-WA (63.8 ± 4.60), Hexane-AO (61.3 ± 3.98), DCM-AO (55.0 ± 3.27), Hexane-WOA (53.8 ± 1.83), DCM-WOA (53.8 ± 3.24), Hexane-UL (38.8 ± 2.95) and DCM-UL (36.8 ± 2.35).

The extracts at higher concentration of 15% have shown reduction in per cent attraction of female beetle, but significantly higher compared to the control. Except, uninfested leaf (DCM-UL and Hexane-UL) and DCM-WOA, all the other extracts showed significant higher attraction over the control (Figure 4.24). Hexane-AO extract has exhibited the highest per cent attraction (57.5 ± 1.64) of female beetle by differing significantly with all other extracts. Remaining extracts, DCM-UL, DCM-WOA, DCM-WA, DCM-AO, Hexane-UL, Hexane-WOA and Hexane-WA have recorded 40.0 ± 3.78 , 41.8 ± 3.20 , 46.3 ± 3.24 , 53.8 ± 2.63 , 43.8 ± 4.20 , 50.0 ± 3.78 and 46.3 ± 1.83 per cent attraction, respectively.

Per cent response of attraction towards different extracts in the order of highest to lowest was DCM-WA (5%), Hexane-WA (5%), DCM-AO (5%), Hexane-AO (5%), DCM-WA (10%), Hexane-WA (10%), Hexane-AO (10%), DCM-WA (1%), Hexane-AO (15%), Hexane-WA (5%), DCM-AO (10%), DCM-WOA (10%), Hexane-WOA (10%), DCM-AO (15%), DCM-WOA (1%), Hexane-WA (1%), Hexane-AO (1%), DCM-WOA (5%), Hexane-WOA (15%), DCM-AO (1%), Hexane-WOA (1%), DCM-WA (15%), Hexane-WA (15%), DCM-WOA (15%), Hexane-UL (15%), Hexane-UL (5%), DCM-UL (1%), DCM-UL (15%), Hexane-UL (1%), DCM-UL (10%), Hexane-UL (10%) and DCM-UL (5%) with the attraction of 83.8 ± 3.24 , 72.5 ± 2.50 , 70.0 ± 2.67 , 66.3 ± 3.24 , 66.3 ± 4.98 , 63.8 ± 4.60 , 61.3 ± 3.98 , 58.8 ± 4.79 , 57.5 ± 1.64 , 56.3 ± 2.63 , 55.0 ± 3.27 , 53.8 ± 3.24 , 53.8 ± 1.83 , 53.8 ± 2.63 , 52.5 ± 1.64 , 52.5 ± 4.53 , 52.5 ± 2.50 , 51.3 ± 3.98 , 50.0 ± 3.78 , 48.8 ± 2.95 , 47.8 ± 2.95 , 46.3 ± 3.24 ,

46.3±1.83, 41.8±3.20, 43.8±4.20, 41.3±2.27, 40.0±2.67, 40.0±3.78, 38.8±2.35, 36.8±2.35, 38.8±2.95 and 37.5±3.66 per cent, respectively (Table 4.20).

When the orientation response of female beetle was compared to the concentration gradient of different extracts, WA and AO of both the extractants (DCM and n-Hexane) have exhibited significant difference, while others (WOA and UL) could not. Extract of DCM-WA has shown the highest per cent attraction at the concentration of 5% (83.8±3.24) followed by 10 (66.3±4.98), 1 (58.8±4.79) and 15 (46.3±3.24) by exhibiting significant differences between each other. In case of DCM-AO, highest per cent attraction was obtained at the concentration of 5% (70.0±2.67) by significantly differing with other concentrations, 10% (55.0±3.27), 15 (53.8±2.63) and 1 % (48.8±2.95). Similar trend was also observed in case of n-Hexane extracts, where the extracts of WA and AO have shown the significant variations among the concentrations for the per cent attraction. Extract of WA has shown significant differences between the concentrations with each other for the attraction of female beetle by resulting highest attraction at 5% (72.5±2.50) followed by 10% (63.8±4.60), 1% (52.5±4.53) and 15% (46.3±1.83). In case of AO, 5% (66.3±3.24) was significantly superior over all others followed by 10% (61.3±3.98), 15% (57.5±1.64) and 1% (52.5±2.50); 10 and 15% were found on par with each other, but differed with 1%. Remaining extracts have not shown the significant variation between the concentration gradient for the attraction (Table 4.21).

Between the sexes, female has shown the significant higher attraction than male to DCM-UL at 15%, DCM-WA at 5%, DCM-AO at 5%, Hexane-UL at 1% and Hexane-WOA at 5%. Whereas, WA extracts of both DCM and n-Hexane have attracted more number of males than females at lowest concentration of 1%. Other extracts have not shown significant difference for attraction between male and female (Table 4.22).

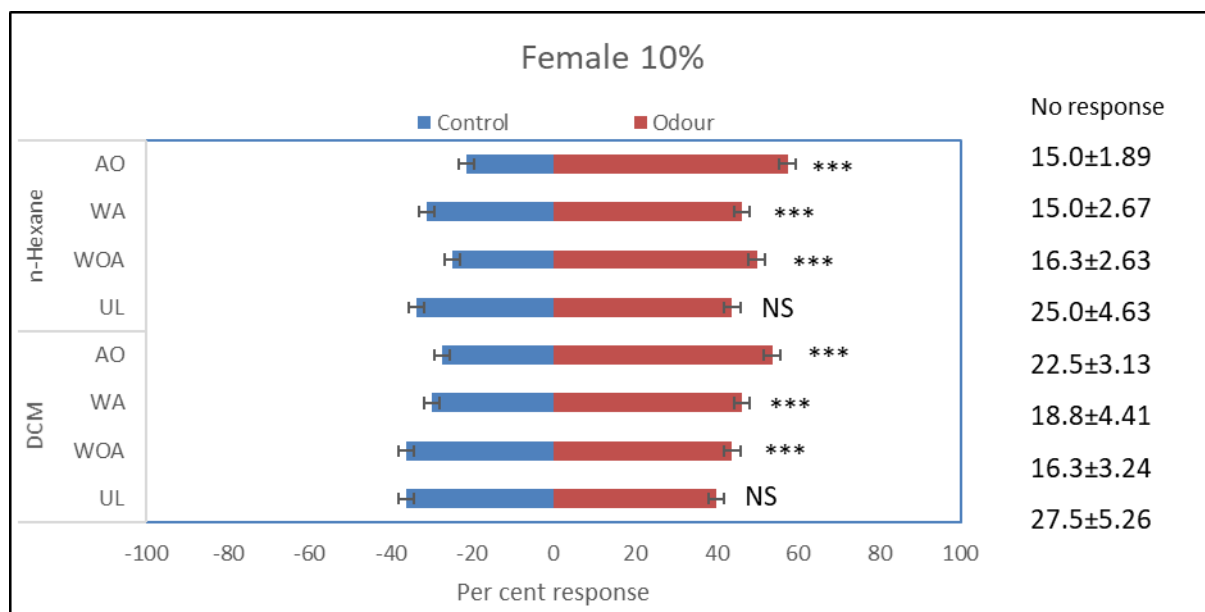
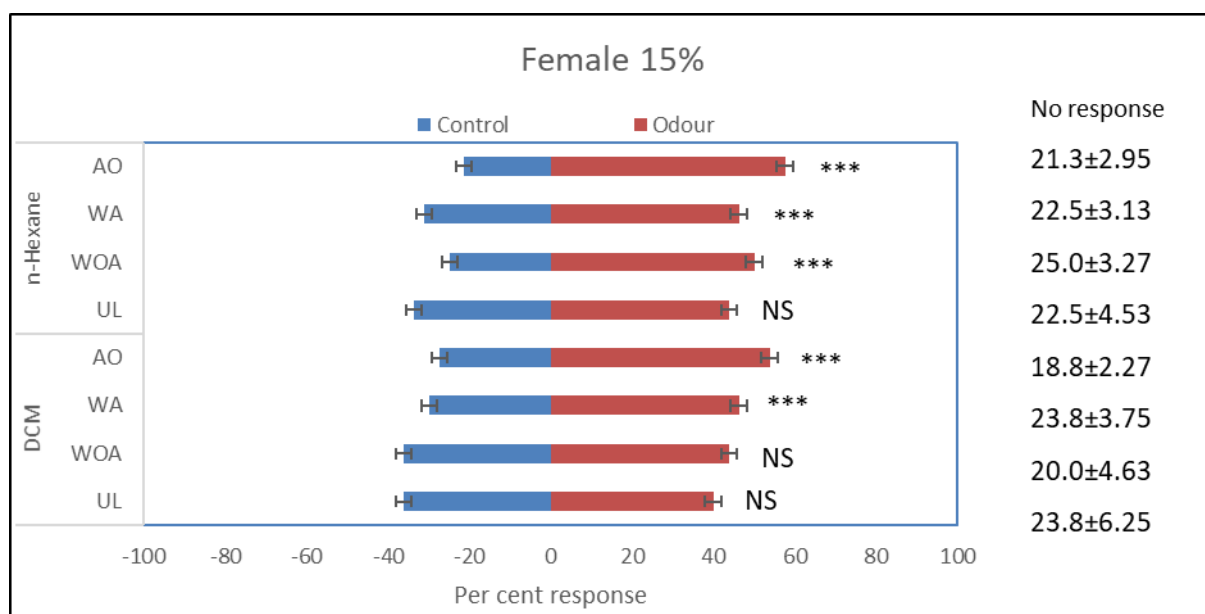


Figure 4.23: Per cent orientation of *C. sexmaculata* female to different extracts at 10% concentration



*Significant at $P<0.05$, ** Significant at $P<0.01$, *** Significant at $P<0.001$, NS-Non-significant.

Figure 4.24: Per cent orientation of *C. sexmaculata* female to different extracts at 15% concentration

Table 4.21: Comparative response of *C. sexmaculata* female to the different concentrations of each extract

Extractant	Extract	Orientation response of female (%±SE) to the extracts at the respective concentration				F cal	P value
		1%	5%	10%	15%		
DCM	UL	40.0±2.67	37.5±3.66	36.8±2.35	40.0±3.78	0.132	0.940NS
	WOA	52.5±1.64	51.3±3.98	53.8±3.24	41.8±3.20	1.730	0.184NS
	WA	58.8±4.79bc	83.8±3.24a	66.3±4.98b	46.3±3.24c	14.303	7.72E-06***
	AO	48.8±2.95b	70.0±2.67a	55.0±3.27b	53.8±2.63b	10.0178	0.000118***
n-Hexane	UL	38.8±2.35	41.3±2.27	38.8±2.95	43.8±4.20	0.570	0.639NS
	WOA	47.8±2.95	56.3±2.63	53.8±1.83	50.0±3.78	1.425	0.256NS
	WA	52.5±4.53bc	72.5±2.50a	63.8±4.60ab	46.3±1.83c	10.63	7.74e-05 ***
	AO	52.5±2.50b	66.3±3.24a	61.3±3.98ab	57.5±1.64ab	3.840	0.0202*

*Significant at $P<0.05$, ** Significant at $P<0.01$, *** Significant at $P<0.001$, NS-Non-significant. Values with the same letter for the respective rows are not significantly different.

Table 4.22: Comparative response of *C. sexmaculata* male and female to different extracts at various concentrations

Extra ctant	Extract	Concen tration	% attraction of male		% attraction of female		T cal	P value
			Mean	SE	Mean	SE		
DCM	UL	1%	37.50	3.66	40.00	2.67	0.552	0.295NS
		5%	38.75	3.98	37.50	3.66	0.231	0.410NS
		10%	38.75	3.98	38.75	2.95	0.000	0.500NS
		15%	31.25	2.95	40.00	3.78	1.825	0.045*
	WOA	1%	50.00	5.35	52.50	1.64	-0.447	0.331NS
		5%	50.00	4.63	51.25	3.98	-0.205	0.420NS
		10%	52.50	4.12	53.75	3.24	-0.239	0.407NS
		15%	46.25	3.24	43.75	4.20	0.471	0.322NS
	WA	1%	68.75	2.27	58.75	4.79	1.886	0.040*
		5%	72.50	3.66	83.75	3.24	-2.302	0.019*
		10%	68.75	3.98	66.25	4.98	0.392	0.350NS
		15%	51.25	2.95	46.25	3.24	1.141	0.273NS
	AO	1%	48.75	5.15	48.75	2.95	0.000	0.500NS
		5%	57.50	2.50	70.00	2.67	-3.416	0.002**
		10%	63.75	4.20	55.00	3.27	1.643	0.061*
		15%	58.75	3.98	53.75	2.63	1.048	0.156NS
n- Hexane	UL	1%	38.75	2.95	53.75	2.63	-3.795	0.002**
		5%	38.75	3.98	41.25	2.27	-0.546	0.297NS
		10%	41.25	4.41	38.75	2.95	0.471	0.322NS
		15%	37.50	2.50	43.75	4.20	-1.279	0.111NS
	WOA	1%	52.50	5.59	48.75	2.95	0.593	0.281NS
		5%	53.75	4.98	56.25	2.63	-0.444	0.332NS
		10%	58.75	2.266	53.75	1.83	1.717	0.054NS
		15%	47.50	3.13	50.00	3.78	-0.509	0.309NS
	WA	1%	67.50	3.66	52.50	4.53	2.575	0.011*
		5%	70.00	4.23	72.50	2.50	-0.509	0.309NS
		10%	68.75	2.95	63.75	4.60	0.914	0.188NS
		15%	47.50	2.50	46.25	1.83	0.403	0.346NS
	AO	1%	56.25	2.63	52.50	2.50	1.033	0.159NS
		5%	61.25	2.95	66.25	3.24	-1.141	0.136NS
		10%	65.00	3.27	61.25	3.98	0.728	0.239NS
		15%	56.25	2.63	57.50	1.64	-0.403	0.346NS

*Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$, NS- Non-significant.

4.5.3 Response of *C. sexmaculata* male beetles in terms of time to reach the odour source

Orientation time (time taken by beetles to reach the odour source) response of male beetles was recorded in Y-tube olfactometer as studied for both DCM and n-Hexane extracts. The data was analysed and compared with control, and different odours at various concentrations.

4.5.3.1 Orientation time response of male beetles to different odours extracted with DCM

Details of orientation time response of male beetle to the different extracts of DCM is given with comparisons between odour vs control and also among the different odour sources (Table 4.23).

Olfactometer studies on male beetles using 1 per cent concentration of different DCM extracts resulted a significant reduction in time to reach the odour source except un-infested leaf (UL). Extracts were found to attract male beetles as it took significantly less time compared to the control. Among the significant extracts, WOA attracted faster and took less time (4.1 ± 0.27 min) followed by WA (4.3 ± 0.19 min) and AO (4.6 ± 0.21 min). However, there was no significant difference among these three extracts for orientation time. Control has witnessed 6.6 ± 0.51 , 6.2 ± 0.23 , 6.8 ± 0.24 and 5.4 ± 0.15 min of orientation time response for UL (un-infested leaf), WOA (infested leaf without aphid), WA (infested leaf with aphid) and AO (aphids only), respectively (Figure 4.25).

When exposed to 5 per cent concentration, all the extracts except UL have shown faster attraction compared to the control. The time to reach the odour source for WA, AO, WOA and UL was recorded as 3.9 ± 0.12 , 4.4 ± 0.14 , 4.4 ± 0.20 and 6.8 ± 0.29 min, respectively against the control with 7.1 ± 0.16 , 6.1 ± 0.28 , 6.3 ± 0.22 and 7.1 ± 0.41 min for the respective extracts. However, there was no significant difference among the three significant extracts (Figure 4.26).

Table 4.23: Time taken to reach the odour source by *C. sexmaculata* male to DCM extracts

Extract concentration	Extract	Mean time to reach the odour and control Aarm of Y-tube olfactometer (min ± SE)		T cal	P value
		Odour Arm	Control Arm		
1 %	UL	6.5±0.27a	6.6±0.51	0.230	0.82014
	WOA	4.1±0.27b	6.2±0.23	5.562	3.51E-06
	WA	4.3±0.19b	6.8±0.24	7.047	3.9E-08
	AO	4.6±0.21b	5.4±0.15	3.035	0.004591
		Fcal	18.94		
		P value	2.53e-09		
5 %	UL	6.8±0.29a	7.1±0.41	0.676	0.504807
	WOA	4.4±0.20b	6.3±0.22	6.412	2.88E-07
	WA	3.9±0.12b	7.1±0.16	11.895	1.15E-13
	AO	4.4±0.14b	6.1±0.28	6.174	4.56E-07
		Fcal	44.99		
		P value	<2e-16		
10 %	UL	6.3±0.47a	6.1±0.32	0.394	0.696254
	WOA	3.7±0.19bc	6.8±0.28	9.315	6.96E-11
	WA	3.5±0.18c	6.3±0.11	7.619	9.08E-09
	AO	4.5±0.19b	7.6±0.27	8.726	4.38E-10
		Fcal	23.16		
		P value	4.74e-11		
15 %	UL	6.9±0.27a	6.4±0.34	1.007	0.324609
	WOA	4.0±0.14c	8.0±0.25	14.363	3.02E-15
	WA	5.3±0.35b	6.9±0.18	2.569	0.016543
	AO	5.7±0.40ab	7.9±0.50	3.414	0.001636
		Fcal	10.04		
		P value	1.43e-05		

Values with the same letter for the respective columns are not significantly different.

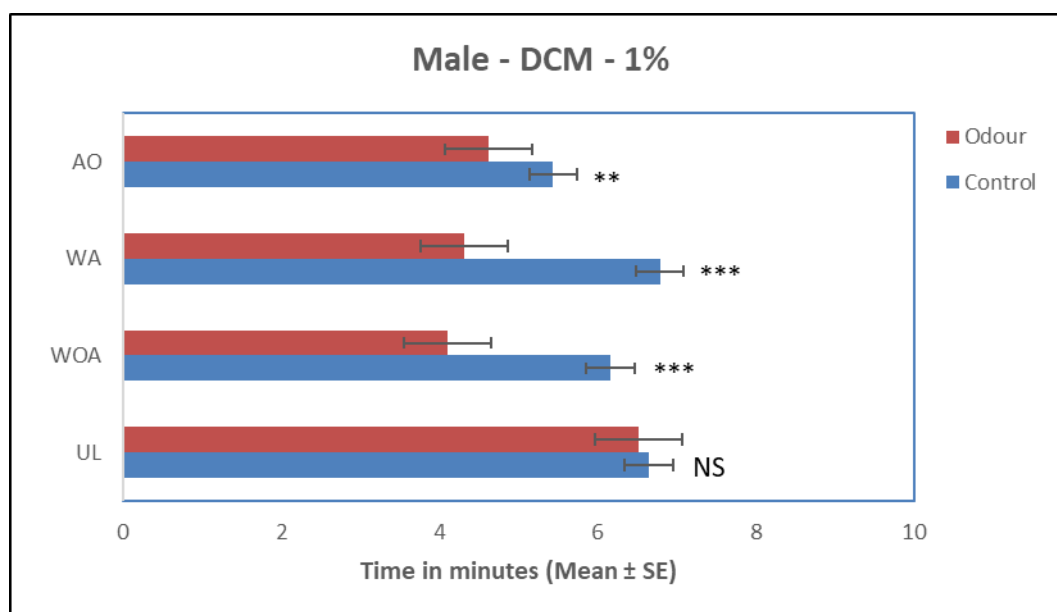
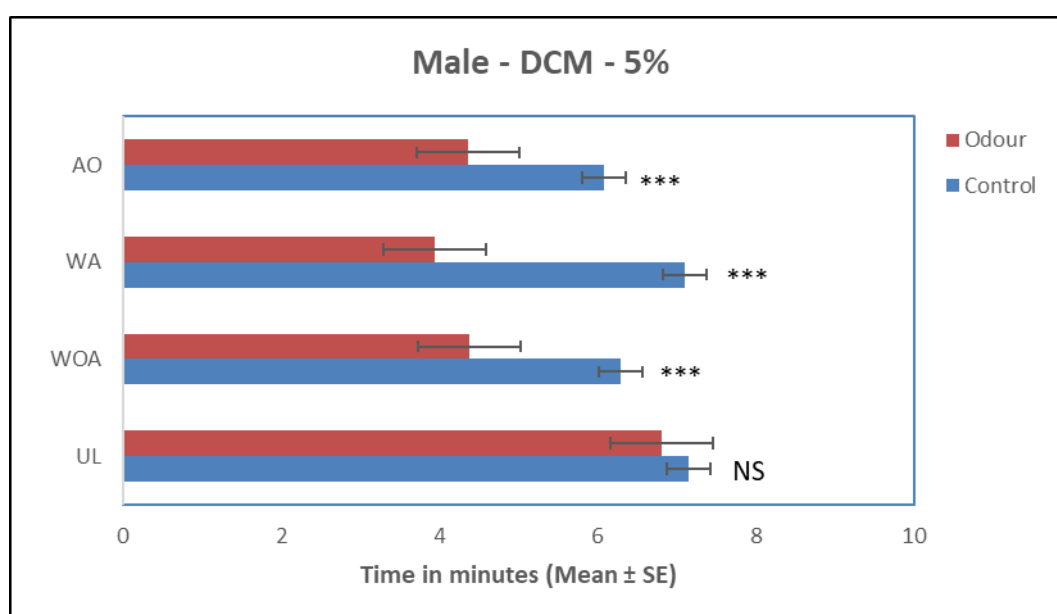


Figure 4.25: Orientation time response of *C. sexmaculata* male to different DCM extracts at 1% concentration



*Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$, NS-Non-significant.

Figure 4.26: Orientation time response of *C. sexmaculata* male to different DCM extracts at 5% concentration

At 10 per cent concentration also similar trend of significant higher attraction was observed for the three extracts as observed earlier with 1 and 5% concentration. Here a demarcation was observed among the three extracts in which WA stood first with least time taken (3.5 ± 0.18 min) followed by WOA (3.7 ± 0.19) and AO (4.5 ± 0.19) with a significant difference (Figure 4.27).

Even at 15 per cent concentration, the un-infested leaf (UL) extract has not shown a significant attraction compared to the control. Whereas, remaining three extracts have shown a higher attraction over control and also exhibited a significant difference among them. Among the three extracts, WOA took least time (4.0 ± 0.14 min) followed by WA (5.3 ± 0.35) and AO (5.7 ± 0.40). However, the time required to reach the odour source was more than the one observed at the lower concentrations (Figure 4.28).

When compared the performance of extracts across the concentration gradient, un-infested leaf (UL) and infested leaf without aphid (WOA) extracts have not shown the significant difference among the concentrations. Whereas, infested leaf with aphid (WA) and aphid only (AO) extract have displayed the significant differences among the concentrations.

WA extract has taken the least time (3.5 ± 0.18 min) at 10% followed by 5 (3.9 ± 0.12), 1 (4.3 ± 0.19) and 15 per cent concentration (5.3 ± 0.35) by displaying significant difference between each other. AO has recorded least time at 5% (4.4 ± 0.14) followed by 10 (4.5 ± 0.19), 1 (4.6 ± 0.21) and 15% (5.7 ± 0.40) concentration without any significant difference between first three concentrations (Table 4.24).

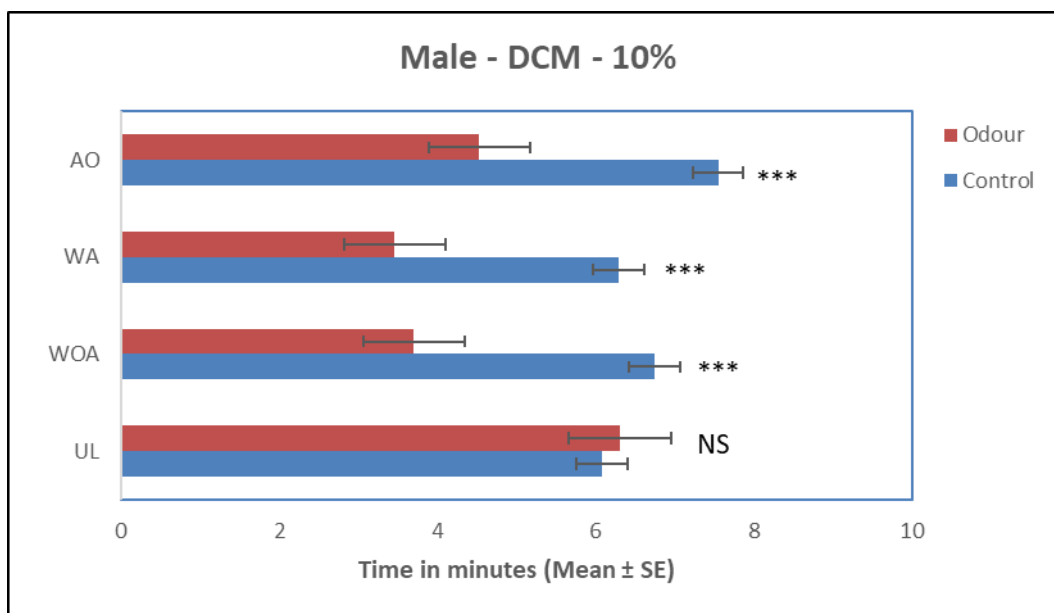
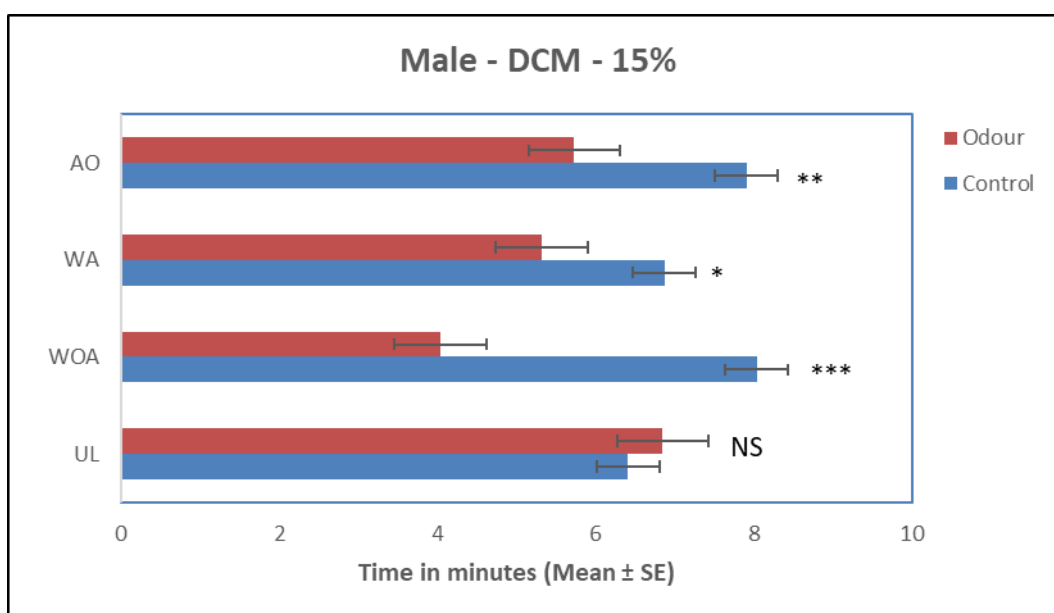


Figure 4.27: Orientation time response of *C. sexmaculata* male to different DCM extracts at 10% concentration



*Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$, NS-Non-significant.

Figure 4.28: Orientation time response of *C. sexmaculata* male to different DCM extracts at 15% concentration

Table 4.24: Comparative orientation time response of *C. sexmaculata* male to the different DCM extracts at various concentrations

Extractant	Extract	Orientation time response of female (min±SE) to the extracts at the respective concentration				F cal	P value
		1%	5%	10%	15%		
DCM	UL	6.5±0.27	6.8±0.29	6.3±0.47	6.9±0.27	0.565039	0.6404NS
	WOA	4.1±0.27	4.4±0.20	3.7±0.19	4.0±0.14	1.840159	0.1471NS
	WA	4.3±0.19b	3.9±0.12bc	3.5±0.18c	5.3±0.35a	13.19895	2.5E-07***
	AO	4.6±0.21b	4.4±0.14b	4.5±0.19b	5.7±0.40a	6.13667	0.0008***

*Significant at $P<0.05$, ** Significant at $P<0.01$, *** Significant at $P<0.001$, NS-Non-significant. Values with the same letter for the respective rows are not significantly different.

4.5.3.2 Orientation time response of male beetles to different odours extracted with n-Hexane

Details of orientation time response of male beetle to the different extracts of n-Hexane is given with comparisons between odour vs control and also among the different odour sources (Table 4.25).

Olfactometer studies on male beetles using 1% concentration of different n-Hexane extracts resulted a significant reduction in time to reach the odour source except un-infested leaf (UL). Extracts were found to attract males as it took significantly less time compared to the control. Among the significant extracts, AO attracted faster as took less time (4.1 ± 0.33 min) followed by WOA (4.3 ± 0.31) and WA (4.3 ± 0.33). However, there was no significant difference among these three extracts for orientation time. Control has witnessed 6.6 ± 0.51 , 6.2 ± 0.23 , 6.8 ± 0.24 and 5.4 ± 0.15 min of orientation time response for, WOA, WA and AO, respectively (Figure 4.29).

When exposed to 5% concentration, all the extracts except UL and AO have shown faster attraction compared to the control. The time to reach the odour source for WA, WOA, AO and UL was recorded as 3.6 ± 0.25 , 4.5 ± 0.21 , 5.9 ± 0.44 and 6.5 ± 0.17 min, respectively against the control with 7.3 ± 0.33 , 6.1 ± 0.41 , 7.3 ± 0.44 and 6.4 ± 0.14 min for the respective extracts. However, there was no significant difference among the three significant extracts (Figure 4.30).

At 10 per cent concentration also similar trend of significant higher attraction was observed for the three extrats. Time recorded with WA was minimum (4.1 ± 0.37 min) followed by WOA (4.2 ± 0.27), AO (4.8 ± 0.44) and UL (7.1 ± 0.31) (Figure 4.31).

Even at 15 per cent concentration, the un-infested (UL) leaf extract has not shown a significant attraction compared to the control. Whereas, remaining three extracts have shown a higher attraction over control and also exhibited a significant difference among them. Among the three extracts, AO took least time (3.6 ± 0.30 min) followed by WA (4.1 ± 0.29 min) and WOA (5.2 ± 0.40). Here, at 15 % concentration, all the extracts have shown a significant difference with each other (Figure 4.32).

Table 4.25: Time taken to reach the odour source by *C. sexmaculata* male to n-Hexane extracts

Extract concentration	Extract	Mean time to reach the odour and control Aarm of Y-tube olfactometer (min ± SE)		T cal	P value
		Odour Arm	Control Arm		
1 %	UL	6.4±0.18a	6.7±0.12	1.394	0.1738NS
	WOA	4.3±0.31b	6.2±0.23	4.637	5.05E-05***
	WA	4.3±0.33b	8.0±0.33	6.012	1.05E-06***
	AO	4.1±0.33b	7.6±0.58	5.597	2.88E-06***
		Fcal	9.995		
		P value	1.12e-05***		
5 %	UL	6.5±0.17a	6.4±0.14	0.565	0.5765NS
	WOA	4.5±0.21b	6.1±0.41	3.833	0.0005***
	WA	3.6±0.25b	7.3±0.33	7.457	1.43E-08***
	AO	5.9±0.44b	7.3±0.44	1.978	0.0561NS
		Fcal	17.47		
		P value	7.03e-09***		
10 %	UL	7.1±0.31a	6.6±0.16	1.467	0.1534NS
	WOA	4.2±0.27b	5.8±0.17	3.882	0.00047***
	WA	4.1±0.37b	7.5±0.33	4.280	0.000176***
	AO	4.8±0.44b	8.8±0.60	4.082	0.000256***
		Fcal	10.71		
		P value	4.71e-06***		
15 %	UL	6.3±0.12a	7.3±0.33	2.722	0.011044*
	WOA	5.2±0.40ab	7.3±0.45	3.567	0.001161**
	WA	4.1±0.29bc	7.0±0.38	5.632	7.34E-06***
	AO	3.6±0.30c	5.9±0.51	4.081	0.000246***
		Fcal	14.00		
		P value	2.9e-07***		

*Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$, NS- Non-significant. Values with the same letter for the respective columns are not significantly different.

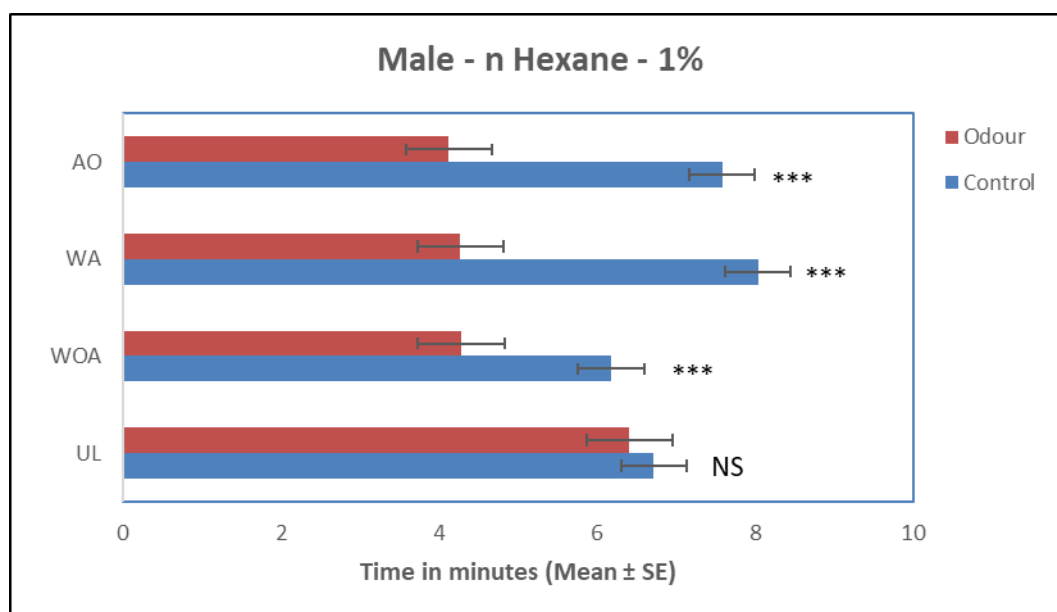
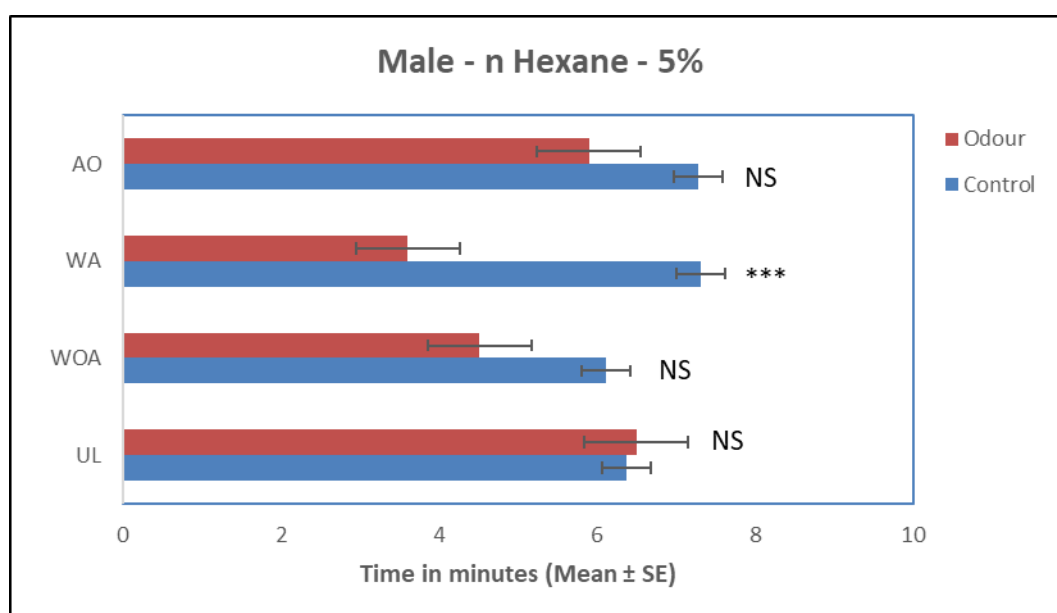


Figure 4.29: Orientation time response of *C. sexmaculata* male to different n-Hexane extracts at 1% concentration



*Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$, NS-Non-significant.

Figure 4.30: Orientation time response of *C. sexmaculata* male to different n-Hexane extracts at 5% concentration

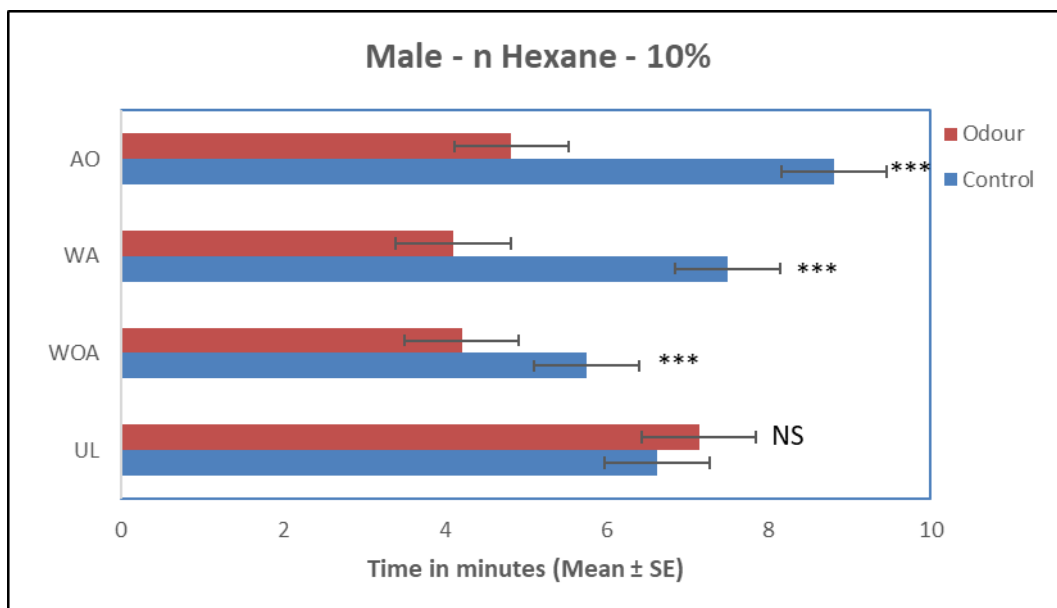
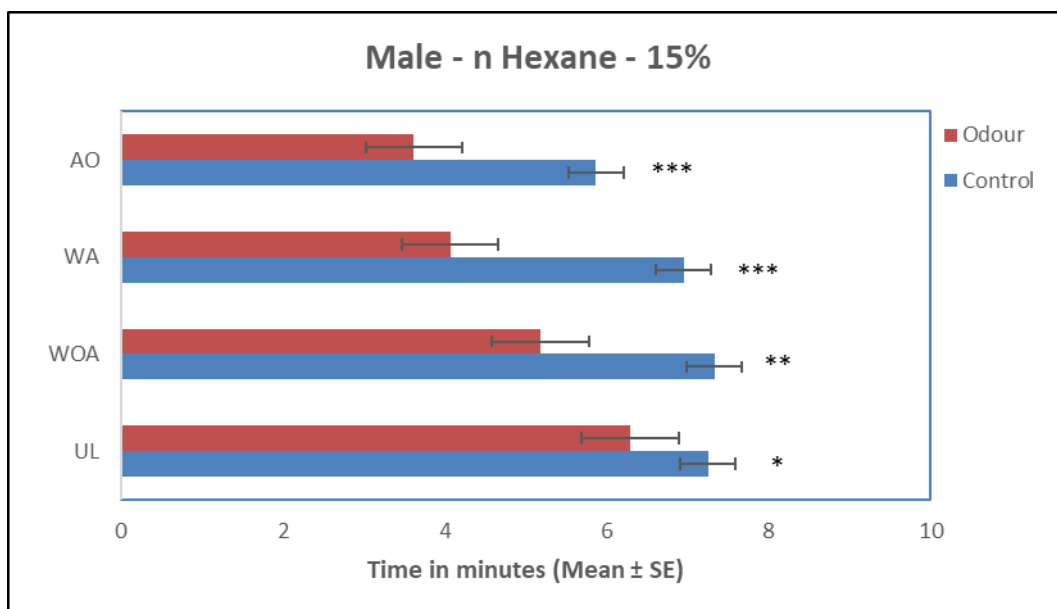


Figure 4.31: Orientation time response of *C. sexmaculata* male to different n-Hexane extracts at 10% concentration



*Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$, NS-Non-significant.

Figure 4.32: Orientation time response of *C. sexmaculata* male to different n-Hexane extracts at 15% concentration

When compared the performance of extracts across the concentration gradient, aphid only (AO) extract showed the significant difference among the concentrations. AO extract has taken least time (3.6 ± 0.30 min) at 15% followed by 1 (4.1 ± 0.33), 10 (4.8 ± 0.44) and 5% (5.9 ± 0.44) concentration; 5% concentration significantly differed with others (Table 4.26).

Time taken to reach the DCM and n-Hexane odour source of different extracts in the order of lowest to highest was: D-WA (10), n-WA (5), n-AO (15), D-WOA (10), D-WA (5), D-WOA (15), D-WOA (1), n-AO (1), n-WA (10), n-WA (15), n-WOA (10), D-WA (1), n-WOA (1), n-WA (1), D-WOA (5), D-AO (5), n-WOA (5), D-AO (10), D-AO (1), n-AO (10), n-WOA (15), D-WA (15), D-AO (15), n-AO (5), D-UL (10), n-UL (15), n-UL (1), D-UL (1), n-UL (5), D-UL (5), D-UL (15) and n-UL (10) with the time recorded as 3.5 ± 0.18 , 3.6 ± 0.25 , 3.6 ± 0.30 , 3.7 ± 0.19 , 3.9 ± 0.12 , 4.0 ± 0.14 , 4.1 ± 0.27 , 4.1 ± 0.33 , 4.1 ± 0.37 , 4.1 ± 0.29 , 4.2 ± 0.27 , 4.3 ± 0.19 , 4.3 ± 0.31 , 4.3 ± 0.33 , 4.4 ± 0.20 , 4.4 ± 0.14 , 4.5 ± 0.21 , 4.5 ± 0.19 , 4.6 ± 0.21 , 4.8 ± 0.44 , 5.2 ± 0.40 , 5.3 ± 0.35 , 5.7 ± 0.40 , 5.9 ± 0.44 , 6.3 ± 0.47 , 6.3 ± 0.12 , 6.4 ± 0.18 , 6.5 ± 0.27 , 6.5 ± 0.17 , 6.8 ± 0.29 , 6.9 ± 0.27 and 7.1 ± 0.31 min, respectively.

4.5.4 Response of *C. sexmaculata* female beetles in terms of time to reach the odour source

Orientation time (time taken by beetles to reach the odour source) response of female beetles was also recorded and analysed in the same way as done for male beetles. The results for response variations between male and female were also compared.

4.5.4.1 Orientation time response of female beetles to different odours extracted with DCM

Details of orientation time response of female beetle to the different extracts of DCM is given with comparisons between odour vs control and also among the different odour sources (Table 4.27).

Table 4.26: Comparative orientation time response of *C. sexmaculata* male to the different n-Hexane extracts at various concentrations

Extractant	Extract	Orientation time response of female (min±SE) to the extracts at the respective concentration				F cal	P value
		1%	5%	10%	15%		
n-Hexane	UL	6.4±0.18ab	6.5±0.17ab	7.1±0.31a	6.3±0.12b	3.281594	0.0272*
	WOA	4.3±0.31	4.5±0.21	4.2±0.27	5.2±0.40	2.084894	0.1087NS
	WA	4.3±0.33	3.6±0.25	4.1±0.37	4.1±0.29	0.87198	0.4585NS
	AO	4.1±0.33b	5.9±0.44a	4.8±0.44ab	3.6±0.30b	6.208831	0.0007***

*Significant at $P<0.05$, ** Significant at $P<0.01$, *** Significant at $P<0.001$, NS-Non-significant. Values with the same letter for the respective rows are not significantly different.

Table 4.27: Time taken to reach the odour source by *C. sexmaculata* female to DCM extracts

Extract concentration	Extract	Mean time to reach the odour and control Aarm of Y-tube olfactometer (min ± SE)		T cal	P value
		Odour Arm	Control Arm		
1 %	UL	5.6±0.37a	6.4±0.52	3.577	0.0013**
	WOA	3.6±0.15c	6.3±0.29	3.941	0.0004***
	WA	5.0±0.33ab	7.1±0.41	7.768	1.45E-08***
	AO	4.0±0.23bc	5.8±0.21	4.767	3.91E-05***
		Fcal	10.08		
		P value	1.18e-05***		
5 %	UL	5.7±0.50a	7.0±0.42	2.065	0.0473*
	WOA	3.7±0.18b	6.3±0.27	3.465	0.0014**
	WA	3.7±0.10b	7.8±0.37	8.954	5.62E-10***
	AO	4.2±0.10b	6.8±0.19	4.719	4.8E-05***
		Fcal	17.21		
		P value	7.03e-09***		
10 %	UL	5.6±0.46a	6.3±0.40	1.020	0.3167NS
	WOA	3.5±0.11b	7.0±0.15	5.144	1.42E-05***
	WA	4.4±0.41ab	7.0±0.52	2.731	0.0103*
	AO	3.9±0.17b	7.1±0.54	4.315	0.0001***
		Fcal	7.642		
		P value	0.0001***		
15 %	UL	6.0±0.41b	7.0±0.41	3.292	0.0027**
	WOA	4.3±0.13c	7.7±0.38	2.592	0.0150*
	WA	7.3±0.24a	7.2±0.18	1.358	0.1852NS
	AO	4.7±0.28c	7.7±0.62	3.866	0.0006***
		Fcal	23.98		
		P value	1.07e-10***		

*Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$, NS-Non-significant. Values with the same letter for the respective columns are not significantly different.

Female beetle of *C. sexmaculata* have shown significant attraction for all the extracts over control. Infested leaf without aphid (WOA) took least time (3.6 ± 0.15 min) followed by AO (4.0 ± 0.23), WA (3.6 ± 0.15) and UL (5.6 ± 0.37). All the extracts displayed significant difference with the control as well as between each other (Figure 4.33).

When exposed to 5 per cent concentration, all the extracts except UL have shown faster attraction compared to the control. The time to reach the odour source in increasing order was WA (3.7 ± 0.10 min), WOA (3.7 ± 0.18), AO (4.2 ± 0.10) and UL (5.7 ± 0.50). However, other treatments differed significantly with un-infested leaf (UL) as well as control, but not among them (Figure 4.34).

At 10% concentration also, similar trend of significant higher attraction was observed for the three extracts (WA, WOA and AO). Time recorded with WOA was the least (3.5 ± 0.11 min) followed by AO (3.9 ± 0.17) and WA (4.4 ± 0.41). However, WOA and AO have not shown significant difference between them (Figure 4.35).

At 15% concentration, un-infested leaf (UL) extract has shown significant attraction with less time over control, but not with aphid (WA) extract. WOA and AO have attracted female beetles in 4.3 ± 0.13 and 4.7 ± 0.28 min, respectively (Figure 4.36).

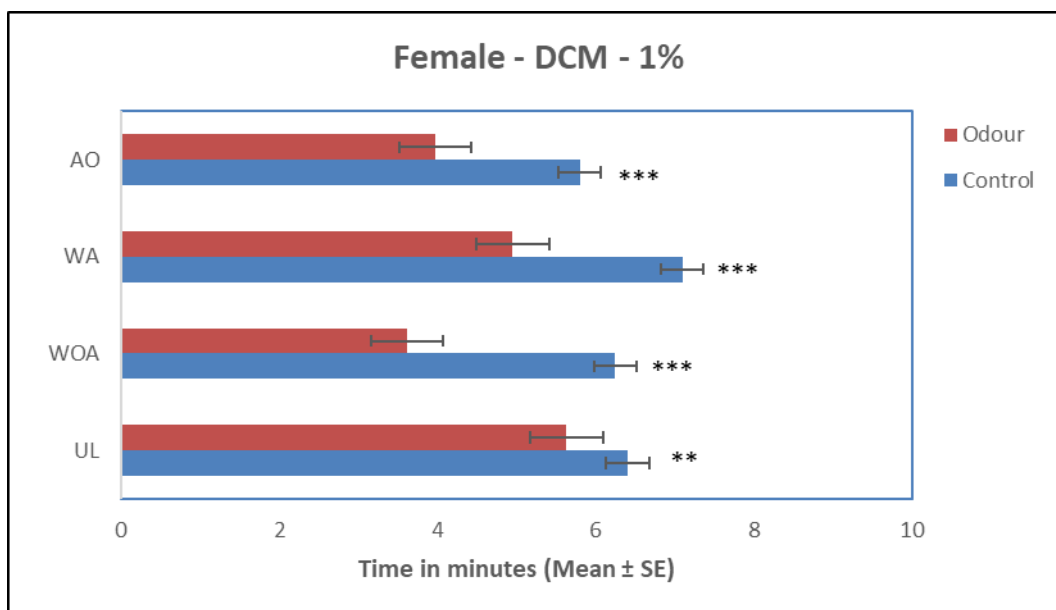
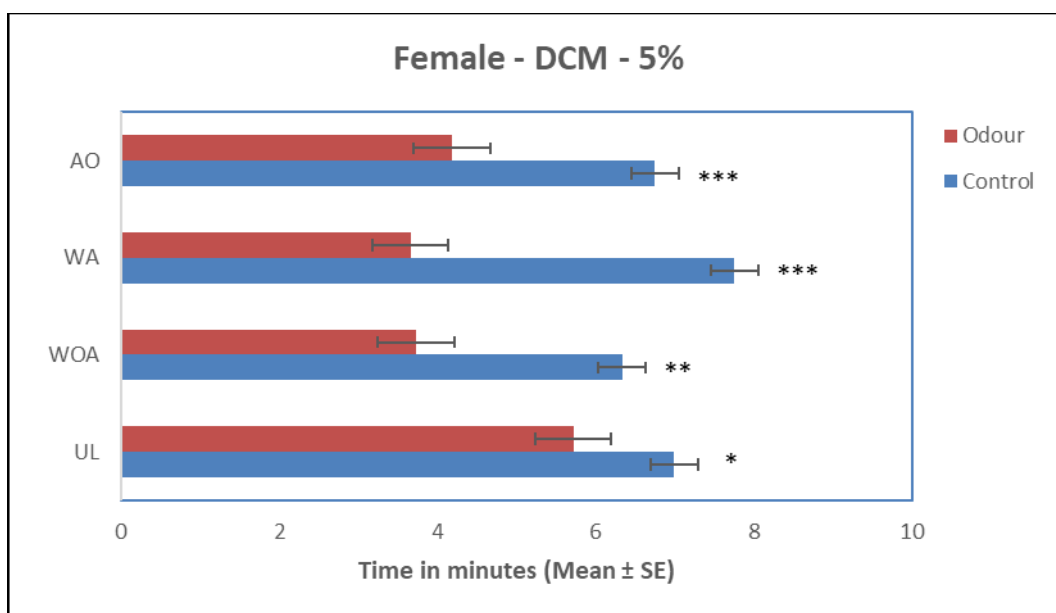


Figure 4.33: Orientation time response of *C. sexmaculata* female to different DCM extracts at 1% concentration



*Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$,
NS-Non-significant.

Figure 4.34: Orientation time response of *C. sexmaculata* female to different DCM extracts at 5% concentration

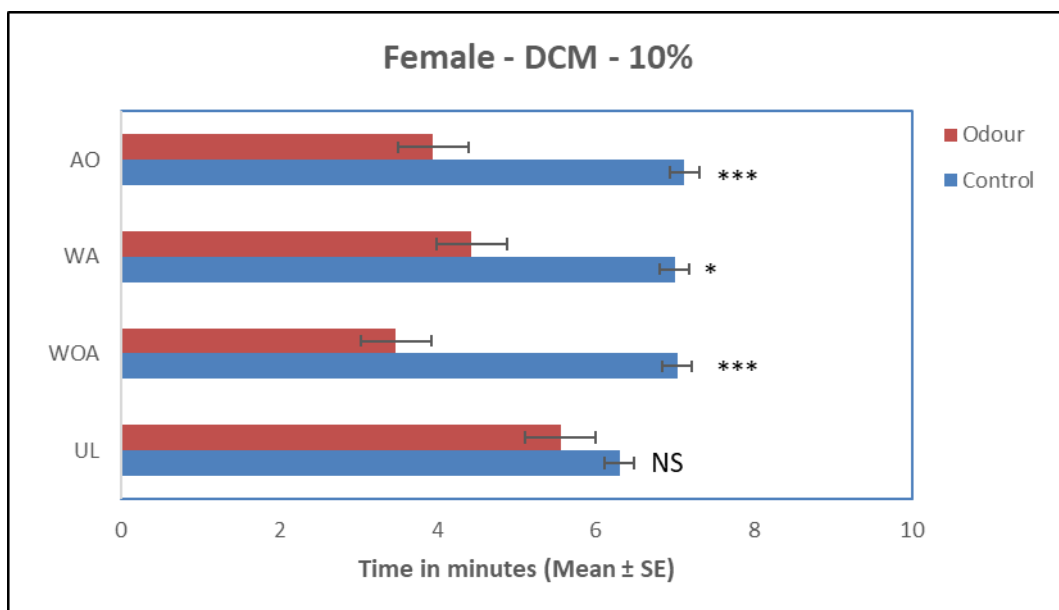
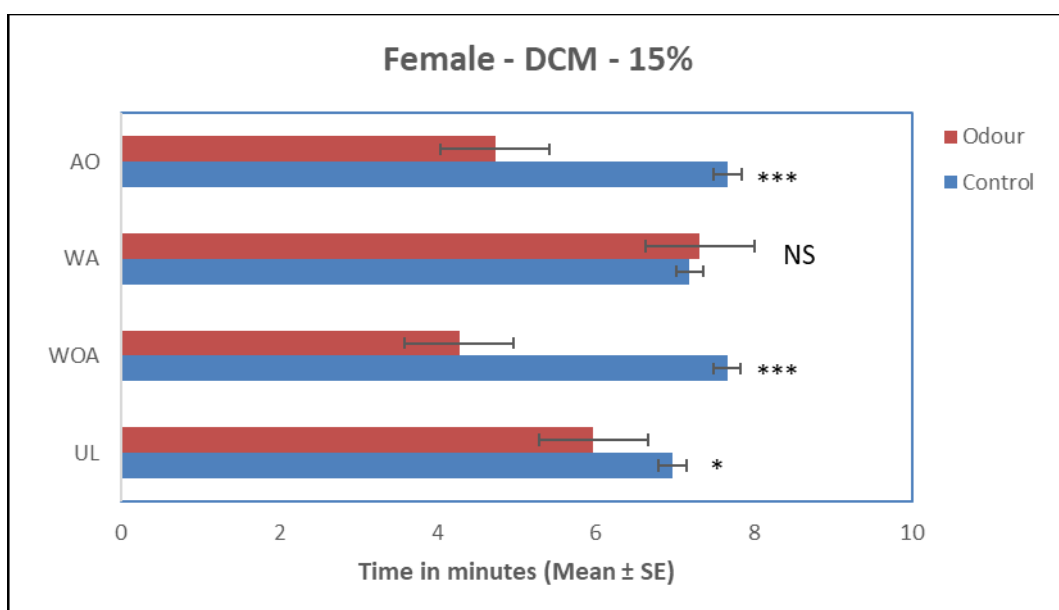


Figure 4.35: Orientation time response of *C. sexmaculata* female to different DCM extracts at 10% concentration



*Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$, NS-Non-significant.

Figure 4.36: Orientation time response of *C. sexmaculata* female to different DCM extracts at 15% concentration

When the performance of extracts across the concentration gradient was compared, except un-infested leaf (UL) all the extracts showed a significant difference. WOA has taken least time (3.5 ± 0.11 min) at 10% concentration followed by 1 (3.6 ± 0.15), 5 (3.7 ± 0.18) and 15% (4.3 ± 0.13). Similar trend was observed for AO with 3.9 ± 0.17 , 4.0 ± 0.23 , 4.2 ± 0.10 and 4.7 ± 0.28 min at 10, 1, 5 and 15% concentration, respectively. Infested leaf with aphid (WA) has shown faster attraction at 5% followed by at 10, 1 and 15% concentration with 3.7 ± 0.10 , 4.4 ± 0.41 , 4.0 ± 0.23 and 4.7 ± 0.28 min of orientation time response, respectively (Table 4.28).

4.5.4.2 Orientation time response of female beetles to different odours extracted with n-Hexane

Details of orientation time response of female beetle to the different extracts of n-Hexane is given with comparisons between odour vs control and also among the different odour sources (Table 4.29).

Olfactometer studies on female beetles using 1 % concentration of different n-Hexane extracts resulted a significant reduction in time to reach the odour source, except un-infested leaf (UL). Extracts were found to attract females as significantly less time was taken compared to control. Time taken to reach the odour source for the extracts of WA, AO, WOA and UL was 3.6 ± 0.22 , 3.8 ± 0.40 , 4.2 ± 0.37 and 5.0 ± 0.43 min, respectively. However, there was no significant difference among the extracts for orientation time (Figure 4.37).

When exposed to 5 % concentration, all the extracts except UL have shown faster attraction compared to the control. The time to reach the odour source for WA, WOA, AO and UL was recorded as 3.5 ± 0.25 , 4.3 ± 0.23 , 4.7 ± 0.40 and 5.4 ± 0.50 min, respectively against the control with 7.6 ± 0.43 , 6.4 ± 0.80 , 8.4 ± 0.15 and 6.5 ± 0.15 min for the respective extracts. WA extract has shown a significant difference with all other extracts (Figure 4.38).

Table 4.28: Comparative orientation time response of *C. sexmaculata* female to the different DCM extracts at various concentrations

Extractant	Extract	Orientation time response of female (min±SE) to the extracts at the respective concentration				F cal	P value
		1%	5%	10%	15%		
DCM	UL	5.6±0.37	5.7±0.50	5.6±0.46	6.0±0.41	0.174085	0.9135NS
	WOA	3.6±0.15b	3.7±0.18ab	3.5±0.11b	4.3±0.13a	5.246244	0.0024**
	WA	5.0±0.33b	3.7±0.10c	4.4±0.41bc	7.3±0.24a	29.55545	2.86E-13***
	AO	4.0±0.23b	4.2±0.10ab	3.9±0.17b	4.7±0.28a	3.323619	0.0234*

*Significant at $P<0.05$, ** Significant at $P<0.01$, *** Significant at $P<0.001$, NS-Non-significant. Values with the same letter for the respective rows are not significantly different.

Table 4.29: Time taken to reach the odour source by *C. sexmaculata* female to n-Hexane extracts

Extract concentration	Extract	Mean time to reach the odour and control Aarm of Y-tube olfactometer (min $\pm$ SE)		T cal	P value
		Odour Arm	Control Arm		
1 %	UL	5.0 $\pm$ 0.43	6.6 $\pm$ 0.08	1.233	0.2276NS
	WOA	4.2 $\pm$ 0.37	6.1 $\pm$ 0.24	9.080	3.05E-10***
	WA	3.6 $\pm$ 0.22	7.3 $\pm$ 0.51	3.314	0.0025**
	AO	3.8 $\pm$ 0.40	7.1 $\pm$ 0.60	5.807	1.9E-06***
		Fcal	2.468		
		P value	0.0691NS		
5 %	UL	5.4 $\pm$ 0.50a	6.5 $\pm$ 0.15	1.926	0.0647NS
	WOA	4.3 $\pm$ 0.23ab	6.4 $\pm$ 0.80	8.182	3.92E-09***
	WA	3.5 $\pm$ 0.25b	7.6 $\pm$ 0.43	14.971	5.25E-16***
	AO	4.7 $\pm$ 0.40ab	8.4 $\pm$ 0.15	10.781	3.49E-12***
		Fcal	4.612		
		P value	0.00483**		
10 %	UL	5.6 $\pm$ 0.58a	6.3 $\pm$ 0.16	1.180	0.2492NS
	WOA	3.8 $\pm$ 0.28b	5.8 $\pm$ 0.19	18.828	1.59E-18***
	WA	5.6 $\pm$ 0.46a	7.9 $\pm$ 0.27	2.903	0.00781**
	AO	5.0 $\pm$ 0.45ab	8.7 $\pm$ 0.78	7.430	3.45E-08***
		Fcal	3.644		
		P value	0.0161*		
15 %	UL	5.9 $\pm$ 0.22a	7.1 $\pm$ 0.28	1.713	0.0978NS
	WOA	6.0 $\pm$ 0.46a	7.9 $\pm$ 0.53	9.148	4.78E-10***
	WA	5.3 $\pm$ 0.37ab	6.0 $\pm$ 0.40	0.393	0.6971NS
	AO	4.1 $\pm$ 0.31b	6.5 $\pm$ 0.58	4.991	2.39E-05***
		Fcal	6.808		
		P value	0.0004***		

*Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$, NS-Non-significant. Values with the same letter for the respective columns are not significantly different.

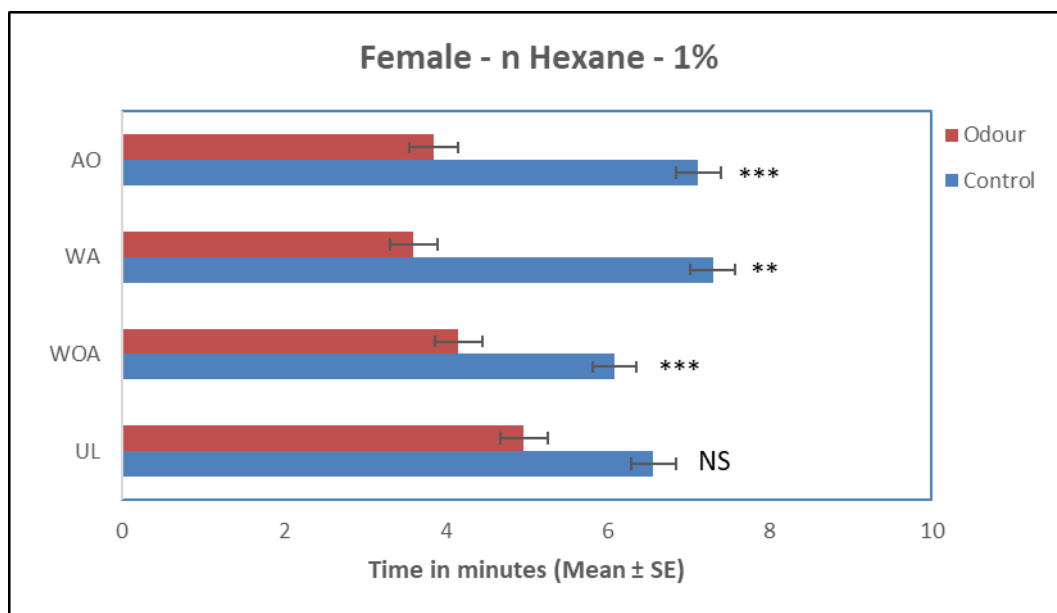
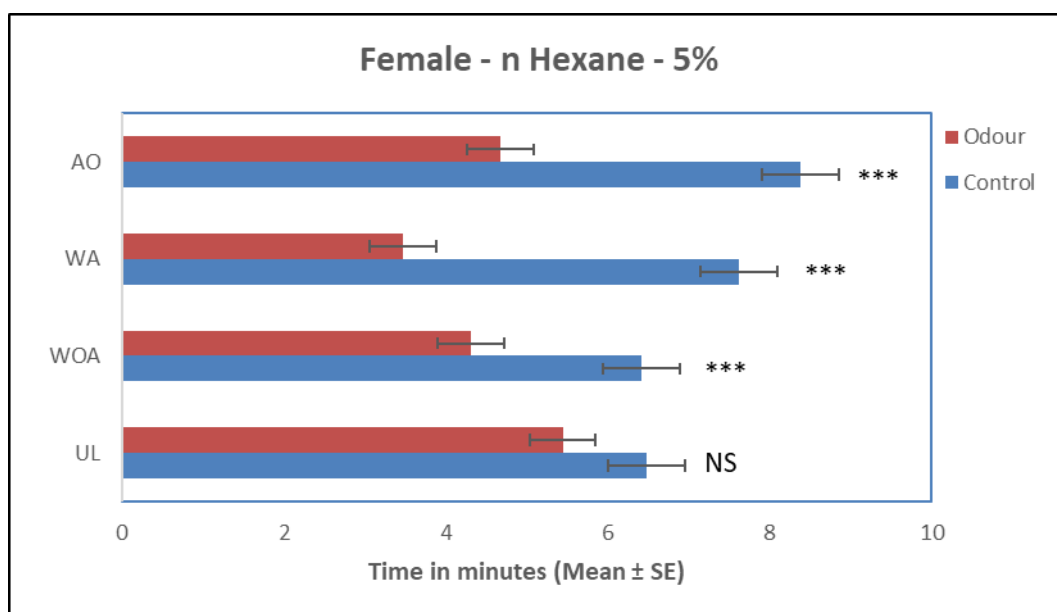


Figure 4.37: Orientation time response of *C. sexmaculata* female to different n-Hexane extracts at 1% concentration



*Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$, NS-Non-significant.

Figure 4.38: Orientation time response of *C. sexmaculata* female to different n-Hexane extracts at 5% concentration

At 10 % concentration also similar trend of significant higher attraction was observed for the three extracts. Time recorded with WOA was minimum (3.8 ± 0.28) followed by AO (5.0 ± 0.45), WA (5.6 ± 0.46) and UL (5.6 ± 0.58). However, WA has not shown significant difference with (UL) [Figure 4.39].

At 15 % concentration, un-infested leaf (UL) extract and WA have not shown significant attraction compared to the control. AO recorded 4.1 ± 0.31 min while WOA recorded 6.0 ± 0.46 min for female beetle to reach the odour source (Figure 4.40).

When the performance of extracts across the concentration gradient compared was recorded, infested leaf without aphid (WOA) and infested leaf with aphid (WA) showed the significant difference among the concentrations. WA has taken least time (3.5 ± 0.25 min) at 5% followed by 1 (3.5 ± 0.25), 15 (5.3 ± 0.37) and 10% concentration (5.6 ± 0.46). WOA took 4.2 ± 0.37 , 4.3 ± 0.23 , 3.8 ± 0.28 and 6.0 ± 0.46 min at 1, 5, 10 and 15% concentration, respectively. Whereas, AO recorded 3.8 ± 0.40 min at 1% followed by 4.1 ± 0.31 , 4.7 ± 0.40 and 5.0 ± 0.45 min at 15, 5 and 10% concentration, respectively (Table 4.30).

Time taken to reach the DCM and n-Hexane odour source of different extracts in the order of lowest to highest was: n-WA (5), D-WOA (10), D-WOA (1), n-WA (1), D-WOA (5), D-WA (5), n-AO (1), n-WOA (10), D-AO (10), D-AO (1), n-AO (15), n-WOA (1), D-AO (5), n-WOA (5), D-WOA (15), D-WA (10), n-AO (5), D-AO (15), D-WA (1), n-UL (1), n-AO (10), n-WA (15), n-UL (5), D-UL (1), D-UL (10), n-UL (10), n-WA (10), D-UL (5), n-UL (15), D-UL (15), n-WOA (15) and D-WA (15) with the times recorded as 3.5 ± 0.25 , 3.5 ± 0.11 , 3.6 ± 0.15 , 3.6 ± 0.22 , 3.7 ± 0.18 , 3.7 ± 0.10 , 3.8 ± 0.40 , 3.8 ± 0.28 , 3.9 ± 0.17 , 4.0 ± 0.23 , 4.1 ± 0.31 , 4.2 ± 0.37 , 4.2 ± 0.10 , 4.3 ± 0.23 , 4.3 ± 0.13 , 4.4 ± 0.41 , 4.7 ± 0.40 , 4.7 ± 0.28 , 5.0 ± 0.33 , 5.0 ± 0.43 , 5.0 ± 0.45 , 5.3 ± 0.37 , 5.4 ± 0.50 , 5.6 ± 0.37 , 5.6 ± 0.46 , 5.6 ± 0.58 , 5.6 ± 0.46 , 5.7 ± 0.50 , 5.9 ± 0.22 , 6.0 ± 0.41 , 6.0 ± 0.46 and 7.3 ± 0.24 min, respectively.

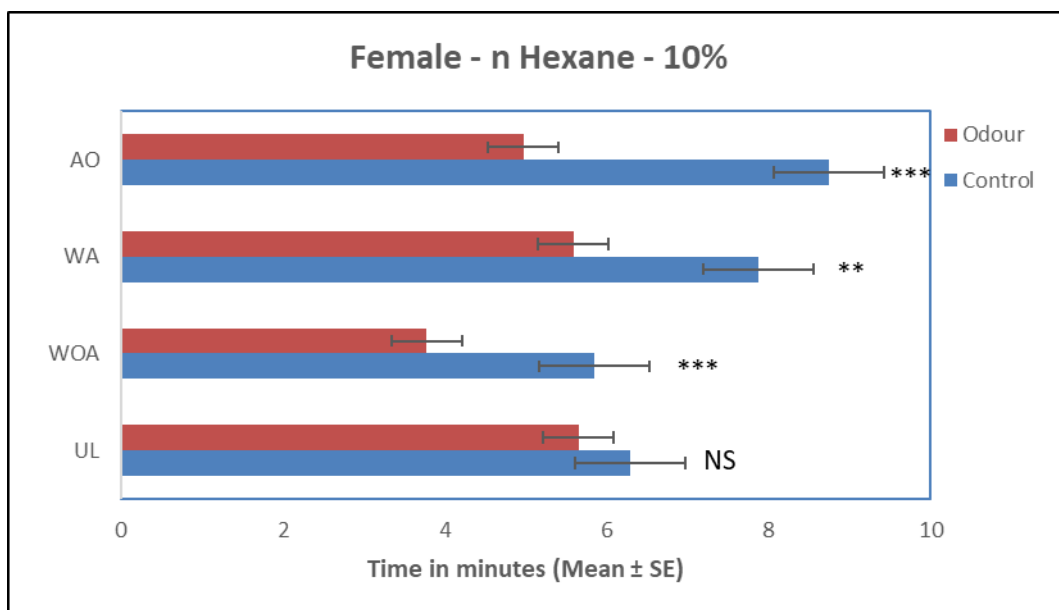
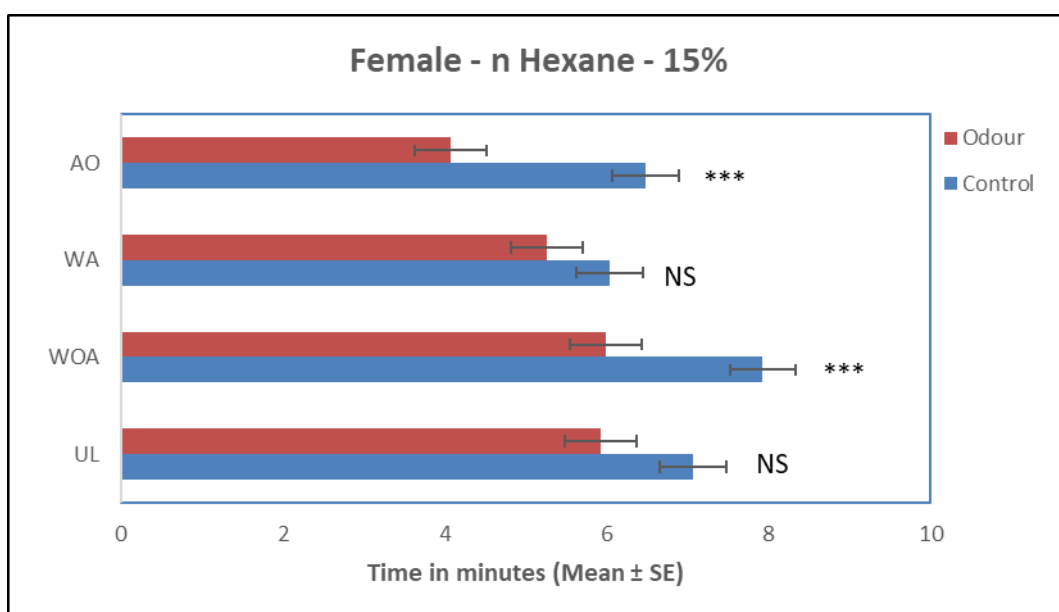


Figure 4.39: Orientation time response of *C. sexmaculata* female to different n-Hexane extracts at 10% concentration



*Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$, NS-Non-significant.

Figure 4.40: Orientation time response of *C. sexmaculata* female to different n-Hexane extracts at 15% concentration

Table 4.30: Comparative orientation time response of *C. sexmaculata* female to the different n-Hexane extracts at various concentrations

Extractant	Extract	Orientation time response of female (min±SE) to the extracts at the respective concentration				F cal	P value
		1%	5%	10%	15%		
n-Hexane	UL	5.0±0.43	5.4±0.50	5.6±0.58	5.9±0.22	0.835768	0.4796NS
	WOA	4.2±0.37b	4.3±0.23b	3.8±0.28b	6.0±0.46a	8.173628	7.65E-05***
	WA	3.6±0.22b	3.5±0.25b	5.6±0.46a	5.3±0.37a	9.580925	1.82E-05***
	AO	3.8±0.40	4.7±0.40	5.0±0.45	4.1±0.31	1.694319	0.1739NS

*Significant at $P<0.05$, ** Significant at $P<0.01$, *** Significant at $P<0.001$, NS-Non-significant. Values with the same letter for the respective rows are not significantly different.

Comparison for the time to reach the odour source between male and female beetles revealed that the female beetle took a significant lesser time than male. Details of comparative orientation time response of *C. sexmaculata* male and female beetle to different extracts of DCM and n-Hexane is furnished in Table 4.31.

Female and male have recorded orientation time to the DCM extracts of UL 1% (5.63 ± 0.37 and 6.51 ± 0.27), UL 5% (5.72 ± 0.50 and 6.81 ± 0.29), UL 15% (5.98 ± 0.41 and 6.85 ± 0.27), AO 1% (3.97 ± 0.23 and 4.62 ± 0.21) and AO 10% (3.94 ± 0.17 and 4.53 ± 0.19 min). However, male has shown faster attraction to DCM-WA at 5 (3.66 ± 0.10), 10 (3.46 ± 0.18) and 15% concentration (5.32 ± 0.35 min).

Similarly, n-Hexane extracts of UL 1, UL 5, UL 10, WA 1, and AO 5% attracted females faster with the orientation time of 4.96 ± 0.43 , 5.44 ± 0.50 , 5.65 ± 0.58 , 3.60 ± 0.22 and 4.68 ± 0.40 min, respectively.

However, Hexane-WA at 10 and 15% concentration attracted males faster than females.

Table 4.31: Comparative orientation time response of *C. sexmaculata* male and female to different extracts at various concentrations

Extra ctant	Extract	Concen tration	Orientation time response (min)				T cal	P value
			Male		Female			
			Mean	SE	Mean	SE		
DCM	UL	1%	6.51	0.27	5.63	0.37	1.91	0.033*
		5%	6.81	0.29	5.72	0.50	1.89	0.036*
		10%	6.31	0.47	5.56	0.46	1.13	0.134NS
		15%	6.85	0.27	5.98	0.41	1.77	0.044*
	WOA	1%	4.10	0.27	3.62	0.15	1.58	0.062NS
		5%	4.38	0.20	3.73	0.18	2.40	0.011*
		10%	3.70	0.19	3.48	0.11	1.01	0.160NS
		15%	4.03	0.14	4.28	0.13	-1.28	0.104NS
	WA	1%	4.31	0.19	4.95	0.33	-1.68	0.051NS
		5%	3.66	0.10	4.95	0.33	-3.73	0.000***
		10%	3.46	0.18	4.43	0.41	-2.16	0.020*
		15%	5.32	0.35	7.32	0.24	-4.73	0.000***
	AO	1%	4.62	0.21	3.97	0.23	2.11	0.021*
		5%	4.36	0.14	4.18	0.10	1.03	0.154NS
		10%	4.53	0.19	3.94	0.17	2.32	0.012*
		15%	5.73	0.40	4.73	0.28	2.07	0.023*
HEX	UL	1%	6.41	0.18	4.96	0.43	3.14	0.003**
		5%	6.50	0.17	5.44	0.50	2.01	0.029*
		10%	7.14	0.31	5.65	0.58	2.25	0.018*
		15%	6.29	0.12	5.92	0.22	1.49	0.075NS
	WOA	1%	4.28	0.31	4.16	0.37	0.25	0.403NS
		5%	4.51	0.21	4.31	0.23	0.65	0.259NS
		10%	4.21	0.27	3.77	0.28	1.11	0.136NS
		15%	5.18	0.40	5.99	0.46	-1.33	0.096NS
	WA	1%	4.27	0.33	3.60	0.22	1.70	0.049*
		5%	3.60	0.25	3.47	0.25	0.37	0.355NS
		10%	4.10	0.37	5.59	0.46	-2.51	0.008**
		15%	4.07	0.29	5.27	0.37	-2.53	0.008**
	AO	1%	4.12	0.33	3.85	0.40	0.53	0.298NS
		5%	5.90	0.44	4.68	0.40	2.05	0.023*
		10%	4.82	0.44	4.97	0.45	-0.23	0.408NS
		15%	3.62	0.30	4.07	0.31	-1.05	0.150NS

*Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$, NS-Non-significant.

4.5.5 GC-MS analysis of different extracts

DCM as extractant: Un-infested leaf (UL) displayed twenty-seven compounds. The number of compounds got increased to 38 for infested leaf with aphid (WA) extract, and infested leaf without aphid (WOA) to 34. However, aphid only (AO) extract could only record 22 components.

The five main components in terms of quantity identified from DCM-UL were (E)- β -Caryophyllene ($28.28 \pm 2.11\%$), α -pinene ($21.87 \pm 1.95\%$), 1,4,7-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z- ($9.58 \pm 0.67\%$), 3-Hexen-1-ol ($5.92 \pm 1.33\%$) and β -Myrcene ($5.90 \pm 2.93\%$) [Table 4.32 & Figure 41]. Similarly, DCM-WOA reported α -pinene ($20.36 \pm 0.91\%$), (E)- β -Caryophyllene ($15.13 \pm 3.18\%$), 1,4,7-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z- ($7.20 \pm 0.60\%$), 3-Hexen-1-ol, (Z)- ($4.04 \pm 1.06\%$) and β -Myrcene ($4.01 \pm 0.82\%$) [Table 4.33 & Figure 42]. Whereas, DCM-WA showed the highest quantity of 3-Carene ($20.15 \pm 1.56\%$) followed by (E)- β -Caryophyllene ($13.06 \pm 1.51\%$), 1,4,7-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z- ($8.98 \pm 0.38\%$), α -Bulnesene ($4.89 \pm 0.96\%$) and 3-Hexenal ($4.88 \pm 0.54\%$) [Table 4.34 & Figure 43].

Aphid only (AO) extract showed the presence of 2-Propenoic acid-tridecyl ester ($16.26 \pm 4.48\%$), Eicosane ($9.68 \pm 1.20\%$), m-Xylene ($9.66 \pm 1.90\%$), 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione ($8.48 \pm 1.60\%$) and Hexadecane ($8.44 \pm 1.18\%$) [Table 4.35 & Figure 44].

n-Hexane as extractant: Extraction with n-Hexane also revealed the similar increase in the number of compounds analysed through GC-MS after aphid infestation. Number of compounds recorded were 22, 29, 31 and 18 from Hexane-UL, Hexane-WA, Hexane-WOA, and Hexane-AO, respectively. Top five compounds identified from different extracts is furnished as, UL: (E)- β -Caryophyllene ($22.10 \pm 2.60\%$), α -Pinene ($15.22 \pm 1.71\%$), 2-Hexenal, (E)- ($8.32 \pm 2.92\%$), 1,4,7-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z- ($6.67 \pm 0.38\%$) and α -Bulnesene ($6.40 \pm 0.58\%$) [Table 4.36 & Figure 44]. WOA: Heptane ($17.98 \pm 3.18\%$), (E)- β -Caryophyllene ($12.19 \pm 1.32\%$), α -pinene ($11.17 \pm 0.83\%$), 3-Hexenal ($10.42 \pm 1.89\%$) and 2-Hexenal, (E)- ($8.07 \pm 2.64\%$) [Table 4.37 & Figure 45].

Table 4.32: Different components in DCM extract of un-infested leaves (UL)
identified through GC-MS analysis

Sl. No.	Name of the component	Ret. time	% Area	SD
1	3-Hexenal	5.469	2.33	1.58
2	2-Hexenal, (E)-	6.856	2.07	1.03
3	3-Hexen-1-ol, (Z)-	6.976	5.92	1.33
4	2-Hexen-1-ol, (E)-	7.243	2.92	0.28
5	Hexyl formate	7.320	1.35	0.12
6	o-Xylene	7.790	1.22	0.10
7	α -Pinene	8.732	21.87	1.95
8	Camphene	9.049	1.16	0.01
9	laevo- β -Pinene	9.662	5.06	0.18
10	β -Myrcene	9.945	5.90	2.93
11	Sylvestrene	10.725	3.07	0.13
12	β -Ocimene	11.073	1.72	0.83
13	Heptadecane, 2,6,10,15-tetramethyl-	11.987	1.19	0.04
14	α -Copaene	16.584	1.94	0.25
15	β -Elemene	16.709	1.17	0.06
16	(E)- β -Caryophyllene	17.278	28.28	2.11
17	α -Guaiene	17.451	3.02	0.52
18	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	17.738	9.58	0.67
19	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.alpha.)]-	17.977	1.45	0.15
20	Bicyclogermacrene	18.395	2.38	0.32
21	α -Bulnesene	18.408	5.38	1.12
22	Hexadecane	19.341	0.27	0.14
23	Globulol	19.862	1.52	0.25
24	Acetic acid, 5-isopropyl-8a,10a-dimethyl-4,6-dioxohexadecahydrodicyclopenta[a,f]naphthalen-1-yl ester	21.121	1.31	0.14
25	trans-Geranylgeraniol	21.705	2.80	0.32
26	Neophytadiene	22.171	1.37	0.10
27	Dibutyl phthalate	23.783	1.32	0.02

Table 4.33: Different components in DCM extract of infested leaves without aphid (WOA) identified through GC-MS analysis

Sl. No.	Name of the component	Ret. time	% Area	SD
1	3-Hexenal	5.470	3.58	1.02
2	2-Hexenal, (E)-	6.862	1.85	0.36
3	3-Hexen-1-ol, (Z)-	6.986	4.04	1.06
4	2-Hexen-1-ol, (E)-	7.250	2.07	0.20
5	Hexyl formate	7.325	0.28	0.05
6	o-Xylene	7.791	0.42	0.10
7	α -Pinene	8.741	20.36	0.91
8	Camphene	9.054	0.16	0.00
9	laevo- β -Pinene	9.667	3.99	0.10
10	β -Myrcene	9.951	4.01	0.82
11	(E)-3-Hexenyl acetate	10.285	0.20	0.03
12	Sylvestrene	10.731	2.16	0.01
13	β -Ocimene	11.075	0.38	0.14
14	α -Copaene	16.586	0.95	0.29
15	β -Elemene	16.788	0.15	0.01
16	(E)- β -Caryophyllene	17.284	15.13	3.18
17	(E)- α -Bergamotene	17.370	0.22	0.02
18	α -Guaiene	17.452	1.97	0.70
19	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	17.744	7.20	0.60
20	β -Sesquiphellandrene	18.070	0.55	0.03
21	Bicyclogermacrene	18.308	2.01	0.55
22	α -Bulnesene	18.408	3.98	1.60
23	(Z)-1-Methyl-4-(6-methylhept-5-en-2-ylidene)cyclohex-1-ene	18.456	1.94	1.45
24	δ -Cadinene	18.593	1.26	0.14
25	Hexadecane	19.345	0.55	0.08
26	(-)-Spathulenol	19.870	0.49	0.13
27	2-Propenoic acid, tridecyl ester	20.436	1.81	0.84
28	Acetic acid, 5-isopropyl-8a,10a-dimethyl-4,6-dioxohexadecahydrodicyclopenta[a,f]naphthalen-1-yl ester	21.130	0.21	0.07
29	Tetradecanoic acid	21.299	0.24	0.10
30	Eicosane	21.711	0.38	0.07
31	trans-Geranylgeraniol	21.940	1.60	0.34
32	Neophytadiene	22.175	0.43	0.08
33	l-(+)-Ascorbic acid 2,6-dihexadecanoate	23.654	1.20	0.02
34	Dibutyl phthalate	23.792	0.37	0.05

Table 4.34: Different components in DCM extract of infested leaves with aphids
(WA) identified through GC-MS analysis

Sl. No.	Name of the component	Ret. time	% Area	SD
1	3-Hexenal	5.478	4.88	0.54
2	2-Hexenal, (E)-	6.862	0.46	0.10
3	3-Hexen-1-ol, (Z)-	6.986	1.76	0.26
4	2-Hexen-1-ol, (E)-	7.250	1.15	0.60
5	Hexyl formate	7.325	0.18	0.06
6	o-Xylene	7.791	0.20	0.07
7	α -Pinene	8.729	0.10	0.03
8	3-Carene	8.751	20.15	1.56
9	Camphene	9.054	0.17	0.01
10	laevo- β -Pinene	9.671	4.26	0.23
11	β -Myrcene	9.961	4.13	0.53
12	α -Thujene	10.235	0.05	0.00
13	(E)-3-Hexenyl acetate	10.285	0.17	0.03
14	Sylvestrene	10.731	2.30	0.08
15	β -Ocimene	11.075	1.04	0.37
16	α -Cubebene	16.161	0.08	0.01
17	α -Copaene	16.586	4.18	0.25
18	Tetradecane	16.724	0.24	0.04
19	β -Elemene	16.788	0.13	0.03
20	(E)- β -Caryophyllene	17.310	13.06	1.51
21	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	17.758	8.98	0.38
22	Valencene	17.986	0.22	0.17
23	Pentadecane	18.070	0.13	0.01
24	Bicyclogermacrene	18.311	1.88	0.15
25	α -Bulnesene	18.408	4.89	0.96
26	δ -Cadinene	18.593	1.58	0.07
27	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	19.158	0.35	0.14
28	Hexadecane	19.339	1.40	0.10
29	Globulol	19.872	0.58	0.02
30	(-)-Spathulenol	19.870	0.44	0.11
31	α -Linolenic acid	20.358	1.88	0.22
32	Acetic acid, 5-isopropyl-8a,10a-dimethyl-4,6-dioxohexadecahydrodicyclopenta[a,f]naphthalen-1-yl ester	21.130	0.46	0.07
33	Tetradecanoic acid	21.299	0.22	0.09
34	Eicosane	21.711	0.28	0.01
35	trans-Geranylgeraniol	21.940	1.22	0.63
36	Neophytadiene	22.175	0.27	0.07
37	l-(+)-Ascorbic acid 2,6-dihexadecanoate	23.654	1.43	0.19
38	Dibutyl phthalate	23.792	0.24	0.10

Table 4.35: Different components in DCM extract of aphids only (AO) identified through GC-MS analysis

Sl. No.	Name of the component	Ret. time	% Area	SD
1	Ethylbenzene	7.021	1.98	1.55
2	m-Xylene	7.215	9.66	1.90
3	Benzene, 1,2,3-trimethyl-	7.227	0.77	0.13
4	o-Xylene	7.782	2.57	1.99
5	Undecane	11.991	1.04	0.25
6	Dodecane	13.691	2.34	0.19
7	Tetradecane	16.699	5.08	0.78
8	10-Methylnonadecane	17.550	2.58	0.77
9	1-Dodecanol	17.759	2.22	0.10
10	1-Decanol, 2-hexyl-	17.969	2.05	0.38
11	Pentadecane	18.070	6.48	1.16
12	Hexadecane	19.339	8.44	1.18
13	2-Propenoic acid, tridecyl ester	20.500	16.26	4.48
14	Heptadecane	20.550	1.06	0.27
15	9-Tricosene, (Z)-	21.625	4.96	0.73
16	Eicosane	21.701	9.68	1.20
17	Phenol, 4,4'-(1-methylethylidene)bis-	21.809	0.87	0.15
18	Tetratetracontane	21.939	1.06	0.07
19	Docosyl heptafluorobutyrate	22.318	1.72	0.21
20	Cyclopropanecarboxylic acid, 1-hydroxy-, (2,6-di-t-butyl-4-methylphenyl) ester	22.760	3.79	0.77
21	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	23.349	8.48	1.60
22	Pentadecanoic acid	23.627	5.59	2.09

Table 4.36: Different components in n-Hexane extract of un-infested (UL) leaves identified through GC-MS analysis

Sl. No.	Name of the component	Ret. time	% Area	SD
1	Heptane	3.243	4.45	2.21
2	3-Hexenal	5.483	5.18	1.62
3	2-Hexenal, (E)-	6.855	8.32	2.92
4	3-Hexen-1-ol, (Z)-	6.976	4.30	0.39
5	Ethylbenzene	7.034	0.70	0.49
6	2-Hexen-1-ol, (E)-	7.250	1.41	0.94
7	α -Pinene	8.725	15.22	1.71
8	laevo- β -Pinene	9.663	3.18	0.29
9	β -Myrcene	9.943	4.03	2.09
10	3-Hexen-1-ol, acetate, (Z)-	10.277	1.07	0.43
11	Sylvestrene	10.723	1.69	0.40
12	β -Ocimene	11.072	0.92	0.65
13	Dodecane	13.691	0.63	0.61
14	α -Copaene	16.585	0.57	0.41
15	(E)- β -Caryophyllene	17.261	22.10	2.60
16	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	17.734	6.67	0.38
17	Bicyclogermacrene	18.303	1.01	0.62
18	α -Bulnesene	18.392	6.40	0.58
19	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	18.593	0.71	0.53
20	Hexadecane	19.345	1.36	0.53
21	Eicosane	21.708	1.12	0.48
22	Dibutyl phthalate	23.785	1.09	0.44

Table 4.37: Different components in n-Hexane extract of infested leaves without aphids (WOA) identified through GC-MS analysis

Sl. No.	Name of the component	Ret. time	% Area	SD
1	Heptane	3.245	17.98	3.18
2	Methylcyclohexane	3.671	0.38	0.07
3	3-Hexenal	5.473	10.42	1.89
4	Cyclohexane, 1,4-dimethyl-	5.603	0.23	0.02
5	2-Hexenal, (E)-	6.862	8.07	2.64
6	3-Hexen-1-ol, (Z)-	6.986	2.07	0.33
7	Ethylbenzene	7.037	0.53	0.13
8	Benzene, (3,3-dimethylbutyl)-	7.249	1.02	0.14
9	α -Pinene	8.729	11.17	0.83
10	laevo- β -Pinene	9.671	2.19	0.14
11	β -Myrcene	9.947	1.69	0.36
12	Undecane	11.991	0.17	0.01
13	Sylvestrene	10.731	0.96	0.68
14	(E)-3-Hexenyl acetate	10.285	1.09	0.13
15	Dodecane	13.701	0.46	0.09
16	α -Copaene	16.586	0.58	0.18
17	Tetradecane	21.939	1.01	0.20
18	(E)- β -Caryophyllene	17.273	12.19	1.32
19	α -Guaiene	17.452	1.00	0.37
20	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	17.738	4.18	0.48
21	1-Decanol, 2-hexyl-	17.969	0.67	0.30
22	Bicyclogermacrene	18.311	1.23	0.35
23	α -Bulnesene	18.408	3.48	1.06
24	Hexadecane	19.345	1.16	0.63
25	β -Bisabolol	20.431	1.11	0.16
26	2-Propenoic acid, tridecyl ester	20.500	1.30	0.28
27	Eicosane	21.701	2.96	1.74
28	Hexatriacontane	22.355	0.73	0.06
29	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	23.356	1.22	0.07
30	l-(+)-Ascorbic acid 2,6-dihexadecanoate	23.654	0.41	0.02
31	Dibutyl phthalate	23.792	1.32	0.87

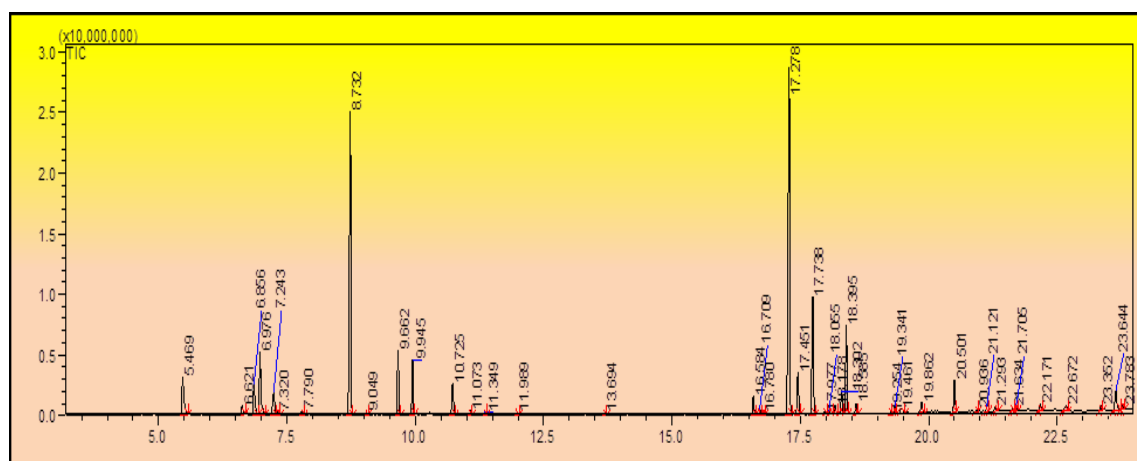


Figure 4.41: GC-MS analysis of DCM extracts: Un-infested Leaves (UL)

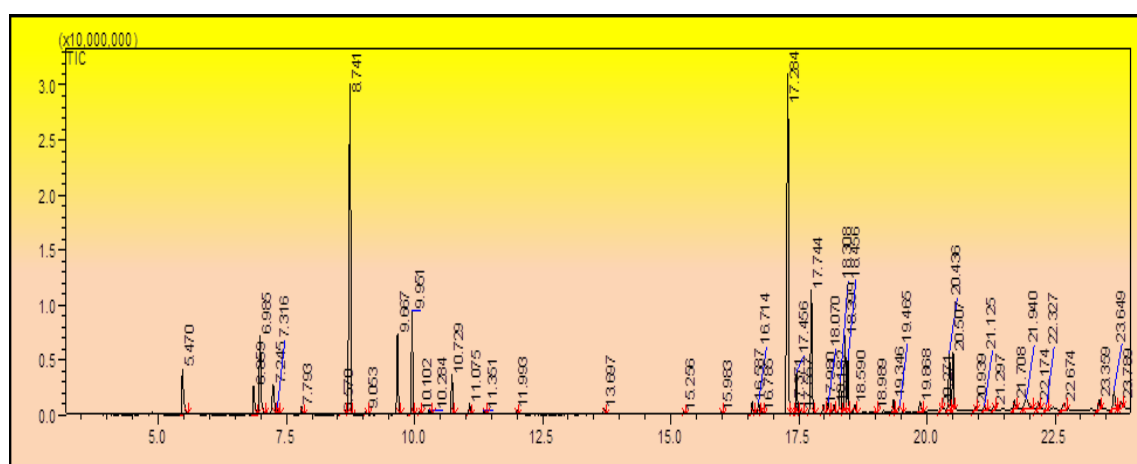


Figure 4.42: GC-MS analysis of DCM extracts: Infested leaves Without Aphis (WOA)

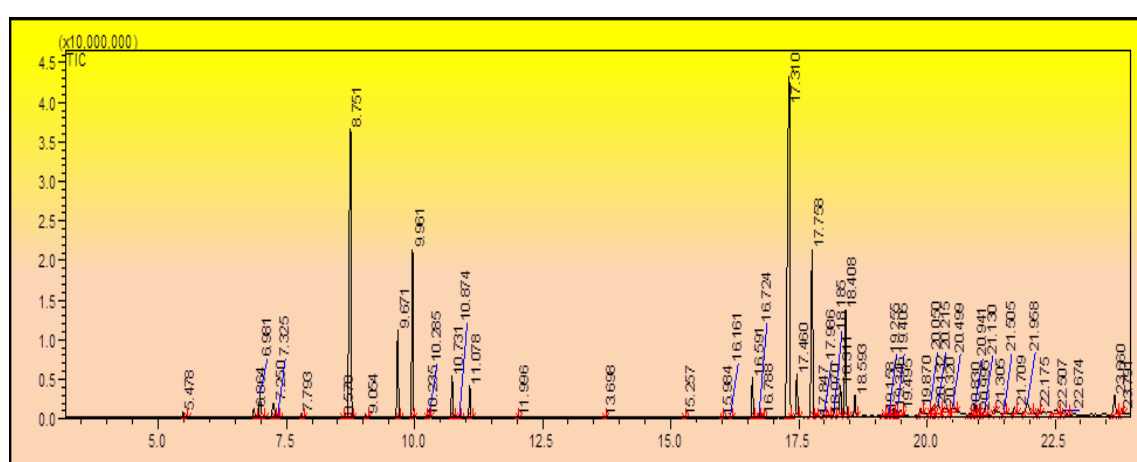


Figure 4.43: GC-MS analysis of DCM extracts: Infested leaves With Aphis (WA)

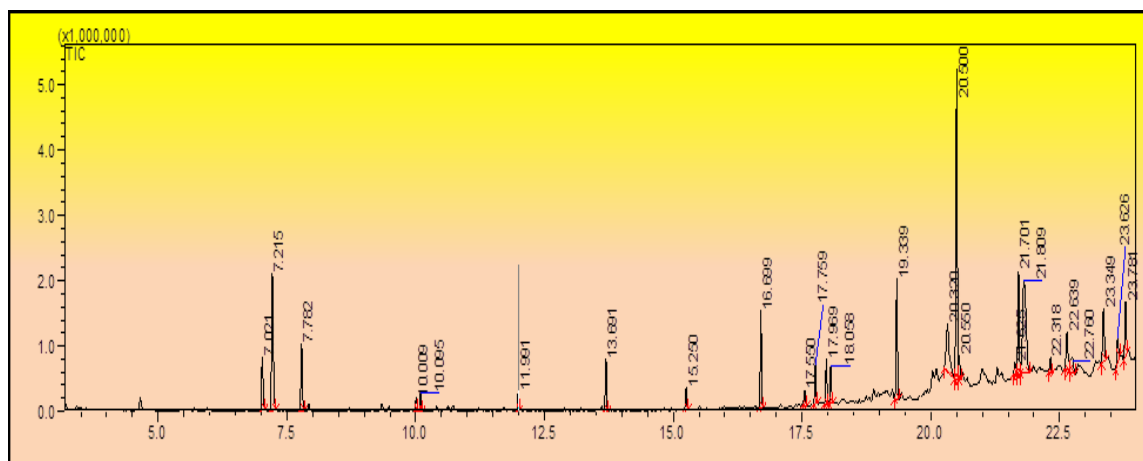


Figure 4.44: GC-MS analysis of DCM extracts: Aphids Only (AO)

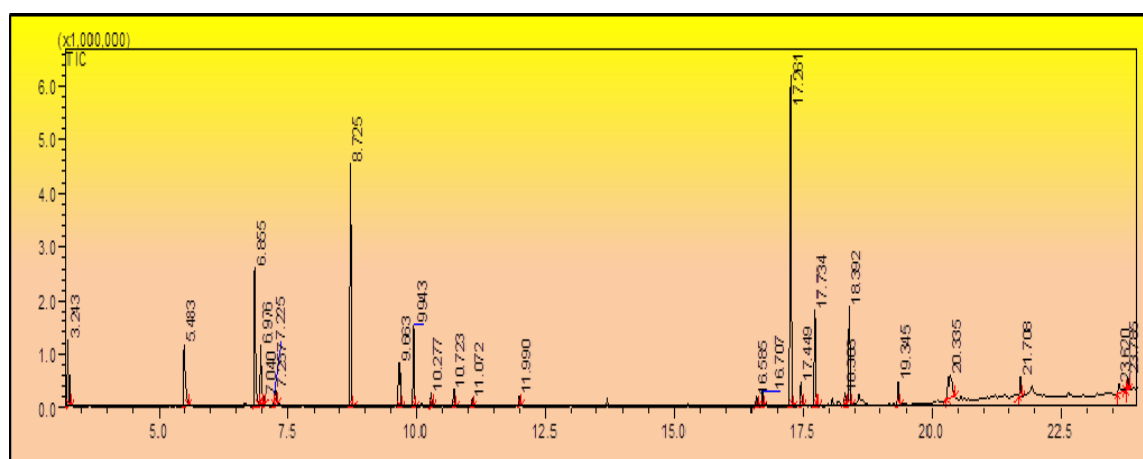


Figure 4.45: GC-MS analysis of n-Hexane extracts: Un-infested Leaves (UL)

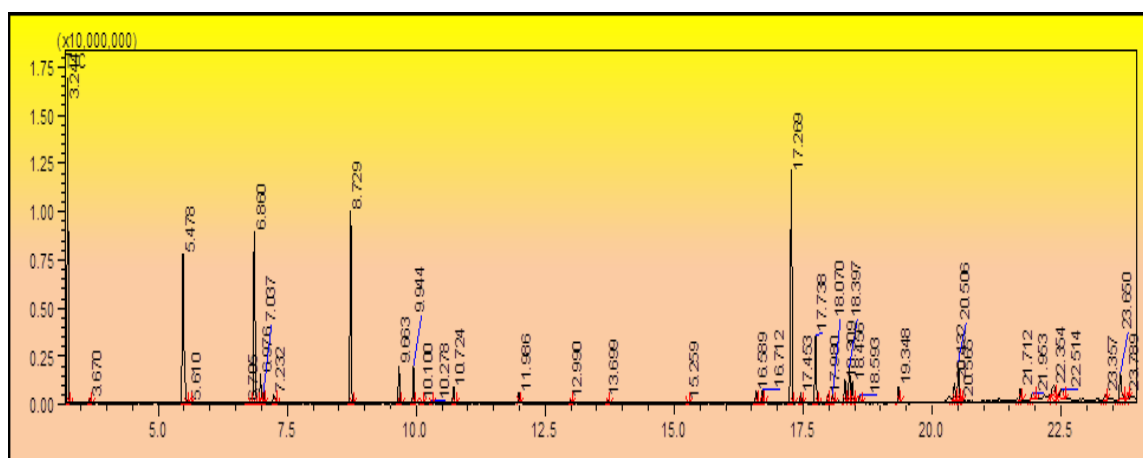


Figure 4.46: GC-MS analysis of n-Hexane extracts: Infested leaves Without Aphids (WOA)

WA: Heptane ($17.36 \pm 5.09\%$), Pentadecane ($10.39 \pm 5.21\%$), 3-Hexenal ($7.93 \pm 1.79\%$), 2-Hexenal, (E)- ($7.09 \pm 0.89\%$) and α -Linolenic acid (2.79 ± 0.99) [Table 4.38 & Figure 47]. AO: Heptane ($26.41 \pm 2.93\%$), α -Linolenic acid ($13.06 \pm 1.37\%$), 2-Propenoic acid-tridecyl ester ($12.05 \pm 0.67\%$), Dibutyl phthalate ($6.12 \pm 1.56\%$) and Dodecane ($5.46 \pm 0.97\%$) [Table 4.39 & Figure 48].

Comparative analysis of DCM extracts revealed that the different compounds identified from extracts of DCM-UL were also reported from infested leaf extracts of DCM-WA & DCM-WOA, except two compounds namely., Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.alpha.)]- and Heptadecane, 2,6,10,15-tetramethyl. DCM-WA had fourteen additional compounds in which 3-Carene was found as predominant. Among the commonly expressed compounds, 3-Hexenal and α -Copaene were observed more compared to DCM-UL, while α -Pinene, (E)- β -Caryophyllene and others got decreased (Table 4.40 & Figure 49).

Comparative analysis of the Hexane extracts revealed that the Sylvestrene, α -Bulnesene and Eicosane were present in Hexane-UL but not in Hexane-WA. Hexane-WA reported eleven additional compounds, in which Pentadecane was highest followed by Hexadecane, α -Linolenic acid, Tetradecanoic acid, Tetradecane, Dihexadecanoate and others. Compounds which were common viz., Heptane, 3-Hexenal and Bicyclogermacrene found in higher from Hexane-WA than Hexane-UL. Whereas, remaining compounds got decreased or remain un-affected. Among the decreased compounds (E)- β -Caryophyllene was most affected followed by α -Pinene, 1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z and others (Table 4.41 & Figure 50).

Table 4.38: Different components in n-Hexane extract of infested leaves with aphids (WA) identified through GC-MS analysis

Sl. No.	Name of the component	Ret. time	% Area	SD
1	Heptane	3.245	17.36	5.09
2	Methylcyclohexane	3.671	0.38	0.10
3	Hexane, 2,5-dimethyl-	3.803	0.15	0.05
4	3-Hexenal	5.473	7.93	1.79
5	Cyclohexane, 1,4-dimethyl-	5.603	0.24	0.08
6	2-Hexenal, (E)-	6.862	7.09	0.89
7	3-Hexen-1-ol, (Z)-	6.986	2.21	0.10
8	Ethylbenzene	7.037	0.73	0.52
9	2-Hexen-1-ol, (E)-	7.250	1.29	0.20
10	α -Pinene	8.729	1.85	0.60
11	laevo- β -Pinene	9.671	1.81	0.42
12	β -Myrcene	9.947	2.61	0.28
13	3-Hexen-1-ol, acetate, (Z)-	10.285	0.91	0.48
14	β -Ocimene	11.075	0.30	0.02
15	2,6-Nonadienal, (E,Z)-	12.993	0.24	0.11
16	Dodecane	13.701	0.70	0.44
17	α -Copaene	16.586	0.93	0.56
18	(E)- β -Caryophyllene	17.273	1.26	0.61
19	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	17.738	1.16	0.45
20	Pentadecane	18.07	10.39	5.21
21	Bicyclogermacrene	18.311	2.64	2.07
22	δ -Cadinene	18.593	0.64	0.08
23	Hexadecane	19.345	1.48	0.75
24	α -Linolenic acid	20.358	2.79	0.99
25	Tetradecanoic acid	21.299	2.57	2.60
26	Tetradecane	21.939	1.91	1.25
27	Hexatriacontane	22.355	0.54	0.03
28	l-(+)-Ascorbic acid 2,6-dihexadecanoate	23.654	2.61	1.15
29	Dibutyl phthalate	23.792	0.87	0.25

Table 4.39: Different components in n-Hexane extract of aphids only (AO)
identified through GC-MS analysis

Sl. No.	Name of the component	Ret. time	% Area	SD
1	Heptane	3.245	26.41	2.93
2	Methylcyclohexane	3.667	1.42	0.38
3	2-Pentanone, 4-hydroxy-4-methyl-	6.606	1.44	0.19
4	Ethylbenzene	7.034	4.10	0.75
5	m-Xylene	7.215	1.70	0.37
6	Dodecane	13.694	5.46	0.97
7	Tetradecane	16.705	2.98	0.12
8	1-Dodecanol	17.769	1.78	0.28
9	2-Hexyldodecyl acetate	17.976	1.69	0.34
10	Pentadecane	18.063	4.69	0.62
11	Hexadecane	19.343	1.67	0.60
12	Cyclooctasiloxane, hexadecamethyl-	20.102	2.66	0.70
13	α -Linolenic acid	20.387	13.06	1.37
14	2-Propenoic acid, tridecyl ester	20.500	12.05	0.67
15	Tetradecyl trifluoroacetate	21.636	1.81	0.46
16	Eicosane	21.706	2.74	0.45
17	Dibutyl phthalate	23.782	6.12	1.56
18	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	23.356	4.95	1.38

Table 4.40: Presence or absence of some volatiles in different extracts of DCM

S.No.	Chemical compound	Ret.time	UL	WA	WOA	AO
1	3-Hexenal	5.473	✓	✓	✓	X
2	2-Hexenal, (E)-	6.862	✓	✓	✓	X
3	3-Hexen-1-ol, (Z)-	6.986	✓	✓	✓	X
4	Ethylbenzene	7.034	X	X	X	X
5	m-Xylene	7.215	X	X	X	✓
6	Benzene, 1,2,3-trimethyl-	7.227	X	X	X	✓
7	2-Hexen-1-ol, (E)-	7.250	✓	✓	✓	X
8	Hexyl formate	7.325	✓	✓	✓	X
9	o-Xylene	7.791	✓	✓	✓	✓
10	α -Pinene	8.729	✓	✓	✓	X
11	3-Carene	8.751	X	✓	X	X
12	Camphene	9.054	✓	✓	✓	X
13	laevo- β -Pinene	9.671	✓	✓	✓	X
14	β -Myrcene	9.947	✓	✓	✓	X
15	α -Thujene	10.235	X	✓	X	X
16	(E)-3-Hexenyl acetate	10.285	X	✓	✓	X
17	Sylvestrene	10.731	✓	✓	✓	X
18	β -Ocimene	11.075	✓	✓	✓	X
19	Undecane	11.991	X	X	X	✓
20	Heptadecane, 2,6,10,15-tetramethyl-	11.997	✓	X	X	X
21	Dodecane	13.691	X	X	X	✓
22	α -Cubebene	16.161	X	✓	X	X
23	α -Copaene	16.586	✓	✓	✓	X
24	Tetradecane	16.699	X	X	✓	✓
25	β -Elemene	16.788	✓	✓	✓	X
26	(E)- β -Caryophyllene	17.273	✓	✓	✓	X
27	(E)- α -Bergamotene	17.370	X	X	✓	X
28	α -Guaiene	17.452	✓	X	✓	X
29	10-Methylnonadecane	17.550	X	X	X	✓
30	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	17.738	✓	✓	✓	X

S.No.	Chemical compound	Ret.time	UL	WA	WOA	AO
31	1-Dodecanol	17.759	X	X	X	✓
32	1-Decanol, 2-hexyl-	17.969	X	X	X	✓
33	Valencene	17.986	X	✓	X	X
34	Pentadecane	18.070	X	X	✓	✓
35	β -Sesquiphellandrene	18.076	X	X	✓	X
36	Bicyclogermacrene	18.311	✓	✓	✓	X
37	α -Bulnesene	18.408	✓	✓	✓	X
38	(Z)-1-Methyl-4-cyclohex-1-ene	18.456	X	X	✓	X
39	δ -Cadinene	18.593	X	✓	✓	X
40	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	19.158	X	✓	X	X
41	Hexadecane	19.345	X	✓	✓	✓
42	(-)-Spathulenol	19.870	X	✓	✓	X
43	Globulol	19.872	✓	✓	X	X
44	α -Linolenic acid	20.358	X	✓	X	X
45	2-Propenoic acid, tridecyl ester	20.500	X	✓	X	✓
46	Heptadecane	20.550	X	X	X	✓
47	Acetic acid, 4,6-... naphthalen-1-yl ester	21.130	X	✓	✓	X
48	Tetradecanoic acid	21.299	X	✓	✓	X
49	9-Tricosene, (Z)-	21.625	X	X	X	✓
50	Eicosane	21.711	X	✓	✓	✓
51	Phenol, 4,4'-(1-methylethylidene)bis-	21.809	X	X	X	✓
52	Tetratetracontane	21.939	X	X	X	✓
53	trans-Geranylgeraniol	21.940	✓	✓	✓	X
54	Neophytadiene	22.175	✓	✓	✓	X
55	Docosyl heptafluorobutyrate	22.318	X	X	X	✓
56	Cyclopropanecarboxylic acid	22.760	X	X	X	✓
57	7,9-Di-tert-butyl-1...-6,9-diene-2,8-dione	23.349	X	X	X	✓
58	Pentadecanoic acid	23.627	X	X	X	✓
59	l-(+)-Ascorbic acid 2,6-dihexadecanoate	23.654	X	✓	✓	X
60	Dibutyl phthalate	23.792	✓	✓	✓	X

Table 4.41: Presence or absence of some volatiles in different extracts of n-Hexane

S.No.	Chemical compound	Ret. time	UL	WA	WOA	AO
1	Heptane	3.245	✓	✓	✓	✓
2	Methylcyclohexane	3.671	X	X	X	✓
3	Methylcyclohexane	3.671	X	✓	✓	X
4	Hexane, 2,5-dimethyl-	3.803	X	✓	X	X
5	Ethylbenzene	4.100	X	X	X	✓
6	Pentadecane	4.690	X	X	X	✓
7	7,9-Di-tert-butyl-1-deca-6,9-diene-2,8-dione	4.950	X	X	X	✓
8	Dodecane	5.460	X	X	X	✓
9	3-Hexenal	5.473	✓	✓	✓	X
10	Cyclohexane, 1,4-dimethyl	5.603	X	✓	✓	X
11	Dibutyl phthalate	6.120	X	X	X	✓
12	2-Pentanone, 4-hydroxy-4-methyl-	6.606	X	X	X	✓
13	2-Hexenal, (E)-	6.862	✓	✓	✓	X
14	3-Hexen-1-ol, (Z)-	6.986	✓	✓	✓	X
15	Ethylbenzene	7.037	✓	✓	✓	X
16	m-Xylene	7.215	X	X	X	✓
17	Benzene, (3,3-dimethylbutyl)-	7.249	X	X	✓	X
18	2-Hexen-1-ol, (E)-	7.250	✓	✓	X	X
19	α -Pinene	8.729	✓	✓	✓	X
20	laevo- β -Pinene	9.671	✓	✓	✓	X
21	β -Myrcene	9.947	✓	✓	✓	X
22	(E)-3-Hexenyl acetate	10.285	X	X	✓	X
23	3-Hexen-1-ol, acetate, (Z)-	10.285	✓	✓	X	X
24	Sylvestrene	10.731	✓	X	✓	X
25	β -Ocimene	11.075	✓	✓	X	X
26	Undecane	11.991	X	X	✓	X
27	2-Propenoic acid, tridecyl ester	12.050	X	X	X	✓
28	2,6-Nonadienal, (E,Z)-	12.993	X	✓	X	X

29	α -Linolenic acid	13.060	X	X	X	✓
30	Dodecane	13.701	✓	✓	✓	X
31	α -Copaene	16.586	✓	✓	✓	X
32	(E)- β -Caryophyllene	17.273	✓	✓	✓	X
33	α -Guaiane	17.452	X	X	✓	X
34	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	17.738	✓	✓	✓	X
35	1-Dodecanol	17.769	X	X	X	✓
36	1-Decanol, 2-hexyl-	17.969	X	X	✓	X
37	2-Hexyldodecyl acetate	17.976	X	X	X	✓
38	Pentadecane	18.070	X	✓	X	X
39	Bicyclogermacrene	18.311	✓	✓	✓	X
40	α -Bulnesene	18.408	✓	X	✓	X
41	δ -Cadinene	18.593	X	✓	X	X
42	1-Isopropyl- 4,7,...hexahydronaphthalene	18.593	✓	X	X	X
43	Hexadecane	19.345	X	X	X	✓
44	Hexadecane	19.345	✓	✓	✓	X
45	Cyclooctasiloxane, hexadecamethyl-	20.102	X	X	X	✓
46	α -Linolenic acid	20.358	X	✓	X	X
47	β -Bisabolol	20.431	X	X	✓	X
48	2-Propenoic acid, tridecyl ester	20.500	X	X	✓	X
49	Tetradecanoic acid	21.299	X	✓	X	X
50	Tetradecyl trifluoroacetate	21.636	X	X	X	✓
51	Eicosane	21.701	✓	X	✓	X
52	Eicosane	21.706	X	X	X	✓
53	Tetradecane	21.939	X	X	X	✓
54	Tetradecane	21.939	X	✓	✓	X
55	Hexatriacontane	22.355	X	✓	✓	X
56	7,9-Di-tert-butyl-1-....diene- 2,8-dione	23.356	X	X	✓	X
57	l-(+)-Ascorbic acid 2,6- dihexadecanoate	23.654	X	✓	✓	X
58	Dibutyl phthalate	23.792	✓	✓	✓	X

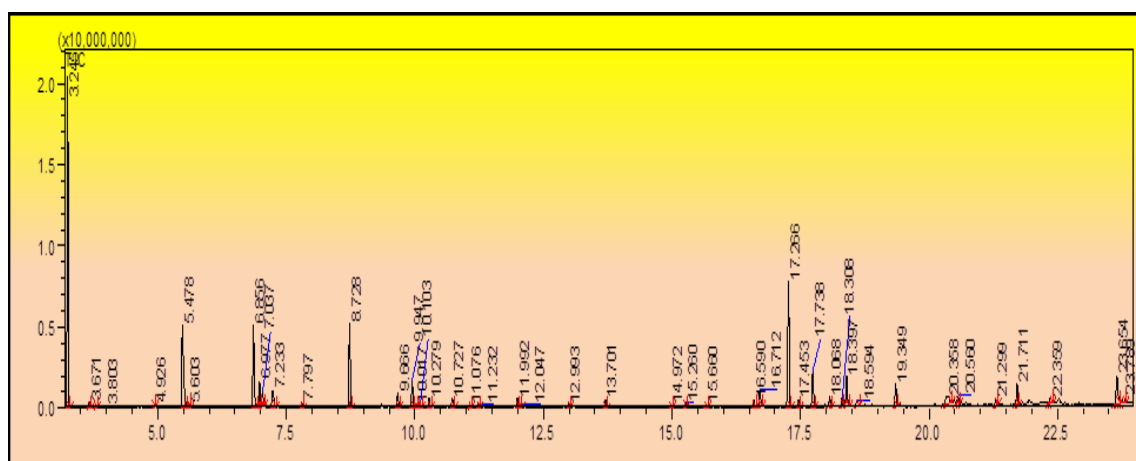


Figure 4.47: GC-MS analysis of n-Hexane extracts: Infested leaves With Aphis (WA)

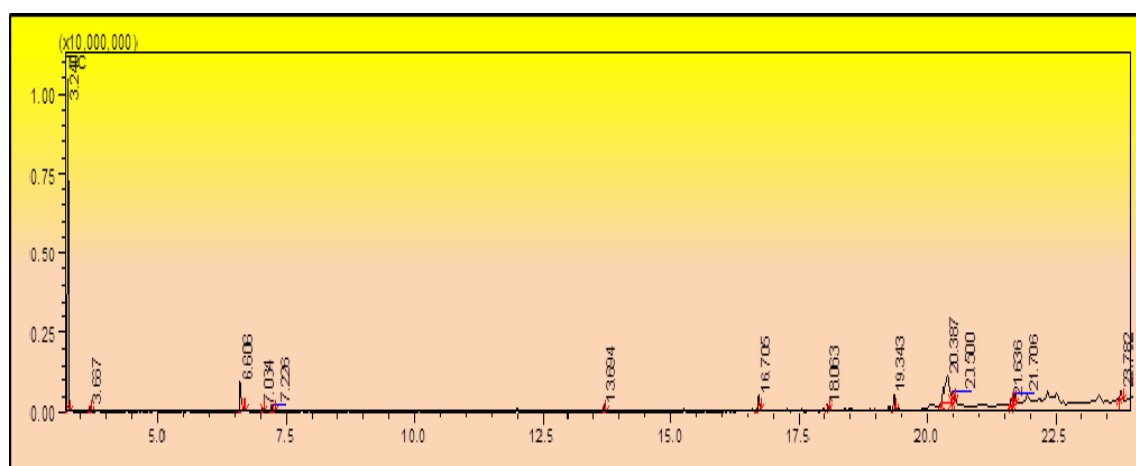


Figure 4.48: GC-MS analysis of n-Hexane extracts: Aphis Only (AO)

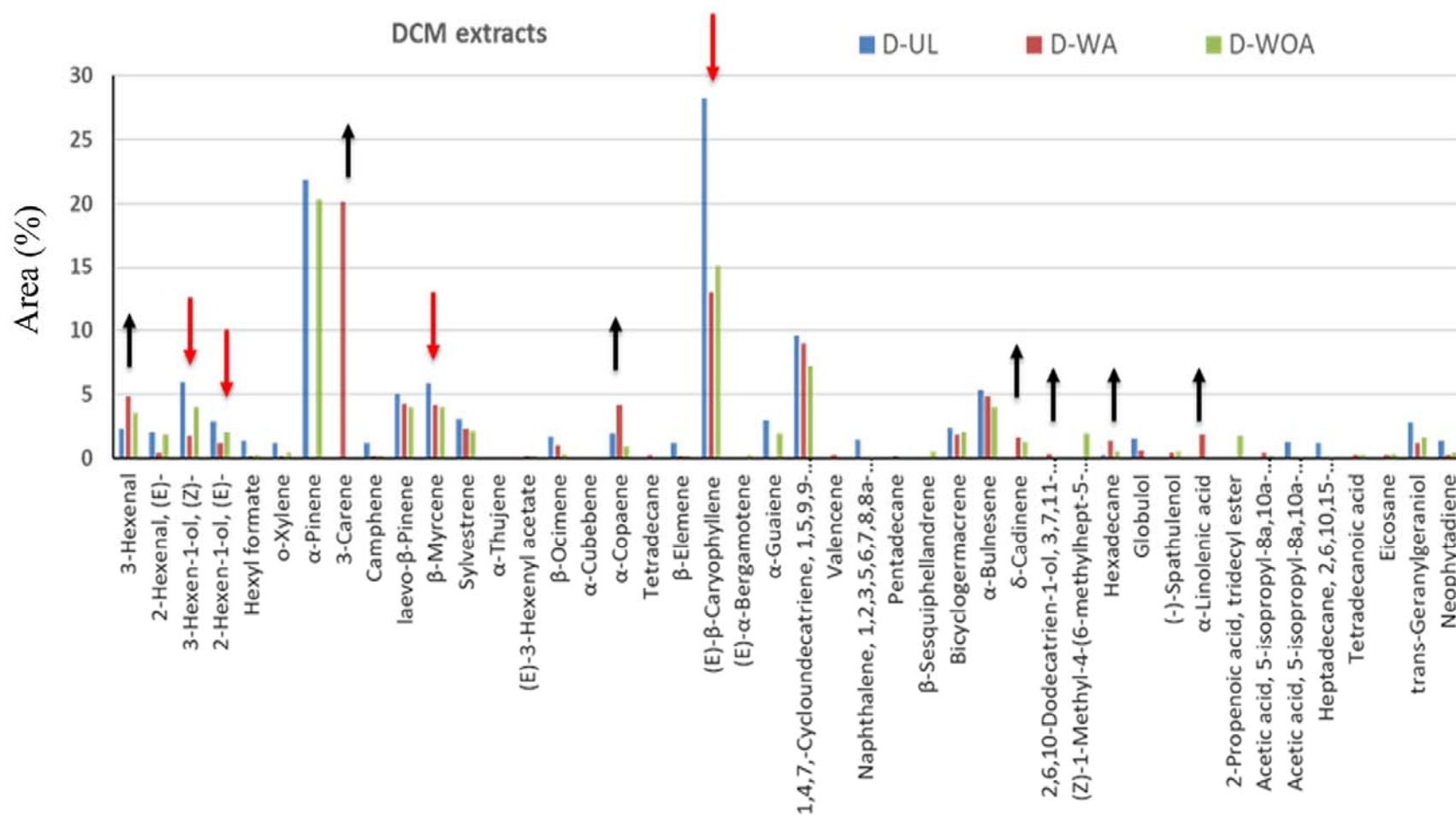


Figure 4.49: Relative abundance of different volatile components from DCM extracts

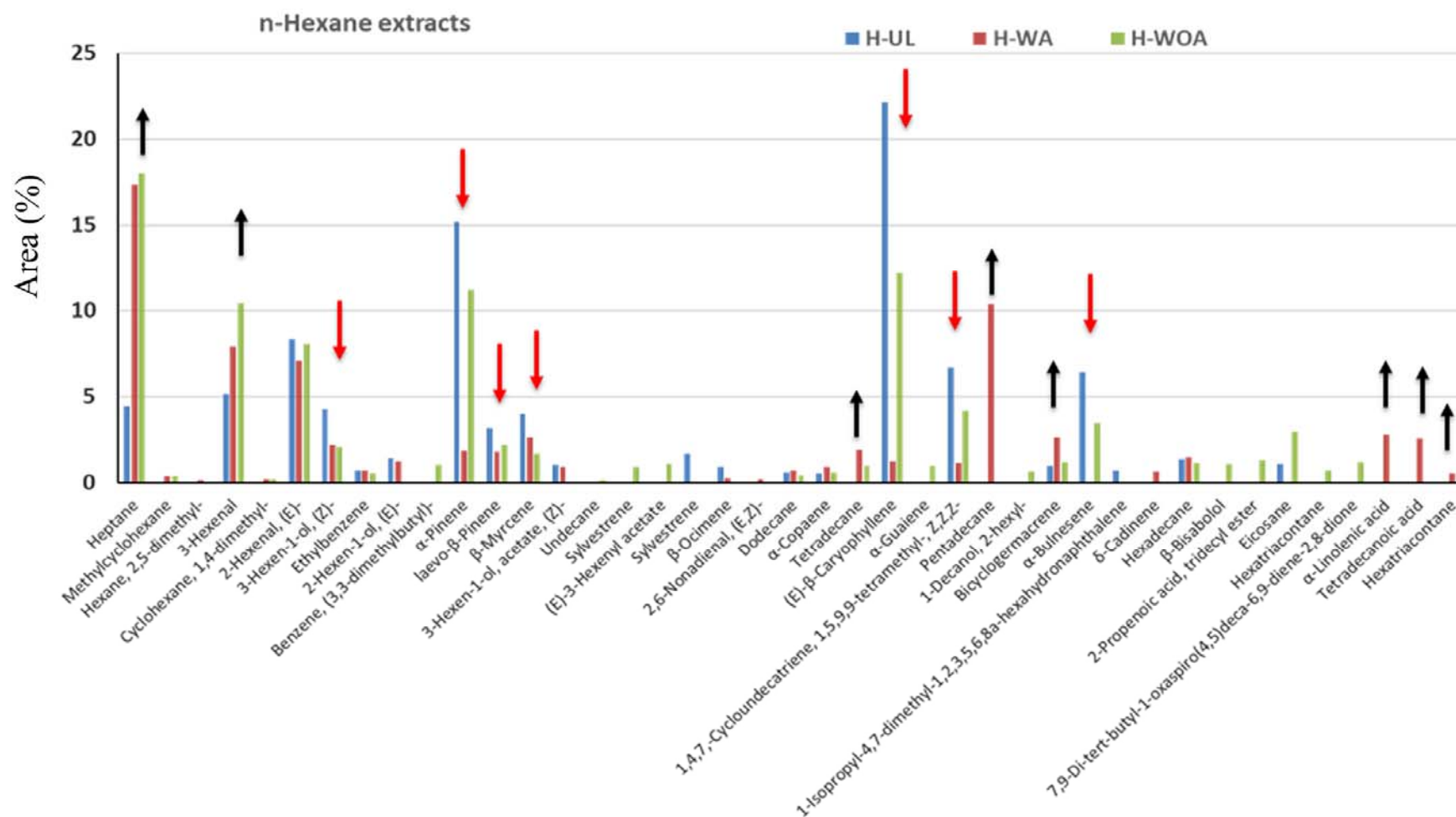


Figure 4.50: Relative abundance of different volatile components from n-Hexane extracts

Volatiles collected through vacuum volatile collection system showed the similar compounds as reported from infested leaf extracts, 3-Carene (2.78%), Pentadecane (3.03%), 3-Henenal (2.24%), Hexatriacontane (10.68%) and Tetradecanoic acid (0.91%) were the major compounds (Table 4.42 & Figure 51).

Extracts collected from un-infested cotton plants after spraying of Methyl salicylic acid showed similarity in number and relative abundance without any additional component at 2 and 4 h of spraying. However, at 10 h of spraying, it has shown additional compounds as well as increase in abundance. At 10 h of spraying, reported caryophyllene (27.8%), 3-Carene (19.6%), 1,4,7,-Cycloundecatriene,1,5,9,9-tetramethyl-, Z,Z,Z- (9.33%), Azulene (5.84%), β -Myrcene (5.31%), 3-Hexen-1-ol (3.87%) and 2-Hexen-1-ol (1.85%) as the major compounds. Some of the compounds viz., 3-Carene, Tetradecanoic acid, 3-Hexenal and Pentadecane were found common in both aphid infested plant and Methyl salicylic acid sprayed plants (Table 4.43 & Figure 52).

With the obtained results and analysis of volatile profile of different extracts some of the compounds were identified as major aphid induced HIPVs namely, 3-Carene, Heptane, Pentadecane, 3-Hexenal, α -Copaene and α -Linolenic acid (Figure 53).

A blend of aphid induced HIPVs consisting of six major [3-Carene (26%), Heptane (22%), Pentadecane (13%), 3-Hexenal (10%), α -Copaene (5%) and α -Linolenic acid (4%)] and eleven minor compounds [Bicyclogermacrene (3%), tetradecanoic acid (3%), tetradecane (3%), δ -cadinene (2%), hexadecane (2%), hexatriacontane (2%), methylcyclohexane (1%), 2,6,10-Dodecatrien-1-ol,3,7,11-trimethyl (<1%), eicosane (<1%), cyclohexane,1,4-dimethyl (<1%) and valencene (<1%)] could be responsible for attraction of ladybird beetle, *C. sexmaculata* (Figure 54).

Table 4.42: Different volatile components collected from aphid infested plant using Vacuum Plant Volatile Collection System

Sl. No.	Name of the component	% Area	
		UL	WA
1	2-Ethyl-1-hexanol	0.73	
2	4-(1-Hydroxyethyl) benzaldehyde	0.57	
3	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester		6.95
4	3-Carene		2.78
5	Acetophenone	8.49	5.28
6	Azulene	1.69	1.84
7	Pentadecane		3.03
8	Benzene, 1,3-dichloro-		1.80
9	3-Hexinal		2.24
10	Benzenemethanol, .alpha.,.alpha.-dimethyl-	8.39	8.31
11	Decanal	0.74	
12	Ethanone, 1-(2,4-dimethylphenyl)-	1.01	4.38
13	Nonanal	2.36	1.18
14	Octanal	0.56	
15	Undecane	0.83	
16	Hexatriacontane		10.68
17	Tetradecanoic acid		0.91

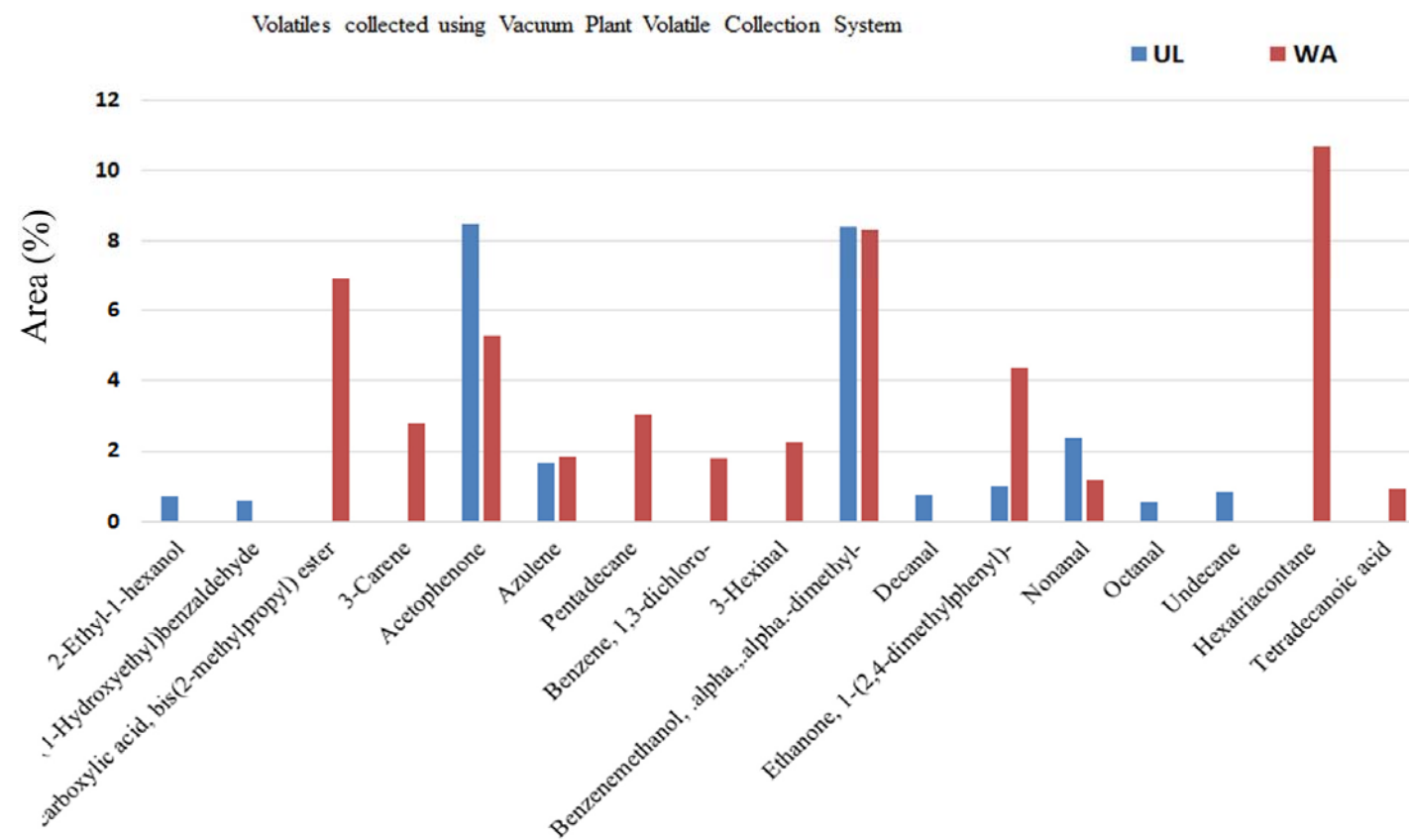


Figure 4.51: Volatiles collected through Vacuum Plant Volatile Collection System from cotton

Table 4.43: Different volatile components collected from un-infested plant sprayed with Methyl Salicylic acid @ 0.1 per cent, extracts were collected from leaves at 2, 4 and 10 h of spray

Sl. No.	Name of the component	% Area		
		At 2 h of spray	At 4 h of spray	At 10 h of spray
1	Caryophyllene	31.94	29.39	27.77
2	α -pinene	19.43	18.96	
3	3-Carene			19.56
4	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	9.77	9.08	9.33
5	Azulene	4.82	5.77	5.84
6	β -Myrcene	4.49	4.99	5.31
7	3-Hexen-1-ol	2.79	3.19	3.87
8	Trans-Geranylgeraniol	1.28	2.61	
9	α -Guaiene	2.23	2.38	2.50
10	Cyclohexene, 1-methyl-5-(1-methylethenyl)-, (R)-	2.08	2.02	2.27
11	(1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo[8.1.0]undeca-2,6-diene	1.92	1.76	1.69
12	α -Copaene	1.32	1.41	1.41
13	l-(+)-Ascorbic acid 2,6-dihexadecanoate	0.92	1.13	0.89
14	β -Ocimene	0.89	0.90	0.89
15	2-Hexen-1-ol, (E)-	1.19	1.25	1.85
16	trans-Geranylgeraniol			1.35
17	(-)-Spathulenol			0.69
18	3-Hexen-1-ol, acetate, (E)-	0.8		
19	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	0.91	0.77	

20	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-			0.49
21	Tetradecanoic acid			0.49
22	Neophytadiene			0.33
23	Formic acid, hexyl ester			0.43
24	3,7,11,15-Tetramethyl-2-hexadecen-1-ol			0.38
25	3-Hexenal		0.62	
26	2-Hexenal, (E)-		0.42	0.34
27	Caryophyllene oxide			0.32
28	Dibutyl phthalate	0.25	0.30	0.28
29	Tetradecane	0.15	0.13	0.18
30	Caryophyllene oxide	0.70		
32	Eicosane			0.19
33	Pentadecane			0.10
34	Pentadecanal-			0.09
35	Dodecane			0.08

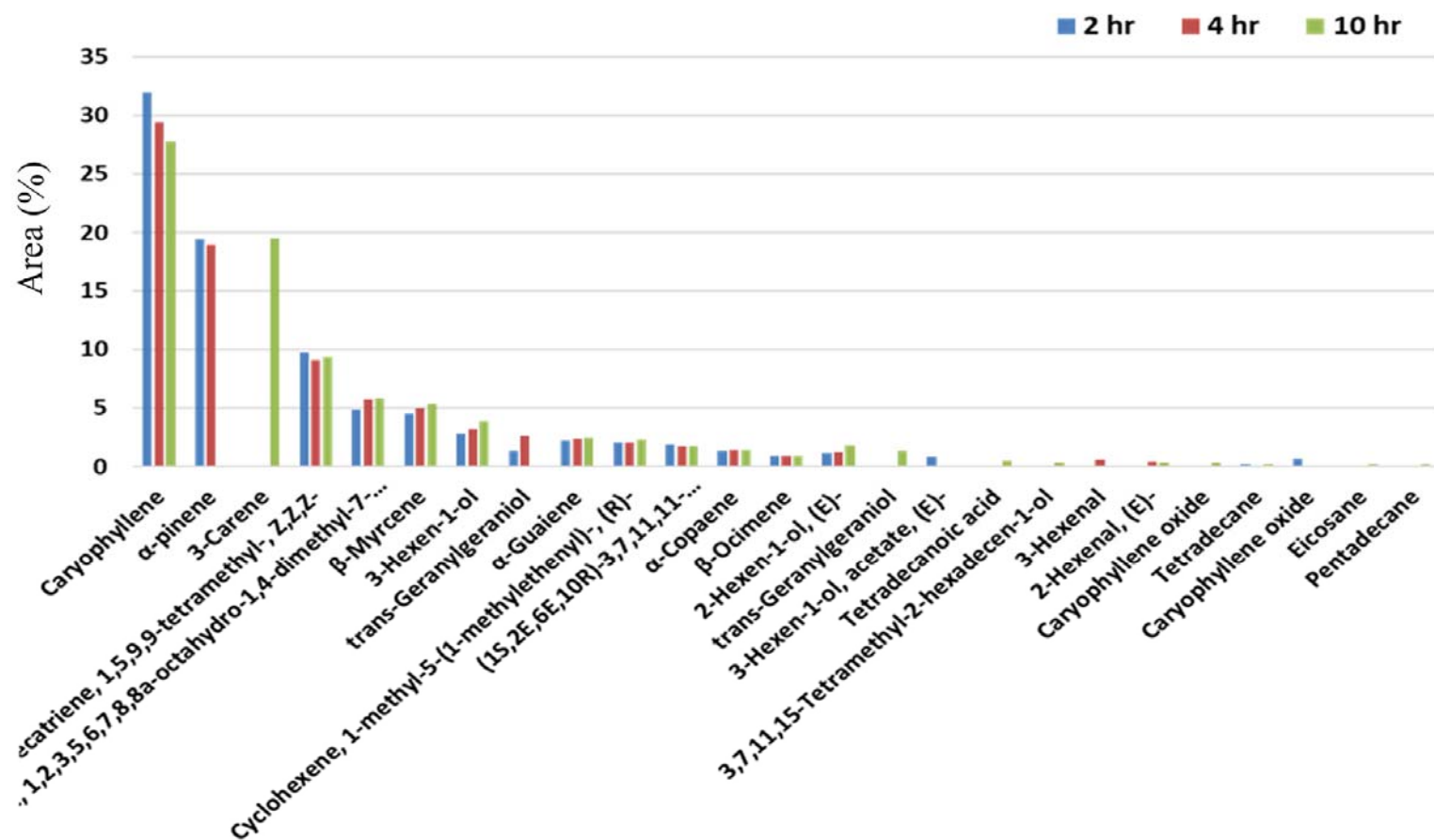


Figure 4.52: Volatiles noticed from cotton plant after spray of Methyl salicylic acid (MeSA)

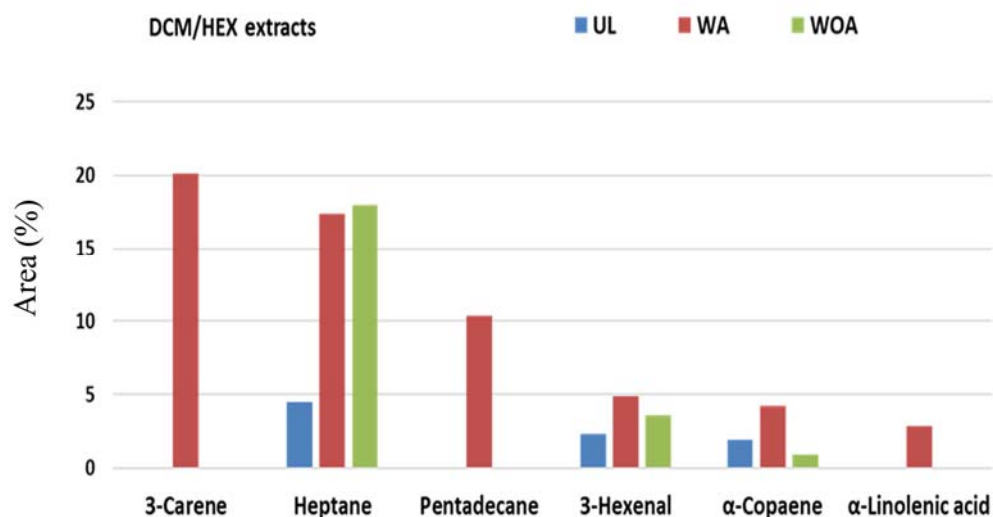


Figure 4.53: Major HIPVs noticed due to aphid infestation in cotton

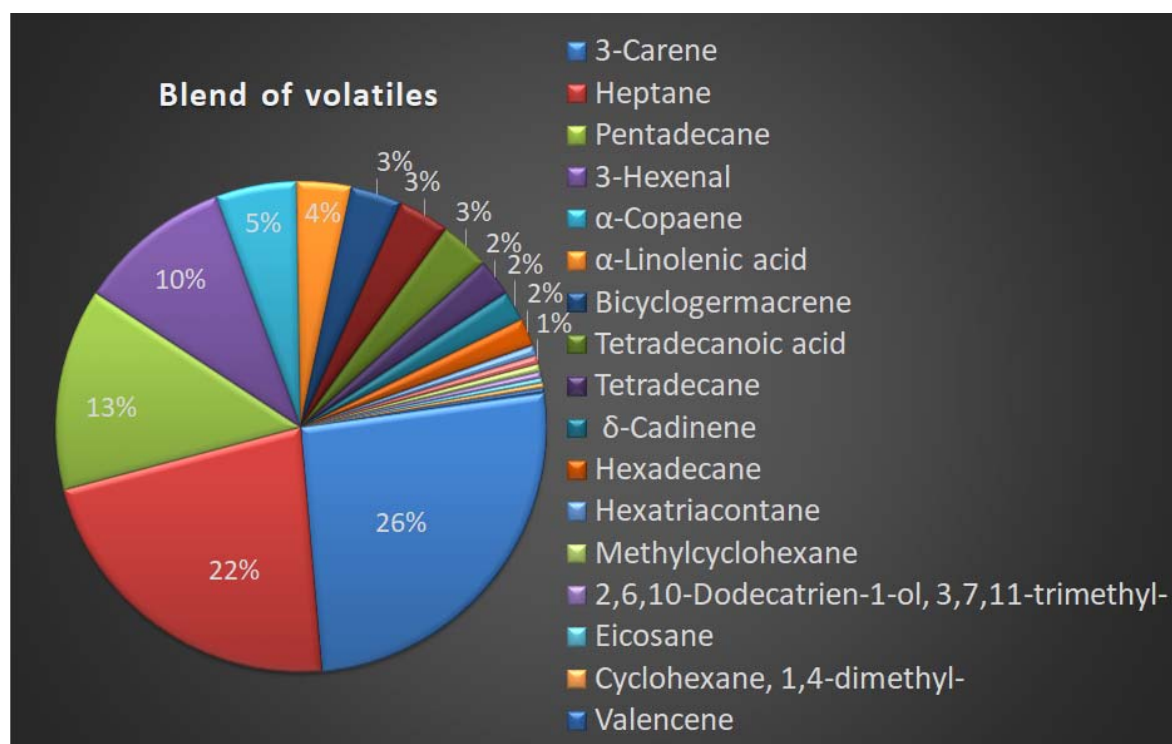


Figure 4.54: Proposed blend of volatile compounds assumed to be responsible for higher attraction of *C. sexmaculata* adults

5. DISCUSSION

5.1 Biology and morphometry of *C. sexmaculata* on cotton aphid, *A. gossypii*

The results of present study signify the suitability of *A. gossypii* for *C. sexmaculata* as faster development of grub in just 6.21 days was achieved. The faster development may be attributed to the quality of prey with essential nutrients for the growth and development of predator. Although, many studies have reported *A. craccivora* as the most suitable prey for ladybird beetles, viz., *C. septempunctata* (Hodek, 1960), *P. dissecta* (Omkar and Mishra, 2005) and *C. sexmaculata* (Omkar and Bind, 2004), the present combination of predator-prey was attempted for the first time in detail. Suitability of prey depends on lady bird species and also varies with predator-prey combinations (Omkar *et al.*, 2005).

It was evidenced that the prey suitability and its developmental period also get influenced by host plant species on which the prey is reared as earlier studied by Rattanapun (2012) who reported significant variation in developmental period of *C. sexmaculata* on *A. gossypii* when reared on two different host species. Similarly, Rakshan and Ahmed (2013) found morphological and developmental variations in *C. sexmaculata* fed on *A. craccivora* reared on different hosts. Faster development of *C. sexmaculata* on *A. gossypii* found in present study was strongly supported by Omkar *et al.* (2009) who found fastest development of ladybird beetle, *A. cardoni* on *A. gossypii* followed by *A. craccivora* and *L. erysimi* and reported *A. gossypii* as most suitable prey.

After adult emergence, three days of pre-mating period was observed. Total longevity of male and female ranged from 19 to 86 and 34 to 87 days with a mean duration of 65.1 ± 7.3 and 67 ± 4.7 days. Despite of shorter duration of grub stage, longevity of adults was found on higher side. Adult longevity was higher when compared with earlier studies which reported it from 30 to 50 days (Tank and Korat, 2007; Priyadarshani *et al.*, 2016; Sanghani *et al.*, 2017). However, Singh *et al.* (2008) reported higher longevity of female (92.25 days) which is in accordance with the present study. Male has shown longer duration compared to female as reported earlier (Tank and Korat, 2007; Priyadarshani *et*

al., 2016). In a total life span of 67 days of female, 6 ± 0.4 days were devoted for pre-oviposition, 42 ± 3.3 days for oviposition and 19 ± 3.1 days for post oviposition. During the mean oviposition period of 42 days, fecundity has been found to increase for first 30 days and thereafter decreased gradually by exhibiting a triangular fecundity function (Dixon and Agarwala, 2002). The highest per day fecundity for individual female was 72 whereas, the mean fecundity was found to be 18.01 ± 0.88 eggs/ day/female. The total fecundity varied between 241 and 1377 with pooled mean of 756.5 ± 128.96 eggs/ female. Higher longevity and oviposition period might have contributed to higher fecundity (756.5). High fecundity and oviposition period of *C. sexmaculata* on *A. craccivora* has already been reported (Omkar *et al.*, 2005). Our results are in closer agreement with Rajamohan and Jayaraj (1973), who reported fecundity of 717.7 for *C. sexmaculata* reared on *A. craccivora*. Total life cycle has completed with a mean duration of 78.06 ± 5.96 days in female and was found higher than male (75.56 ± 8.76). Survival percentage of egg, larva and pupa were 94.9, 90.0 and 92.59, respectively. Morphometrical observations recorded in the present study are in line with the earlier reports (Rai *et al.*, 2003; Tank and Korat, 2007; Rakshan and Ahmed, 2013) without much deviation, indicates proper growth and development of *C. sexmaculata* on *A. gossypii*.

5.2 Foraging behaviour of *C. sexmaculata* on *A. gossypii*

Predation efficiency of *C. sexmaculata* on *A. gossypii* increased with the age of the grub which might have contributed to faster development. Even though, the consumption was comparatively low for early instars, the faster development indicates quality and quantity of nutrition attained by the grub. Similar findings have already been reported for feeding potential of *C. sexmaculata* grub with respect to mean daily consumption on *A. gossypii* (Saha, 1987; Patro, 2004; Sanghani *et al.*, 2017). However, some other studies showed little deviation for total consumption which might be due to variations in the developmental period of grub (Bukero *et al.*, 2014).

Despite of shorter duration of grub, adult has shown high longevity with substantially longer oviposition period. Adults of both sexes exhibited high feeding potential throughout the life except for last few days. High feeding potential of *C. sexmaculata* adult on *A. gossypii* has been reported earlier with a mean daily consumption

of 34.24 (Sanghani *et al.*, 2017) and 35.25 aphids (Kumar *et al.*, 2016) which is in conformity with the present findings. However, the total consumption of adult has shown the variation with earlier findings (Sanghani *et al.*, 2017), which might be due to the differences in longevity. Hence, per day consumption must be more appropriate parameter rather than total consumption to express feeding potential of adult, when the developmental period differs. Range of age-specific consumption and irregular peaks in age-specific fecundity (m_x) were found in conformity with earlier works on coccinellids (Yu *et al.*, 2005; Boopathi *et al.*, 2020).

All the stages of *C. sexmaculata* displayed Type-II functional response which is considered more effective than type-III. Type-II functional response of all instars of grub and adults might be a result of their innate capacity to search and predate even at low prey densities. It shows the predation efficiency of *C. sexmaculata* and its affinity towards *A. gossypii* as it's preferential prey, and the same was reflected with faster development of grub and higher longevity of adults. Earlier studies on different species have established the predominance of Type-II functional response of ladybird beetles to the different aphid species (Pervez and Omkar, 2005; Gupta *et al.*, 2012). Few species of *Cheilomenes* viz., *C. propingua*, *C. lunata* and *C. sulphurea* predation behaviour against *A. gossypii* also showed Type-II response (Mrosso *et al.* 2013). Although, Type-II functional response was observed in many ladybird beetles on various preys, the studies on functional response of immature stages is limited. In few cases, type-III response was noticed, specifically in fourth instar grub and female of *N. includens* against *A. gossypii* (Bayoumy 2011) and in *H. axyridis* against pine aphid, *Cinara* sp (Hu *et al.* 1989).

Coccinellid predator, *S. gilvifrons* has exhibited a modified Type-II response for a mite, *T. urticae*. A decelerating curve (Type-II) at low to medium mite density and an accelerating curve (Type-III) at high density are seen (Osman and Bayoumy, 2011). We too observed a similar waving of polynomial regression curve in case of second instar grub as other stages have showed gradual decline. Even though, it appeared to be a shift between Type-II and III, many studies confirmed Type-II as a best fit (Bayoumy, 2011; Osman and Bayoumy, 2011; Kumar *et al.*, 2014)

Increasing coefficient of attack rate with the advancement of stage signifies the order of predator stages for their aggressiveness. Higher values of coefficient of attack rate recorded for adults indicates their superior predation efficiency which is also proved by earlier findings for *C. sexmaculata* adults against *A. craccivora* and *M. persicae* (Pervez and Omkar, 2005); adults of *C. propingua*, *C. lunata* and *C. sulphurea* against *A. gossypii* (Mrosso *et al.*, 2013), and for other aphidophagous ladybird beetles against various pests (Shah and Khan, 2013).

Lower handling time of IV instar grub and female observed in the present study is in line with the works conducted on three different species of *Cheilomenes* sp., has witnessed lower handling time for female (Mrosso *et al.*, 2013) and IV instar grub (Pervez and Omkar, 2005). The similar results were also reported for *A. tetraspilota*, *H. variegata* and *C. undecimpunctata* on *A. gossypii* (Al-Deghairi *et al.*, 2014).

In spite of moderate coefficient of attack rate, the IV instar grub has shown highest theoretical maximum predation as a result of lower handling time, which indicates its consistency in consumption of prey. The differences among various stages for the parametric values for functional response could be due to differences in predator size, its physical strength, voracity, satiation time, digestion ability, walking speed and learned behaviour for predation (Pervez and Omkar, 2005; Mills, 1982). Even though attack rate is a key factor, many workers have explained the efficiency of a predator using handling time (Veeravel and Baskaran, 1997).

The present study tried to explain the effectiveness of a predator by thriving on functional response equation as a whole which includes both the parameters *i.e.*, attack rate and handling time. In addition to this, the other parameters *viz.*, numerical response of predator and the natural increase of prey were worked out. By simulating the obtained equations for total response of predator and natural increase of prey, the change in aphid population in response to predation by different stages was depicted. It clearly exhibited the prey elimination by female, male and IV instar grub of predator. Among the three stages, female was the most effective as it resulted in rapid elimination of prey by keeping it below 25, while male and IV instar grub could eliminate prey by keeping it below 30.

Predator-prey interaction in the field could be influenced by other physical and biological factors such as weather, alternate prey, migration, intra-guild predation etc. The present study is the first little step to understand predator-prey interaction between *C. sexmaculata* and *A. gossypii*. The practical implication of this study is that the female, male and IV instar grub are found an effective and efficient stages of *C. sexmaculata* as they exhibit rapid reduction of prey with high attack rate and low handling time. Female beetles, in addition to their immediate functional response also contribute to the prolonged numerical response. So, the use of female and IV instar grub could be advocated for the release towards the augmentative biological control of *A. gossypii*.

5.3 Rearing technique for cotton aphid, *A. gossypii* on mesta, *H. cannabinus*

The present study ended up with a simple rearing technique for *A. gossypii* on *H. cannabinus* as a host. It is proved for its advantage over earlier methods as it is a quick, easy to handle, requires less space, ensure longer duration of host for continuous rearing up to three generations without disturbing flow and quality of nutrients. High population build-up of nymphs and adults proved the suitability of *H. cannabinus* as a host for rearing *A. gossypii*, with ensured nutrition for aphid without any defense.

The host suitability of *H. cannabinus* for *A. gossypii* on potted plants was already worked long ago (Norman *et al.*, 1968). Decrease in developmental period with an increase in temperature is already an established fact, many researchers have reported 25 °C as the most suitable temperature for aphid mass rearing (Steenis and El-Khawass, 1995; Oliveira *et al.*, 2010; Hosseini-Tabesh *et al.*, 2015).

Present results regarding the developmental period are in conformity with the earlier works who reported the developmental period of 4.3 days at 27.5 °C and 8.1 days at 17.5 °C for *A. gossypii* (Satar *et al.*, 2005). Intergenerational variation observed for the developmental period of nymph might be due to variations in the nutritional status of host plant as it grows. However, there is no intergenerational variation for developmental period at 27 °C which might be due to faster growth of host plant at that temperature which paved the way for the required nutrition to aphid right from the first generation. Intergenerational variation for the developmental period of *A. gossypii* on citrus has already been reported

(Satar *et al.*, 1998). Among different instars, the I instar took higher developmental period at all the temperatures as newly laid nymph required time to settle, feed and prepare for molting. In accordance with the present findings the developmental period of I instar nymphs was reported significantly higher than other instars when *A. gossypii* was reared in the laboratory (Hosseini-Tabesh *et al.*, 2015).

Maximum populations of both the nymphs and adults of *A. gossypii* obtained in the present study at 24 °C signify it as an optimum temperature for growth and reproduction of cotton aphid which is in close agreement with the earlier work on okra which reported high population of *A. gossypii* on different okra varieties at 24 °C (Hail *et al.*, 2007). Similarly, the temperatures between 22 and 27 °C was reported as the most favorable for *A. gossypii* when reared on the cotton (Nagrare *et al.*, 2019). However, the highest population growth was observed at 25 °C, but 28.6 °C was found optimum for survival and development of *A. gossypii* (Parajulee, 2007). The high population increase of nymphs proved the efficiency of present method which supported high reproductive performance of *A. gossypii* within the suitable range of temperature. These results are in conformity with the earlier works who reported an intrinsic rate of increase of cotton aphid as 0.302 (Satar *et al.*, 1998), 0.31 (Elegbede, 2014) and highest net reproductive rate was obtained at 25 °C (Satar *et al.*, 2005). However, the nymph population was dropped during last 6 days (24 to 30 days after release) due to deterioration of host plant which could be compensated by transfer of aphid population to fresh seedlings at 24 days after release.

The minimal nymphal population noticed at 27 °C might be due to nutritional stress, as yellowing of leaves was observed earlier at this temperature compared to other. Similarly, large number of winged adults obtained at 27 °C might be the result of crowding and nutritional stress. Lower population of wingless adults observed at 18 °C might be due to slower development and high mortality (Satar *et al.*, 2005).

This rearing technique of cotton aphid, *A. gossypii* is easy and simple as it does not require pot culture of host plant. Rearing of *A. gossypii* on seedlings of *H. cannabinus* could be an alternative method that we have found to produce different morphs of aphid in large numbers throughout the year. Assessments made of different temperatures on its impact on population build-up of different morphs of aphid provided all the necessary

information and clues for designing rearing conditions to yield desired morphs. The reared aphids found to readily adopt to cotton without any host incompatibility.

5.4 Predation risk effect of *C. sexmaculata* on *A. gossypii*

Predation risk exposure results confirmed the oxidative stress with increased SOD levels in cotton aphid which induced through the olfactory cues transferred from the predator. Our findings showed that the persistence of oxidative stress even after removal of predator has shown sustainable higher SOD levels up to 24 hours after removal of predator. Interestingly, the short duration exposure (1 hr) also induced significantly higher oxidative stress as increased SOD levels for a longer period even after removal of predator (24 hours) was observed indicating the consistency of risk effect on aphid even in the absence of predator once exposed. However, a peak in SOD level on 1 hr exposure at 6 hours after removal of predator might be due to a very high stress during initial period and which got stabilized in due course of time but higher than un-exposed. This indicates potentiality of short-term exposure similar to the long-term exposures. Superoxide dismutase (SOD) was reported as a major antioxidant enzyme in many insects as a first line of defence by converting superoxide into oxygen and hydrogen peroxide (Aucoin *et al.*, 1991; Wang *et al.*, 2001; Purushottam *et al.*, 2015). Upregulation of SOD activity under other stresses has also been reported in insects (Krishnan and Kodrik, 2006; Wang *et al.*, 2012). No or insufficient production of SOD might lead in to the accumulation of superoxide responsible for oxidative damage.

When the enzyme activity was compared, similar increase in SOD and Catalase (CAT) under both the active predation and risk predation, confirmed that the olfactory cues played a prime role in the induction of predation risk effect. It also indicates the underlying antioxidant mechanism of cotton aphid through up-regulation of these two antioxidant enzymes particularly under predation risk. Failing in up-regulation of either SOD or CAT may lead to increased oxidative damage, thereby a reduced growth rate and fecundity is expected as seen in damselfly naids, *E. cyathigerum*, where it was associated with lower levels of SOD (Slos and Stoks, 2008; Janssens and Stoks, 2013). In the present studies, higher MDA content in cotton aphid clearly indicated lipid peroxidation that might be due to insufficient reduction of oxidative stress by SOD and CAT (Janssens and Stoks, 2013).

The maintenance of antioxidant enzymes is also a costly affair as reported earlier in damselflies (De Block and Stoks, 2008; Slos *et al.*, 2009). This explains possible lipid damage in aphids that might influence the growth and reproduction (Ahmad, 1992).

We have seen that cotton aphid showed significant reduction in reproduction performance with reduced fecundity and reproductive period without affecting the longevity of exposed cotton aphid under predation risk. Trade-offs between production and maintenance cost of antioxidant enzymes might have compensated with the reduced reproductive performance was noticed. However, there was no compulsion on the growth reduction in prey when exposed to the predation (Benard, 2004).

Even though, the predation risk has not affected either the longevity of exposed adult or the developmental period of off springs, it has strongly affected the winged polyphenism in the offsprings. Three-fold increase in the number of winged aphids obtained emphatically confirmed the winged polyphenism as an important biological indicator in the cotton aphid *A. gossypii* under predation risk. Although, the production of winged morphs under the influence of crowding and the poor nutrient quality has already been confirmed in some aphid species (Kawada, 1987). It was also reported that the predation risk can also induce the production of winged offspring in the pea aphid, *A. pisum* (Dixon and Agarwala, 1999; Weisser *et al.*, 1999; Sloggett and Weisser, 2002; Kunert and Weisser, 2003) and the cotton aphid, *A. gossypii* (Mondor *et al.*, 2005). Predator-induced production of winged morphs would be a complementary to protection measures by facilitating dispersal of aphids to another locality (Weisser *et al.*, 1999).

Unlike other aphids (pea aphid) which exhibits different anti-predation behaviors viz., dropping, kicking, moving etc. are not displayed by the cotton aphid. Hence, the cotton aphid responded with “sit and wait” strategy, as no prominent anti-predation behavior is exhibited, and the resulted stress was mainly combated through the production of antioxidant enzymes (SOD and CAT). This cost of production of antioxidant enzymes might have affected physiology through reduction in reproductive performance. While, it exhibited “sit and wait” strategy in the adult stage, might have adopted “fight or flight” strategy by its progeny through the induction of winged morphs. Underlying mechanism of winged polyphenism could be debatable as the crowding and low nutritional status are the

factors which induce winged morphs. There is a possibility of low nutritional status under predation risk due to reduced feeding as more time is devoted towards vigilance, and the extra energy is required for the production of antioxidant enzymes. Pea aphid has experienced higher activity of the antioxidant enzymes in winged adults than the wingless (Lukasik, 2007).

Predation risk effect might decline aphid population directly through the reduced reproduction and may also indirectly through the induction of winged morphs. Production of winged morphs has already been seen in the pea aphid under predation risk due to phenotypic plasticity which led to their dispersal from the site of predator (Kunert and Weisser, 2003; Mondor *et al.*, 2005). It was also reported that the aphid prey under predation risk of ladybird beetle got scattered on the plant canopy which costs it more energy leading to reduced reproductive success and also induction of winged morphs (Mackay and Wellington, 1975; Roitberg *et al.*, 1979; Keiser and Mondor, 2013).

In the present investigations we found that the induction of predation risk effect of *C. sexmaculata* on cotton aphid *A. gossypii* might get transmitted through the olfactory cues as even short duration exposure could also raised the antioxidant enzyme levels. Adult aphids exhibited antioxidant mechanism through the upregulation of SOD and CAT but, not sufficient to avoid oxidative damage which might have led to increased MDA content. As a result, the cost of maintenance of SOD and CAT might have been trade-off with the reduced reproduction by adopting “sit and wait” strategy, while triggering winged morphs production of offsprings for “fight or flight” strategy to disperse.

5.5 Orientation behaviour of *C. sexmaculata* towards prey and host plant extracts

Orientation response of *C. sexmaculata* male and female was studied towards different odour sources. Attraction per cent of beetles was recorded using Y-tube olfactometer which could depict simplified picture of decision making by insects as it revealed the effect of various volatile concentrations on behavioural response of beetles (Ballhorn and Kautz, 2013).

Orientation response studies on adult beetle of *C. sexmaculata* to different extracts clearly exhibited a significant higher attraction compared to the control. Aphid (*A.*

craccivora) induced cowpea plant extracts led to attraction of ladybird beetle, *C. transversalis* for four different concentrations (Rakshit *et al.*, 2018). The attraction of *H. variegata* to the olfactory stimuli from pest infested plants was proved using Y-tube olfactometer (Tapia *et al.*, 2010; Li *et al.*, 2013).

Although, the knowledge on orientation response of *C. sexmaculata* is limited and under progress, it was observed that the *C. sexmaculata* adults got more attracted to the *A. craccivora* infested cowpea plants. It showed increase in response to the increase in number of aphids due to the presense of E- β -farnesene responsible for change in behaviour (Jaba *et al.*, 2013). Present investigations on *A. gossypii* have not found alarm pheromone, E- β -farnesene through GC-MS analysis of aphid only extracts. It indicates more reliability of predatory beetle on volatile chemicals released by the host plant than from aphid body.

Among the different extracts, the highest attraction of both male and female beetle was recorded for infested leaf with aphid (WA) for both DCM and n-Hexane as extractants. Out of 24 variants of odours tested, WA extracts occupied first six places for the highest attraction per cent of male beetle. It clearly indicates the strong orientation response to WA extracts. Among those top six WA extracts, 5 % concentration of DCM extract was the number one attractant ($72.5 \pm 3.66\%$) followed by 5 % n-Hexane extract of WA ($70.0 \pm 4.23\%$). It further confirms that the WA extract at 5% concentration is the most effective for both DCM and n-Hexane. Whereas, at 15% concentration, WA extracts of DCM as well as n-Hexane have shown decline in attraction per cent, but maintained significant difference with the control. Higher concentrations not necessarily be effective in attracting the coccinellids. In case of female, similar trend of higher attraction by infested leaf with aphid (WA) at 5 % concentration of both DCM and n-Hexane was noticed.

Many researchers have proved that the volatile compounds released by plants under biotic stress are used by insect-pests to locate host plant and also by their natural enemies to locate insect pests (Dick *et al.*, 2009). Predators of insect pests are also sensitive to chemical ecology of the multitrophic environment for finding their host location (Poppy, 1997) by getting used to numerous chemical cues emitted from prey and host plants alone or from both (Vet and Dicke, 1992).

Findings of the present study are inline with the findings on cowpea aphid, *A. craccivora* predator, *C. transversalis* which reported that the extracts of cowpea plant and aphid only have shown higher attraction of male and female beetles at different concentrations viz., 5, 10, 15 and 20 %. However, infested cowpea leaf with aphid (WA) extract was found the most effective in attracting both male and female beetle (Rakshit *et al.*, 2018).

Aphid only (AO) extracts have also been reported to attract higher number of male beetles at the concentration of 10 % of both DCM ($63.8 \pm 4.20\%$) and n-Hexane ($65.0 \pm 3.27\%$). Ninkovic *et al.* (2001) demonstrated that the seven-spotted ladybird, *C. septempunctata*, responded positively to volatiles from the aphid, *R. padi* as well as from infested barley plants. It is very important for a predator ultimately to locate its prey either through cues from plant or prey or from both. It was observed that the specialist parasitoid *A. ervi* recognized its host *A. pisum* (pea aphid) over the non-host *A. fabae* (bean aphid) on *V. faba* (broad bean) which indicates the ability of parasitoid to recognize its favoured prey and also to discriminate close related non-host aphid species (Blande *et al.*, 2017).

Male beetles have shown significant variations for attraction to the concentration gradient of plant extracts, while females have shown for both plant and aphid extracts. It indicate the ability of female beetle to assess the source of prey and its richness more precisely than male. A positive correlation between attraction of *C. sexmaculata* and number of aphids has already been worked out (Jaba *et al.*, 2013). A volatile dose-dependent response of another coccinellid beetle, *C. septempunctata* has also been observed (Han and Chen, 2002).

Synthetic compounds resembling HIPVs such as benzaldehyde, methyl salicylate significantly affected the dose-dependent selection choices of *P. japonica* in Y-tube olfactometer tests (Qi *et al.*, 2008).

Neither the un-infested (UL) leaf extracts of DCM nor the n-Hexane have shown significant higher attraction compared to the control. Ninkovic and Pettersson (2003) also demonstrated that the ladybird beetle, *C. septempunctata* got attracted to odour sources from only two common weeds (*C. arvense* and *E. repens*) among the different plants tested.

Another study with the same predator species exposed to ethanol extracts from 22 plant species showed volatiles from the weeds, *B. vulgares* and *T. inodorum* were attractive while the others were not (Schaller and Nentwig, 2000).

The present findings and earlier studies clearly infer that the isolated plants are not the universal sources of infochemicals for entomophagous natural enemies (Francis *et al.*, 2004). The low response of predators and parasitoids to the un-infested plant volatiles might be due to absence of reliable signal about the prey or host.

Francis *et al.* (2004) found higher attraction of female than male of *C. septempunctata* to the odor of aphid-infested plants, indicating the ability of female to exploit olfactory cues for prey detection. Similarly, higher attraction of *C. transversalis* female than male was recorded for the odours of aphid infested cowpea leaves extracted with DCM with 86 and 75% attraction of female and male, respectively (Rakshit *et al.*, 2018).

Ciarla *et al.* (2005) also reported higher attraction in less time of three coccinellid beetles viz., *A. conoxa*, *C. sanguinea* and *H. hexalis* to the volatiles taken from aphid, *M. persicae* and host plant *C. annuum*. Glinwood *et al.* (2011) also demonstrated the preference of *C. septempunctata* to aphid infested barley cultivar over un-infested cultivar.

5.7 GC-MS analysis of different extracts

More number of compounds in the infested leaf extract confirmed the induction of volatile due to aphid infestation in cotton. The GC-MS profile of infested leaf extracts against un-infested leaf has shown alteration of certain compounds, some of it either up-regulated or down regulated with some additional compounds. Similar trend in number of compounds was observed for both the DCM and n-Hexane extracts. The number of compounds were limited in aphid only extracts still the orientation response was significant for AO extracts than WOA. The lower orientation response of beetles to WOA also got reflected in GC-MS profile, as certain compounds such as 3-carene, α -copaene, hexadecane, α -linolenic acid, tetradecane and pentadecane were down regulated

immediately after removal of aphid from the infested leaf (WOA). It also speak about the ability of plants to immediately shift the resources as the pest infestation come down.

Essien *et al.* (2011) has also observed the similar composition of volatile chemical compounds in un-infested cotton leaf extract. They reported the composition of tricyclene (29.6%), bornyl acetate (18.6%), α -Pinene (12.8%), α -Terpinene (11.1%), isodene (6.0%), pinene (5.4%), p -Cymene (2.1%), terpinolene (2.0%), sabinene (1.4%), γ -Cadinene (1.1%), β -myrcene (0.6%), (E)-2-hexenal (0.5%), (E)- β -Ocimene (0.3%), γ -Terpinene (0.3%), β -Caryophyllene (0.2%), α -Thujene (0.2%) camphene (0.2%), limonene (0.1%) and α -humulene (0.1%).

Among the different volatile compounds of cotton leaf extracts, (E)- β -Caryophyllene got reduced on infestation and maintained low level even after removal of aphids, similar peaks were seen for WA and WOA. Whereas, the other compounds viz., α -pinene, (E)2-Hexenal, α -Guaiene, 1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-, and α -Bulnesene were seen in low levels in WA, but gained its peaks, immediately after removal of aphid (WOA) and attained equal to UL. These compounds must be closely linked to aphid infestation *i.e.*, compounds might be getting converted to other compounds acting against aphid attracting natural enemies. One of such possibility was notably observed in case of α -pinene. It was higher in UL and WOA, while very low in WA by matching peaks with 3-carene which was expressed only on infestation (WA). Thus, 3-carene might be playing dual role as a synomone *i.e.*, defence against aphids and also attracting natural enemies like aphidophagous ladybird beetles. Similar observation was also observed in case of green leaf volatiles (GLVs) such as (E)-2-hexenal and 3-hexenal.

Costello *et al.* (2008) who worked on wood borer of pine trees found that the trap catches of cerambycids increased with the addition of host monoterpene, 3-carene as efficiently as ipsenol or ipsdienol pheromone components. Similarly, (E)-2-hexenal found as one of the nine components of tea aphid/tea shoot volatiles showed a positive orientation response of *C. septempunctata* in wind tunnel bioassays (Han and Chen, 2002). The negative effect of 3-hexenal on aphid performance in a transgenic potato plant was also worked out (Vancanneyt *et al.*, 2001). Based on identified compounds, synthetic HIPVs

have been developed and verified for the attraction of coccinellids under laboratory and field conditions for their potential in biological control programmes (Yu *et al.*, 2008; Lee, 2010).

The present investigations revealed that the composition of volatile compounds released by the cotton plant has changed after aphid infestation. Based on GC-MS profile of different extracts and their relative composition, we identified some of the crucial compounds responsible for the attraction of coccinellid beetles. The identified compounds were either upregulated or found present on the infestation of aphids are arranged in the descending order as 3-carene (20.15%), Heptane (17.36%), Pentadecane (10.39%), 3-Hexenal (7.93%), α -copaene (4.18%), α -Linolenic acid (2.79%), Bicyclogermacrene (2.64%), 1-(+)-Ascorbic acid 2,6-dihexadecanoate (2.61%), Tetradecanoic acid (2.57%), Tetradecane (1.91%), δ -Cadinene (1.58%), Hexadecane (1.4%), Hexatriacontane (0.54%), Acetic acid-5-isopropyl-8a,10a-dimethyl-4,6-dioxohexadecahydrodicyclopenta[a,f]naphthalen-1-yl ester (0.46%), Methylcyclohexane (0.38%), 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl- (0.35%), Eicosane (0.28%), Cyclohexane, 1,4-dimethyl- (0.24%) and valencene (0.22%).

Some of the components reported earlier such as δ -cadinene, caryophyllene oxide and α -copaene as major compounds of *G. barbadense* also goes with our findings (Hedin *et al.*, 1972a). Aliphatic alcohols such as cis-3-hexen-1-ol, trans-2-hexen-1-ol, 1-penten-3-ol and 6-octen-4-ol and terpenoids viz., terpineol, bisabolol and bisabolene oxide were found as characteristic compounds of *G. hirsutum* (Hedin *et al.*, 1972b).

The emission profiles of HIPVs from wild tobacco, *N. attenuata* in response to tobacco horn worm, *M. sexta* revealed that the volatile blends had GLVs and sesquiterpenes responsible for the attraction of predator species. Manipulation of these biochemically mediated tritrophic interactions could be explored to improve the field efficiency of augmentative biological control through induction of indirect plant defense indirectly (Joo *et al.*, 2017). Instead of using single compound, synthetic blend consisting of two or more chemicals might attract predatory beetles more precisely and effectively (Szendrei and Rodriguez Saona, 2010).

Development of synthetic formulations through blending identified species-specific HIPVs could act as a cue in an agro-ecosystem to aggregate predatory species in to infested crop (Rodriguez-Saona *et al.*, 2011).

Of late, methyl salicylate (MeSA) has been used to attract some coccinellid species in vineyards, sweet corn, soybean, tea and cranberry fields (Zhu and Park, 2005; Qi *et al.*, 2008; Rodriguez-Sauna *et al.*, 2011; Mallinger *et al.*, 2011; Gadino *et al.*, 2012; Maeda *et al.*, 2015). However, Salamanca *et al.* (2017) reported that MeSA attracted *H. convergens* and *C. rufilabris* adults under laboratory and greenhouse conditions resulting in higher predation and oviposition, but not observed under field conditions. Thus, the predator responses to HIPVs could be a complex process in a real agricultural scenario which could be set right through a suitable blend of volatiles rather than single compound.

Based on the knowledge gained and comprehensive observations from the results obtained, we proposed a possible blend of volatiles to be used as an effective attractant to enhance the foraging behavior of aphidophagous coccinellid beetles in the cotton crop. Proposed blend of volatile consists 17 compounds, which includes, 3-carene (20.15%), Heptane (17.36%), Pentadecane (10.39%), 3-Hexenal (7.93%) and α -copaene (4.18%) as major compounds.

6. SUMMARY & CONCLUSIONS

Predatory ladybird beetles (Coccinellidae: Coleoptera) are important natural enemies in agri-horticultural ecosystem responsible for the biological pest suppression of aphids and other soft bodied insect pests. The six spotted zigzag ladybird beetle, *C. sexmaculata* is one of the important native species reported for huge appetite against aphids. A detailed study, covering consumptive and non-consumptive effects of coccinellid predator on its prey, expected population dynamics of prey, and orientation response of ladybird beetles to the aphid induced HIPVs was lacking. Therefore, the present investigations were carried out on predatory behaviour of *C. sexmaculata* and its predation risk on prey. The prominent findings of present study using predator-prey system of *C. sexmaculata* and *A. gossypii* on the cotton host plant is summarized as follows.

6.1 Biology and morphometry of *C. sexmaculata* on cotton aphid, *A. gossypii*

Female beetle completed its life cycle with a duration of 78.06 ± 5.96 days, which is higher than the male (75.56 ± 8.76). Cluster of smooth shiny yellow cigar shaped eggs have shown 89.9 ± 4.7 % hatching with an incubation period of 3.2 ± 0.1 days. Grub has passed through four instars with the developmental period of 1.729 ± 0.018 , 1.161 ± 0.022 , 1.323 ± 0.072 and 2 ± 0.027 days for I, II, III and IV instar grub, respectively. Developed grub transformed to pupa with a pre-pupation period of 1.318 ± 0.05 days. Adults emerged from pupa after 3.585 ± 0.043 days.

Longevity of male beetle ranged from 19 to 86 days with a mean of 65.1 ± 7.3 . Female longevity ranged from 34 to 87 days with a mean of 67 ± 4.7 of which 6 ± 0.4 days were devoted for pre-oviposition, 42 ± 3.3 days for oviposition and 19 ± 3.1 days for post oviposition period. Fecundity was found to increase for the first 30 days and thereafter decreased gradually. The highest per day fecundity for individual female was recorded as 72. The mean fecundity was found as 18.01 ± 0.88 eggs/day/female. The total fecundity varied between 241 and 1377 with a pooled mean of 756.5 ± 128.96 eggs/female.

Under life table studies, age-specific survival rate (l_x) ranged from 0.03 to 0.91. The age-specific fecundity (m_x), age-specific maternity ($l_x m_x$) and age-stage-specific fecundity (f_x) of *C. sexmaculata* preyed on *A. gossypii* was maximum at 27 days of age with the values of 14.63, 13.3 and 27.44, respectively. Life table studies showed intrinsic rate of increase (r) of 0.2156, net reproductive rate of 383.6, mean fecundity of 790.19 and the mean generation time of 27.29 days.

Length and breadth (L:B) of egg, I, II, III, IV instar grub, pupa, male and female was recorded as $1.06 \pm 0.06 : 0.38 \pm 0.02$, $1.49 \pm 0.05 : 0.42 \pm 0.01$, $4.13 \pm 0.09 : 0.68 \pm 0.03$, $5.74 \pm 0.15 : 0.83 \pm 0.02$, $7.37 \pm 0.12 : 1.36 \pm 0.02$, $3.83 \pm 0.13 : 2.32 \pm 0.1$, $4.23 \pm 0.09 : 3.75 \pm 0.09$ and $5.35 \pm 0.25 : 4.06 \pm 0.15$ mm, respectively.

6.2 Foraging behaviour of *C. sexmaculata* on *A. gossypii*

Ladybird beetle, *C. sexmaculata* consumed 16.24 ± 1.26 , 20.3 ± 1.89 , 37.13 ± 2.10 and 101.43 ± 3.04 cotton aphids during its I, II, III and IV instar grub stage with a mean daily consumption of 9.74 ± 0.74 , 18.61 ± 1.31 , 26.04 ± 2.33 and 36.66 ± 2.02 aphids, respectively.

The total consumption of the cohort size 33 was 96842.5 aphids with net predation rate of 2934.62 aphids/predator. Female beetle has consumed a total of 3389.84 ± 70.26 aphids, while the male consumed 3036.93 ± 109.37 aphids. Mean daily consumption of female (43.13 ± 0.54) was significantly higher than the male (36.62 ± 1.01).

When sex and stage differentiation was taken in to consideration and analysed using CONSUME-MSChart, the age-specific consumption rate, k_x (mean number of prey consumed by the predator at age x) and age-specific net consumption rate, q_x (weighted number of prey consumed by the predator at age x) was obtained maximum at the age of 33 days with a net predation rate (C_o) of 2934.62 aphids/predator.

The functional response (FR) studies revealed that all the grub stages and adults displayed a Type-II FR. Fitted lines using Roger's functional response model exhibited a decelerative polynomial curve with highest slope for female followed by IV instar grub, male and other instars. Among all the stages, female has exhibited highest coefficient of

attack (3.45) followed by IV instar grub (2.60), male (2.54), III (1.41) II (0.75) and I instar grub (0.23). The IV instar grub has shown the lowest handling time with highest maximum predation (96.1aphids/day), followed by female (94.6), male (82.6) and other stages.

The efficiency of conversion of ingested food (ECI) was high at prey density of 20 with $47.6 \pm 8.1\%$ and then decreased with increasing prey density to $33.9 \pm 3.1\%$ by displaying a linear regression relationship. Whereas, the numerical response study revealed the mean number of eggs laid per day as 0, 0, 8.4 ± 1.2 , 13.2 ± 1.4 , 19.4 ± 2.9 , 21.4 ± 0.7 and 21.8 ± 2.2 at the prey density of 5, 10, 20, 40, 80, 120 and 160 aphids, respectively. Best fitted regression line exhibited a logarithmic equation ($7.173 \log_e N_0 - 13.42$). Considering an oviposition period of 32 days, female longevity of 67 days and female to adult ratio of 0.533, a numerical response equation was worked out as given below.

$$NR = (7.173 \log_e N_0 - 13.42) 32/67 \times 0.533$$

In the absence of predator, fecundity life table study of parthenogenetic aphid population has resulted in the finite rate of increase (λ) of 2.162 per day.

Drawn the curves by simulating respective equations as the prey increase with the finite rate of increase, while it depletes at the rate of total response of predator (FR X NR). Simulated curve depicted female as the most effective with rapid elimination of prey by keeping it below 25, while male and fourth instar grub could keep it below 30.

6.3 Rearing technique for cotton aphid, *A. gossypii* on mesta, *H. cannabinus*

Mesta, *H. cannabinus* seedlings in a Petri plate has come up well on absorbent cotton and was ready just in three days after sowing. Each Petri plate (10 cm Θ) could accommodate a stand of 25 seedlings. Mesta seedlings supported the population buildup of *A. gossypii* up to 33 days after sowing. Aphids completed three generations within 30 days of sowing at all the temperatures, except 18 °C, where it took 35 days. Developmental period increased with the reduction in temperature. Fastest development was recorded at 27 °C with a mean duration of 4.22 ± 0.69 days.

Nymphal population increased geometrically from first day to 12 days of releasing, then increased exponentially up to 21-24 days and thereafter a faster decline was observed. The highest number of 568.9 ± 24.8 nymphs per seedling was recorded at 24 °C.

The wingless adult population has started appearing from sixth day of releasing at 24 and 27 °C whereas, it was observed from 12th day after releasing at 18 and 21 °C. The adult population was noticed highest at 24 °C (30 ± 1.8) followed by 21 (27.3 ± 0.9), 27 (22.2 ± 3.4) and 18 °C (19.2 ± 3.0) at 21, 24, 15 and 24 days after release, respectively.

Winged adults were observed early in case of 27 °C where, they started appearing from 18th day after release whereas, observed from 21 days after release for other temperatures. The number of winged aphids gradually increased from the first appearance till the end of experiment (up to 30 days after initial release) at all the temperatures. The cumulative total of winged aphids at 30 days after release was noticed highest at 27 °C (33.3 ± 1.8 /seedling), which is significantly higher than other temperatures. Among the other temperatures, the cumulative total was highest at 21 °C (22.5 ± 2.1) followed by 18 (17.8 ± 2.6) and 24 °C (14.7 ± 1.6).

6.4 Predation risk effect of *C. sexmaculata* on *A. gossypii*

Superoxide dismutase (SOD) activity was observed significantly higher for both the active predation and risk predation over control. Activity levels of SOD were recorded under active predation and risk predation as 9.75 ± 0.603 and 9.30 ± 0.406 units per mg protein and both have shown significant difference with the control (5.39 ± 0.13) with the minimum significant difference of 1.07. Whereas, MDA content was obtained 3.41 ± 0.312 , 2.73 ± 0.786 and 1.40 ± 0.0398 nM per mg protein under active predation, risk predation and no-predation, respectively. MDA content has shown higher under predation with a significant difference between each other.

Similarly, catalase (CAT) activity was recorded as 78.12 ± 5.266 , 62.62 ± 5.222 and 26.48 ± 5.999 H₂O₂ decomposed per min. per mg protein under active predation, risk predation and no-predation with significant difference with each other, respectively.

Predation risk did not affect the longevity of exposed aphids but reduced the reproductive period and the number of nymphs produced per female during the total life span. Both the active predation and risk predation exhibited similar trend in inducing lower reproduction compared to the control, without showing significant difference between them. However, there was no significant difference between the treatments for the number of nymphs produced in first 24 hours of exposure period.

Female longevity was recorded as 17.03 ± 4.191 , 16.63 ± 3.253 and 18.31 ± 3.382 days under active predation, risk predation and no-predation, respectively. Number of nymphs produced per female was least under risk predation (30.00 ± 4.402) followed by active predation (32.35 ± 4.666) and control (41.62 ± 5.602).

Although, there was no influence on mortality and developmental period of nymphs laid by the females exposed to predator, it clearly paved the way for production of winged morphs. There was more than three-fold increase in the number of winged adults in both active and risk risk predation compared to no-predation. Per cent winged morphs produced to the total adults was highest under active predation (21.53 ± 1.05) followed by risk predation (18.28 ± 4.87) and control (6.00 ± 2.99).

6.5 Orientation behaviour of *C. sexmaculata* towards prey and host plant extracts

The DCM extract of infested leaf with aphid (DCM-WA) responded with 68.8 ± 6.39 , 72.5 ± 3.66 , 68.8 ± 3.98 and 51.3 ± 2.95 % attraction of male beetles at 1, 5, 10 and 15 % concentration, respectively. Similarly, with n-Hexane it was recorded as 67.5 ± 3.66 , 70.0 ± 4.23 , 68.8 ± 2.95 and 47.5 ± 2.50 % attraction, respectively.

When the orientation response of male beetle was compared to the concentration gradient of different extracts, infested leaf with aphid (WA) has exhibited significant difference for both the extractants (DCM and n-Hexane). Attraction of beetle to DCM-WA and Hexane-WA was highest and consistent among the concentrations of 1, 5 and 10 % and was also found on par with each other. Whereas, the per cent attraction got significantly reduced at higher concentration of 15%.

Per cent response of male beetle towards different extracts in the order of highest to lowest was DCM-WA (5%), Hexane – WA (5%), DCM-WA (1%), DCM-WA (10%), Hexane-WA (10%), Hexane-WA (1%), Hexane-AO (10%), DCM-AO (10%), Hexane-AO (5%), Hexane-WOA (10%), DCM-AO (15%), DCM-AO (5%), Hexane-AO (1%), Hexane-AO (15%), Hexane-WOA (5%), Hexane-WOA (1%), DCM-WOA (10%), DCM-WA (15%), DCM-WOA (1%), DCM-WOA (5%), DCM-AO (1%), Hexane-WOA (15%), Hexane-WA (15%), DCM-WOA (15%), Hexane-UL (10%), Hexane-UL (1%), DCM-UL (5%), Hexane-UL (5%), DCM-UL (10%), DCM-UL (1%), Hexane-UL (15%) and DCM-UL (15%) with the attraction of 72.5 ± 3.66 , 70.0 ± 4.23 , 68.8 ± 6.39 , 68.8 ± 3.98 , 68.8 ± 2.95 , 67.5 ± 3.66 , 65.0 ± 3.27 , 63.8 ± 4.20 , 61.3 ± 2.95 , 58.8 ± 2.27 , 58.8 ± 3.98 , 57.5 ± 2.50 , 56.3 ± 2.63 , 56.3 ± 2.63 , 53.8 ± 4.98 , 52.5 ± 5.59 , 52.5 ± 4.12 , 51.3 ± 2.95 , 50.0 ± 5.35 , 50.0 ± 4.63 , 48.8 ± 5.15 , 47.5 ± 3.13 , 47.5 ± 2.50 , 46.3 ± 3.24 , 41.3 ± 4.41 , 40.0 ± 4.63 , 38.8 ± 3.98 , 38.8 ± 3.98 , 38.8 ± 2.95 , 37.5 ± 3.66 , 37.5 ± 2.50 and 31.3 ± 2.95 %, respectively.

When the orientation response of female beetle was compared to the concentration gradient of different extracts, WA and AO of both the extractants (DCM and n-Hexane) have exhibited significant difference, while others (WOA and UL) could not. Extract of DCM-WA has shown the highest per cent attraction at the concentration of 5% (83.8 ± 3.24) followed by 10 (66.3 ± 4.98), 1 (58.8 ± 4.79) and 15 (46.3 ± 3.24) by exhibiting significant differences between each other. In case of DCM-AO, highest per cent attraction was obtained at the concentration of 5% (70.0 ± 2.67) by significantly differing with other concentrations like, 10 (55.0 ± 3.27), 15 (53.8 ± 2.63) and 1 % (48.8 ± 2.95). Similar trend was also observed in case of n-Hexane extracts, where the extracts of WA and AO have shown the significant variations among the concentrations for the per cent attraction. Extract of WA has shown significant differences between the concentrations with each other for the attraction of female beetle by resulting highest attraction at 5% (72.5 ± 2.50) followed by 10 (63.8 ± 4.60), 1 (52.5 ± 4.53) and 15 (46.3 ± 1.83). In case of AO, 5% (66.3 ± 3.24) was significantly superior over all others followed by 10 (61.3 ± 3.98), 15 (57.5 ± 1.64) and 1 (52.5 ± 2.50); 10 and 15% were found on par with each other, but differed with 1%. Remaining extracts have not shown the significant variation between the concentration gradient for the attraction.

Per cent response of female beetle towards different extracts of DCM and n-Hexane in the order of highest to lowest was DCM-WA (5%), Hexane-WA (5%), DCM-AO (5%), Hexane-AO (5%), DCM-WA (10%), Hexane-WA (10%), Hexane-AO (10%), DCM-WA (1%), Hexane-AO (15%), Hexane-WA (5%), DCM-AO (10%), DCM-WOA (10%), Hexane-WOA (10%), DCM-AO (15%), DCM-WOA (1%), Hexane-WA (1%), Hexane-AO (1%), DCM-WOA (5%), Hexane-WOA (15%), DCM-AO (1%), Hexane-WOA (1%), DCM-WA (15%), Hexane-WA (15%), DCM-WOA (15%), Hexane-UL (15%), Hexane-UL (5%), DCM-UL (1%), DCM-UL (15%), Hexane-UL (1%), DCM-UL (10%), Hexane-UL (10%) and DCM-UL (5%) with the attraction of 83.8 ± 3.24 , 72.5 ± 2.50 , 70.0 ± 2.67 , 66.3 ± 3.24 , 66.3 ± 4.98 , 63.8 ± 4.60 , 61.3 ± 3.98 , 58.8 ± 4.79 , 57.5 ± 1.64 , 56.3 ± 2.63 , 55.0 ± 3.27 , 53.8 ± 3.24 , 53.8 ± 1.83 , 53.8 ± 2.63 , 52.5 ± 1.64 , 52.5 ± 4.53 , 52.5 ± 2.50 , 51.3 ± 3.98 , 50.0 ± 3.78 , 48.8 ± 2.95 , 47.8 ± 2.95 , 46.3 ± 3.24 , 46.3 ± 1.83 , 41.8 ± 3.20 , 43.8 ± 4.20 , 41.3 ± 2.27 , 40.0 ± 2.67 , 40.0 ± 3.78 , 38.8 ± 2.35 , 36.8 ± 2.35 , 38.8 ± 2.95 and 37.5 ± 3.66 %, respectively.

Between the sexes comparison revealed that, female has shown the significant higher attraction than male to DCM-UL at 15%, DCM-WA at 5, DCM-AO at 5, Hexane-UL at 1 and Hexane-WOA at 5%. Whereas, WA extracts of both DCM and n-Hexane have attracted more number of males than females at lowest concentration of 1%. Other extracts have not shown significant difference for attraction between male and female.

GC-MS analysis of DCM-extracts revealed 27 compounds for un-infested leaf (UL), 34 for infested leaf without aphid (WOA) and 38 for infested leaf with aphid (WA). Aphid only (AO) extract could only record 22 compounds.

Hexane extracts also revealed the similar increase in the number of compounds was recorded as 22, 29 and 31 compounds from UL, WOA and WA. Whereas, AO displayed only 18 compounds.

DCM extract has shown (E)- β -Caryophyllene ($28.28 \pm 2.11\%$), α -pinene ($21.87 \pm 1.95\%$), 1,4,7-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z- ($9.58 \pm 0.67\%$), 3-Hexen-1-ol ($5.92 \pm 1.33\%$) and β -Myrcene ($5.90 \pm 2.93\%$) as major compounds for un-infested leaf (UL), while the infested leaf with aphid (WA) exhibited 3-Carene

(20.15±1.56%), (E)- β -Caryophyllene (13.06±1.51%), 1,4,7-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z- (8.98±0.38%), α -Bulnesene (4.89±0.96%) and 3-Hexenal (4.88±0.54%).

n-Hexane extract has shown (E)- β -Caryophyllene (22.10±2.60%), α -Pinene (15.22±1.71%), 2-Hexenal, (E)- (8.32±2.92%), 1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z- (6.67±0.38%) and α -Bulnesene (6.40±0.58%) for un-infested leaf (UL), while WA displayed Heptane (17.36±5.09%), Pentadecane (10.39±5.21%), 3-Hexenal (7.93±1.79%), 2-Hexenal, (E)- (7.09±0.89%) and α -Linolenic acid (2.79±0.99%).

Volatiles collected through Vacuum Plant Volatile Collection System displayed 3-Carene (2.78%), Pentadecane (3.03%), 3-Hexenal (2.24%), Hexatriacontane (10.68%) and Tetradecanoic acid (0.91%) as the major compounds.

Extracts collected after spraying of Methyl salicylic acid has shown additional compounds as well as increase in its abundance at 10 h of spraying. Compounds like β -caryophyllene (27.8%), 3-Carene (19.6%), 1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z- (9.33%), Azulene (5.84%), β -Myrcene (5.31%), 3-Hexen-1-ol (3.87%) and 2-Hexen-1-ol (1.85%) were noticed as the major compounds.

Comparative analysis of compounds exhibited by un-infested leaf (UL) and infested leaf with aphid (WA) revealed that certain compounds either get induced or enhanced by the aphid infestation. These aphid induced HIPVs were 3-Carene (26%), Heptane (22%), Pentadecane (13%), 3-Hexenal (10%), α -Copaene (5%), α -Linolenic acid (4%), Bicyclogermacrene (3%), tetradecanoic acid (3%), tetradecane (3%), δ -cadinene (2%), hexadecane (2%), hexatriacontane (2%), methylcyclohexane (1%), 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl (<1%), eicosane (<1%), cyclohexane, 1,4-dimethyl (<1%) and valencene (<1%). This blend could be responsible for attraction of *C. sexmaculata* and would be helpful in developing formulation for the attraction of predatory beetles in crop ecosystem for the biological suppression of insect pests.

CONCLUSIONS

Successful completion of life cycle and establishment of *C. sexmaculata* on *A. gossypii* confirmed that the cotton aphid is one of the preferred host for it. Faster development of grub, higher longevity of adults and increased fecundity of *C. sexmaculata* on *A. gossypii* indicates it's predatory efficiency on cotton aphid. The data generated on bionomics would help in facilitating laboratory rearing, handling and mass multiplication of *C. sexmaculata* on cotton aphid.

Predator-prey interaction in the field are generally influenced by the physical and biological factors such as weather, alternate prey, migration, intra-guild predation etc. The present study is the first little step to understand predator-prey interaction between *C. sexmaculata* and *A. gossypii*. The practical implication of this study is that the female, male and IV instar grub are found an effective and efficient stages of *C. sexmaculata* as it exhibited rapid reduction of prey with high attack rate and low handling time. Female beetles, in addition to their immediate functional response also contribute to the prolonged numerical response. So, the use of female and IV instar grub could be advocated for the release towards the augmentative biological control of *A. gossypii*.

The present study revealed a simple technique of *A. gossypii* rearing using *H. cannabinus* as a host. This technique is easy and simple as it does not require pot culture of host plant. It proved advantageous over earlier methods as it is quick, easy to handle, requires less space, ensure longer duration of host for continuous rearing up to three generations without disturbing flow and quality of nutrients. High population buildup of nymphs and adults proved the host suitability of *H. cannabinus* for rearing of *A. gossypii*, which indicate sustained nutrition coupled with little or no defense by the host. It is an alternative and effective method that we have found to produce different morphs of aphid in large numbers throughout the year. Assessments made at different temperatures on population buildup of different morphs of aphid provided necessary information to design rearing conditions to yield desired morphs. The reared aphids readily adopted cotton without any host incompatibility issues.

We also found that the predation risk effect of *C. sexmaculata* on cotton aphid *A. gossypii* must have been transmitted through the olfactory cues, and even short duration exposure also could raise the antioxidant enzyme levels. Adult aphids exhibited antioxidant mechanism through the up-regulation of SOD and CAT but, not sufficient to avoid oxidative damage which might have led to increased MDA content. As a result, the cost of maintenance of SOD and CAT might have been trade-off with the reduced reproduction by adopting “sit and wait” strategy, while triggering winged morphs production of offsprings for “fight or flight” strategy to disperse.

Infested leaf with aphid (WA) extracted with DCM and n-Hexane attracted maximum number of beetles at 5% concentration. Among all, DCM-WA 5% concentration attracted more number of female followed by Hexane-WA 5, DCM-AO 5, Hexane-AO 5, DCM-WA 10, Hexane-WA 10, Hexane-AO 10 and DCM-WA 1% concentration. Similarly, male has shown maximum attraction to DCM-WA 5% concentration followed by Hexane-WA 5, DCM-WA 1, DCM-WA 10, Hexane-WA 10, Hexane-WA 1, Hexane-AO 10, DCM-AO 10, Hexane-AO 5, Hexane-WOA 10 and DCM-AO.

GC-MS analysis of infested plant extract has shown a different profile with increase in number and relative abundance of volatile compounds. Adult of *C. sexmaculata* might have majorly attracted to this Herbivore Induced Plant Volatiles (HIPVs) blend consisting of 17 compounds of which 3-Carene, Heptane, Pentadecane, 3-Hexenal, α -Copaene and α -Linolenic acid are the major ones. Volatiles collected through vacuum plant volatile collection system have shown similarity with the one reported from WA extract (DCM) and reported Hexatriacontane, 3-Carene, Pentadecane, 3-Hexenal and Tetradecanoic acid as major compounds. Extracts collected after 2 and 4 h of spraying MeSA (0.1%) have not shown differences for their volatile compounds, but the number and abundance of volatiles have increased at 10 h of spraying. This MeSA induced plant volatiles also recorded some of the major aphid induced compounds viz., 3-Carene, Tetradecanoic acid, 3-Hexenal and Pentadecane.

Development of synthetic formulations through blending identified species-specific HIPVs could act as a cue in an agro-ecosystem to aggregate predatory species in to infested

crop. The present findings provide a detail information about the olfactory responses of predator, *C. sexmaculata* towards the *A. gossypii* infested cotton crop. Based on the knowledge gained and comprehensive observations from the results obtained, we proposed a possible blend of volatiles to be used as an effective attractant to enhance the foraging behavior of aphidophagous coccinellid beetles in the cotton crop. Proposed blend of volatile consists 17 compounds, which includes, 3-carene (20.15%), Heptane (17.36%), Pentadecane (10.39%), 3-Hexenal (7.93%) and α -copaene (4.18%) as major compounds. Identified effective volatile compounds could be exploited for developing a possible synthetic blend of attractant for *C. sexmaculata*. This blend would be helpful in the attracting of predatory beetles in crop ecosystem for the biological suppression of insect pests.

Predatory behaviour of coccinellid beetle, *Cheilomenes sexmaculata* (Fab.) and its predation risk on prey

ABSTRACT

Predatory ladybird beetles (Coccinellidae: Coleoptera) are considered to be of immense importance in agri-horticultural ecosystem due to their successful deployment in the biological pest suppression programmes and kinds of ecosystem services they provide. The six spotted zigzag ladybird beetle, *Cheilomenes sexmaculata* Fabricius is one of the important native species widely distributed all over the country and known for the effective suppression of aphids and other soft bodied insects. Although, there are many studies being conducted on predatory behavior of *C. sexmaculata* on different hosts, a detailed study including bionomics, feeding potential, functional response, numerical response, predation risk effect (non-consumptive effect), orientation behavior and its ultimate influence on prey population dynamics was lacking. Cotton aphid, *Aphis gossypii* Glover, a cosmopolitan pest damages many crops leading to a huge economic loss all over the world. Considering the economic importance of cotton aphid and round the year availability of six spotted zigzag ladybird beetle in cotton ecosystem, a comprehensive study on predation behavior, predation risk effect and orientation behaviour of *C. sexmaculata* on cotton aphid, *A. gossypii* was taken up in the Biological Control Laboratory (BCL), Division of Entomology, ICAR-Indian Agricultural Research Institute (IARI), Pusa, New Delhi during the year 2017 to 2020.

Field collected adults of cotton aphid and six spotted ladybird beetles were used to create stock cultures on potted cotton plants in the separate screen cages at the controlled conditions of $25\pm1^{\circ}\text{C}$, $65\pm5\%$ RH and 16 h of photo period in the environmental growth chamber. Bionomics, feeding potential, functional response and numerical response was studied by providing prey (*A. gossypii*) on a cotton leaf kept as substrate in a Petri plate and the prolonged freshness of leaf was ensured by inserting cut end of leaf petiole in to agar-based Hoaglan's media (1.5 %) poured at one side of Petri plate. Biology and feeding

potential was observed from the first instar till the end of subsequent life stages by providing prey @ 160 aphids per day.

Adult beetles of *C. sexmaculata* laid eggs in clusters which hatched ($89.9 \pm 4.7\%$) in 3.2 ± 0.1 days. Grub went through four instars with a mean duration of 1.73 ± 0.02 , 1.16 ± 0.02 , 1.3 ± 0.07 and 2 ± 0.03 days for I, II, III and IV instar, respectively. Compared to earlier such reports on *A. gossypii* and other aphids this beetle has shown faster development of grub (6.21 ± 0.03 days). Despite of shorter duration of grub stage, longevity of adults was found on higher side. Out of a total adult life span of 67.0 ± 4.7 days for female, 6 ± 0.4 days were devoted for pre-oviposition, 42 ± 3.3 days for oviposition and 19 ± 3.1 days for post oviposition period. Cotton aphid, *A. gossypii* was found to be the most suitable host for *C. sexmaculata* as evidenced by faster development, more longevity of female and high fecundity (756.5 eggs/female). These positive biological attributes of *C. sexmaculata* on *A. gossypii* could contribute to its mass production for augmentative biological control.

Feeding potential and foraging behaviour are two important factors which determine the predator's efficiency of managing prey. Predator-prey system of *C. sexmaculata* and *A. gossypii* was evaluated to find out the predation efficiency. Feeding potential was observed for different stages of *C. sexmaculata* on *A. gossypii* whereas, functional and numerical responses were ascertained as a function of prey/host density. For the grub stage, highest feeding potential (101.43 ± 3.04 aphids) was observed for the fourth instar which was higher than the gross feeding potential of all other instars. Among the adults, female recorded high feeding potential with a mean daily consumption of 43.13 ± 0.54 aphids. Life table studies showed intrinsic rate of increase (r) of 0.22 with the mean generation time of 27.29 days. The foraging behaviour studies revealed that all the grub stages and adults displayed a type-II FR. Type-II FR of all instars of grub and adults might be a result of their innate capacity of searching ability and consumption capability even at low prey density. Fitted lines using Roger's functional response model exhibited a decelerative polynomial curve with highest slope for female followed by fourth instar, male and other instars. Among all the stages, female has exhibited highest coefficient of attack (3.45) followed by IV instar grub (2.60), male (2.54), III (1.41) II (0.75) and I instar grub

(0.23). The fourth instar grub has shown the lowest handling time with highest maximum predation (96.1aphids/day), followed by female (94.6), male (82.6) and other stages. However, female depicted a rapid extinction of aphid by keeping it below 25, as against fourth instar grub and male which affected extinction by keeping it below 30. So, the releases of fourth instar grub and female of *C. sexmaculata* could be advocated for the augmentative biological control of *A. gossypii*.

Continuous rearing of host insect is a key in biological control experiments. Of late, potted cotton plants are being extensively used for the rearing of cotton aphid. This method is cumbersome and requires more time and space. The other methods viz., rearing on excised cotton leaves and artificial diet are not suitable for rearing in large numbers. Hence, the present studies were aimed to find out an easy and reliable method for rearing. A simple rearing technique for cotton aphid based on a host, *Hibiscus cannabinus* L. (Malvaceae) is developed for quick and continuous rearing of cotton aphid throughout the year. Here, we assessed the developmental periods and population change of aphid as temperature dependent parameters. Among the different temperatures tested, 24 °C is found most suitable to produce nymphs and wingless adults in large numbers. For the production of winged adults, rearing at 27 °C is observed most suitable. This technique has certain advantages as it requires less space, easy to maintain, incurs less cost and ensures clear visibility of all the instars for counting. This new technique would help researchers in continuous rearing of *A. gossypii* for use in biological control and other studies.

Predation risk effects are non-lethal changes in prey that alter its behavior and physiology on receiving threat signals from the predator. On perceiving fear of predation, prey responds with increased oxidation that lead to oxidative stress. Prey exhibits a complex of antioxidant mechanisms to reduce or balance the oxidative stress which might be lethal to them. Cotton aphid, *A. gossypii* is an important polyphagous pest predated by a variety of coccinellids and very much preferred by six spotted zigzag ladybird beetle, *C. sexmaculata* in cotton ecosystem. Predation risk effect of *C. sexmaculata* was studied to understand the underlying anti-oxidative mechanisms in the cotton aphid. We found that predation risk effect of *C. sexmaculata* on *A. Gossypii* is transmitted through the olfactory cues, and even short duration exposure could raise the antioxidant enzyme levels in cotton

aphids. Adult aphids exhibited antioxidant mechanism through the up regulation of SOD and CAT, but not sufficient to offset the oxidative damage that led to increased MDA content. SOD, CAT and MDA were recorded significantly higher under predation risk (9.30 ± 0.4 units/mg protein, 62.62 ± 5.22 μM H_2O_2 decomposed/min/mg protein and 2.7 ± 0.4 nM/mg protein, respectively) compared to the control (5.39 ± 0.13 , 26.48 ± 5.99 and 1.40 ± 0.04). Predation risk has reduced reproductive performance of adult aphid without affecting its longevity. Similarly, it has not imparted any influence on survival and developmental period of nymphs but their development in to alate forms was enhanced as it led to significantly higher number of winged adults under active predation (18.3 ± 3.0) compared to the predator-free environment (6.0 ± 1.0). These antioxidant costs were compensated by the reduced reproduction in adult aphids following “sit and wait” strategy; while triggering winged morphs production indicating a “fight or flight” strategy. Predation risk effect might be directly leading to decline in aphid population through the reduced reproduction and may also indirectly through the induction of winged morphs for dispersal to new locality.

Cotton plant volatiles were extracted from un-infested leaf (UL), infested leaf along with aphid (WA), infested leaf without aphid (WOA) and from aphid only (AO) through DCM and n-Hexane. Orientation behavior of *C. sexmaculata* adults towards different extracts was studied with Y-tube olfactometer. Volatile components were identified through GC-MS analysis. Volatiles were also collected after spraying of Methyl salicylic acid (MeSA), and by using vacuum plant volatile collection system for the comparison and further interpretation. GCMS analysis of un-infested leaf (UL) extracts showed the presence of twenty seven compounds which rise to 38 for infested leaf along with aphid (WA) extracts and infested leaf without aphid (WOA) extract to 34. However, the aphid body (AO) extracts could show presence of only 22 volatile compounds. Olfactometer experiment revealed female beetle is highest attraction of 83.8 ± 3.2 per cent to 5% WA extract (DCM) followed by $72.5 \pm 2.5\%$ attraction to 5% WA extract (n-Hexane) and $70.0 \pm 2.7\%$ attraction to 5% AO extract (DCM). However, male got attracted most ($72.5 \pm 3.7\%$) to 5% WA extract (DCM) followed by $68.8 \pm 4.0\%$ attraction to 10% WA extract (DCM) and $68.8 \pm 2.9\%$ attraction to 10% WA extract (n-Hexane).

Infested plant extract has shown a different profile with increase in number and relative abundance of volatile compounds. Adult of *C. sexmaculata* might have majorly attracted to this Herbivore Induced Plant Volatiles (HIPVs) blend consisting of 17 compounds of which 3-Carene, Heptane, Pentadecane, 3-Hexenal, α -Copaene and α -Linolenic acid are the major ones. Volatiles collected through vacuum plant volatile collection system have shown similarity with the one reported from WA extract (DCM) by exhibiting Hexatriacontane, 3-Carene, Pentadecane, 3-Hexenal and Tetradecanoic acid as major compounds. Extracts collected after 2 and 4 h of spraying MeSA (0.1%) have not shown differences for their volatile compounds, but the number and abundance of volatiles have increased at 10 h of spraying. This MeSA induced plant volatiles also recorded some of the major aphid induced compounds viz., 3-Carene, Tetradecanoic acid, 3-Hexenal and Pentadecane. The present findings provide a detail information about the olfactory responses of predator, *C. sexmaculata* towards the *A. gossypii* infested cotton crop. Identified effective volatile compounds could be exploited for developing a possible synthetic blend of attractant for *C. sexmaculata* for its use in conservation and augmentative biological control.

कॉक्सीनेलिड बीटल, *चीलोमेन्स सेक्समैक्यूलेटा* (फैब.) का परभक्षी व्यवहार तथा शिकार पर इसके द्वारा परभक्षण का जोखिम

सार

कृषि-बागवानी पारिस्थितिक प्रणाली में, परभक्षी लेडीबर्ड बीटल (कॉक्सीनेलिडी: कोलियोप्टेरा) को जैविक नाशीजीव संदमन कार्यक्रमों में उनकी सफलतापूर्वक तैनाती और उनके द्वारा उपलब्ध कराई जाने वाली पारिस्थितिक प्रणाली सेवाओं के प्रकारों के कारण अत्यधिक महत्वपूर्ण समझा जाता है। छह धब्बे वाली जिगजैग लेडीबर्ड बीटल, *चीलोमेन्स सेक्समैक्यूलेटा* फैब्रीसियस महत्वपूर्ण देशी प्रजातियों में से एक है जिसका देश भर में व्यापक रूप से वितरण है और इसे एफिड्स एवं अन्य मुलायम शरीर वाले कीटों का प्रभावी रूप से संदमन करने वाला माना जाता है। यद्यपि, विभिन्न पोषी पौधों पर *सी. सेक्समैक्यूलेटा* के परभक्षी व्यवहार के संबंध में अनेक अध्ययन किए गए हैं किन्तु जीव संबंध विज्ञान (बायोनोमिक्स), भोजन क्षमता, कार्यात्मक अनुक्रिया, संख्यात्मक अनुक्रिया, परभक्षण-जोखिम प्रभाव (गैर-उपभोगी प्रभाव), उन्मुखीकरण व्यवहार तथा शिकार आबादी गतिकी पर इसके चरम प्रभाव सहित एक विस्तृत अध्ययन नहीं किया गया था। कपास एफिड, *एफिस गौसिपाई* ग्लोवर, एक सार्वलौकिक नाशीजीव अनेक फसलों को क्षति पहुंचाता है जिसके कारण विश्वभर में भारी आर्थिक हानि होती है। कपास एफिड द्वारा होने वाली आर्थिक हानि तथा कपास पारिस्थितिक प्रणाली में छह धब्बे वाली जिगजैग बीटल की वर्ष भर उपलब्धता को देखते हुए, वर्ष 2017-2020 के दौरान भाकृअप-भारतीय कृषि अनुसंधान

संस्थान (आईएआरआई), पूसा, नई दिल्ली के कीट विज्ञान संभाग की जैविक नियंत्रण प्रयोगशाला (बीसीएल) में कपास एफिड, *ए. गौसीपाई* पर *सी. सेक्समैक्यूलेटा* के परभक्षी व्यावहार, परभक्षण जोखिम प्रभाव एवं उन्मुखीकरण व्यवहार के संबंध में एक व्यापक अध्ययन किया गया है।

पर्यावरणीय वृद्धि कक्ष में $25 \pm 1^{\circ}$ सें., $65 \pm 5\%$ आपेक्षिक आर्द्रता एवं 16 घंटे की प्रकाश अवधि वाली नियंत्रित परिस्थितियों में फील्ड से एकत्रित वयस्क कपास एफिड एवं छह धब्बों वाली लेडीबर्ड बीटल को अलग स्क्रीन केज में गमले के भीतर लगे कपास के पौधों पर छोड़ा गया और स्टॉक कल्चर का सृजन किया गया। एक पेट्री प्लेट में क्रियाधार के रूप में एक कपास की पत्ती को रखा गया और उसके ऊपर खाद्य शिकार को रखा गया तथा पत्ती की ताजगी लम्बे समय तक बनी रहे, यह सुनिश्चित करने के लिए पर्णवृंत के कटे भाग को पेट्री प्लेट की एक साइड में डाले गए अगर - आधारित होगलान संवर्धन माध्यम (1.5%) में डुबो कर रखा गया। 160 एफिड प्रतिदिन की दर से शिकार उपलब्ध करा कर प्रथम इन्स्टार से लेकर तदुपरांत सभी जीवन-अवस्थाओं के पूरा हाने ते इस कीट का जीव विज्ञान एवं भोजन क्षमता देखे गए।

सी. सेक्समैक्यूलेटा की वयस्क बीटल ने समुच्चयों में अंडे दिए जिनकी हैचिंग ($89.9 \pm 4.7\%$) 3.2 ± 0.1 दिन में हुई। इस भृंगक (ग्रब) ने I, II, III एवं IV इन्स्टार के लिए क्रमशः 1.73 ± 0.02 , 1.16 ± 0.02 , 1.3 ± 0.07 एवं 2 ± 0.03 दिन की औसत अवधि के साथ चार इन्स्टार उत्पन्न किए। *ए. गौसीपाई* एवं अन्य एफिड के संबंध में पूर्ववर्ती ऐसी सूचनाओं की तुलना में इस बीटल ने ग्रब का अधिक तेजी से विकास (6.21 ± 0.03 दिन) दर्शाया है। ग्रब अवस्था की अल्पावधि के बावजूद, वयस्कों की जीवनक्षमता अधिक पाई

गई। मादा के कुल 67.0 ± 4.7 दिन वयस्क जीवन में से पूर्व-अंड निक्षेपण (प्री-ओवीपोजीशन) में 6 ± 0.4 दिन, ओवीपोजीशन के लिए 42 ± 3.3 दिन तथा पोस्ट-ओवीपोजीशन के लिए समयावधि 19 ± 3.1 दिन थी। *सी. सेक्समैक्यूलेटा* के लिए *ए. गौसीपाई* सर्वाधिक उपयुक्त पोषी पौधा पाया गया जैसा कि त्वरित विकास, मादा की अधिक जीवनक्षमता एवं अधिक जनन क्षमता (फीकंडिटी) (756.5 अंडे/मादा से साक्ष्य मिलता है। *ए. गौसीपाई* पर *सी. सेक्समैक्यूलेटा* के ये सकारात्मक जीव विज्ञानी गुण, जैविक नियंत्रण में बढ़ोतरी करने हेतु इसका बड़े पैमाने पर प्रगुणन करने में योगदान कर सकते हैं।

भोजन क्षमता तथा भोजन ढूंढने संबंधी व्यवहार, दो महत्वपूर्ण कारक हैं जो शिकार-प्रबंधन में शिकारी की क्षमता का निर्धारण करते हैं। परभक्षण क्षमता ज्ञात करने के लिए *सी. सेक्समैक्यूलेटा* एवं *ए. गौसीपाई* की परभक्षी-शिकार प्रणाली का मूल्यांकन किया गया। *ए. गौसीपाई* पर *सी. सेक्समैक्यूलेटा* की विभिन्न अवस्थाओं के लिए भक्षण क्षमता का मूल्यांकन किया गया जबकि कार्यात्मक एवं संख्यात्मक अनुक्रियाएं, शिकार घनत्व के एक फलन के रूप में सुनिश्चित की गई। भृंगक अवस्था के लिए, चौथे इन्सटार की भक्षण क्षमता अधिकतम (101.43 ± 3.04 एफिड) देखी गई जो अन्य सभी इन्सटार को भक्षण क्षमता की तुलना में अधिक थी। वयस्कों में, मादा के द्वारा 43.13 ± 0.54 के औसत दैनिक उपभोग के साथ अधिक भक्षण क्षमता दर्शाई गई। जीवन तालिका संबंधी अध्ययनों के 27.29 दिन के औसत संतति समय के साथ 0.22 की वास्तविक वृद्धि दर (r) दर्शाई। भोजन ढूंढने व्यवहार संबंधी अध्ययनों ने दर्शाया कि सभी भृंगक अवस्थाएं एवं वयस्क एक टाइप-II कार्यात्मक अनुक्रिया दर्शाते हैं। भृंगक के सभी इन्सटार एवं वयस्कों की टाइप-II

कार्यात्मक अनुक्रिया, कम शिकार-घनत्व पर भी उनकी जन्मजात ढूँढने की योग्यता एवं उपभोग क्षमता का परिणाम हो सकती है। रोजर कार्यात्मक अनुक्रिया मॉडल का उपयोग कर फिटेड लाइन्स ने मादा के लिए उच्चतम ढलान सहित एक विघटित बहुपद वक्र (डेसेलेरेटिव पॉलीनोमियल कर्व) दर्शाया जिसके पश्चात चौथे इन्सटार, नर एवं अन्य इन्सटार का स्थान रहा। सभी अवस्थाओं में से, मादा ने अधिकतम आक्रमण गुणांक (3.45) दर्शाया जिसके पश्चात IV इन्सटार ग्रब (2.60), नर (2.54), III (1.41), II (0.75) एवं I इन्सटार ग्रब (0.23) का स्थान रहा। चतुर्थ इन्सटार ग्रब ने अधिकतम परभक्षण (96.1 एफिड/दिन) के साथ न्यूनतम हैंडलिंग टाइम दर्शाया जिसके पश्चात मादा (94.6), नर (82.6) एवं अन्य अवस्थाओं का स्थान रहा। तथापि, चतुर्थ इन्सटार ग्रब तथा नर को 30 से कम रखने, जिसने विलुप्त होने को प्रभावित किया, की तुलना में मादा ने, 25 से कम रखने पर तेजी से विलुप्त होना दर्शाया। इसलिए, *ए. गौसिपाई* के वृद्धिकारी जैविक नियंत्रण हेतु *सी. सेक्समैक्यूलेटा* के चौथे इन्सटार और मादा की सिफारिश की जा सकती है।

पोषी कीट का सतत रूप से पालन, जैविक नियंत्रण प्रयोगों की कुंजी हैं। कपास एफिड को पालने के लिए गमलों में लगे कपास के पौधों का व्यापक रूप से उपयोग किया जा रहा है। यह विधि कठिन है और इसमें अधिक समय एवं स्थान की आवश्यकता होती है। अन्य विधियां यथा, कपास की तोड़ी गई पत्तियों एवं कृत्रिम आहार, इनके बड़ी संख्या में पालन के लिए उपयुक्त नहीं हैं। इसलिए, प्रस्तुत अध्ययन का उद्देश्य पालन की एक आसान एवं विश्वसनीय विधि-ज्ञात करना था। कपास एफिड के वर्ष भर त्वरित एवं सतत रूप से पालन के लिए एक पोषी पौधे, *हिबिस्कस कैनाबिनस* एल. (मालवेसी) पर आधारित

एक सरल पालन तकनीक विकसित की गई। यहां हमने तापमान-निर्भर प्रचलों (पैरामीटर) के रूप में एफिड की विकास अवधियों एवं आबादी-परिवर्तन का मूल्यांकन किया। परीक्षण किए गए विभिन्न तापमानों में से 24⁰ सें., बड़ी संख्या में निम्फ एवं पंखरहित वयस्क तैयार करने के लिए सर्वाधिक उपयुक्त तापमान पाया गया है। पंखों वाले वयस्क तैयार करने के लिए 27⁰ सें. पर पालन सबसे उपयुक्त पाया गया है। इस तकनीक के कुछ लाभ हैं जैसे कि, इसमें कम स्थान की आवश्यकता होती है, रख-रखाव आसान है, कम खर्च होता है और गणना हेतु सभी इन्सटार की सुस्पष्ट दृश्यता सुनिश्चित होती है। यह नई तकनीक से अनुसंधानकर्ताओं को जैविक नियंत्रण तथा अन्य अध्ययनों हेतु *ए. गौसिपाई* के सतत रूप से पालन में सहायता मिलेगी।

परभक्षण जोखिम, शिकार में होने वाले गैर-घातक परिवर्तन हैं जो परभक्षी से चुनौती मिलने पर इसके व्यवहार एवं कार्यिकी में बदलाव करते हैं। परभक्षण का भय होने पर, शिकार प्रवर्धित ऑक्सीकरण के साथ अनुक्रिया देता है जिसके परिणामस्वरूप ऑक्सीकारक प्रतिबल उत्पन्न होता है। ऑक्सीकारक प्रतिबल को कम करने या संतुलित करने के लिए शिकार ऑक्सीकारक क्रियाविधियों का एक कॉम्प्लेक्स प्रदर्शित करता है जो उसके लिए घातक हो सकता है। कपास एफिड, *ए. गौसिपाई* एक महत्वपूर्ण बहुभोजी नाशीजी है जिसका शिकार, कॉक्सीनैलिड की अनेक किस्मों द्वारा किया जाता है और कपास पारिस्थितिक प्रणाली में छह धब्बों वाली जिगजाग लेडीबर्ड बीटल, *सी. सेक्समैक्यूलेटा* इसे खाना बहुत पसंद करती है। कपास एफिड में प्रमुख प्रति-ऑक्सीकारक क्रियाविधियों को समझने के लिए *सी. सेक्समैक्यूलेटा* के परभक्षण जोखिम प्रभाव का अध्ययन किया गया। हमने पाया कि *ए. गौसिपाई* पर *सी. सेक्समैक्यूलेटा* का परभक्षण

जोखिम प्रभाव, ऑल्लैक्ट्री क्यूज के माध्यम से पारेषित होता है और यहां तक कि अल्पावधि अनुभव भी कपास एफिड में ऑक्सीकारक एंजायम स्तरों को बढ़ा सकता है। वयस्क एफिड ने एसओडी एवं सीएटी के अपरेगुलेशन के माध्यम से प्रतिऑक्सीकारक क्रियाविधि दर्शाई किन्तु वह ऑक्सीकारक क्षति को पूरा करने के लिए पर्याप्त नहीं थी जिसके परिणामस्वरूप एमडीए की मात्रा बढ़ गई। कंट्रोल (5.39 ± 0.13 , 26.48 ± 5.99 एवं 1.40 ± 0.04) की तुलना में, बड़े खतरे की स्थिति में एसओडी, सीएटी एवं एमडीए की अधिक मात्राएं रिकार्ड की गई (क्रमशः 9.30 ± 0.4 यूनिट/मि.ग्रा. प्रोटीन, $62.62 \pm 5.22 \mu\text{M}$ H_2O_2 अपघटित/मिनट/मि.ग्रा. प्रोटीन एवं 2.7 ± 0.4 nM/मि.ग्रा. प्रोटीन)। परभक्षण जोखिम से वयस्क की जीवनक्षमता को प्रभावित किए बिना, प्रजनन प्रदर्शन कम हो गया। इसी प्रकार से, निम्फ की उत्तरजीविता एवं विकासीय अवधि पर इसका कोई प्रभाव नहीं पड़ा किन्तु सपक्षक (एलेट) अवस्थाओं में उनके विकास में बढ़ोतरी हुई क्योंकि इसके परिणामस्वरूप परभक्षी-मुक्त पर्यावरण (6.0 ± 1.0) की तुलना में सक्रिय परभक्षण (18.3 ± 3.0) के अंतर्गत पंखों वाले वयस्क अधिक संख्या में उत्पन्न हुए। “सिट एंड वेट” रणनीति को अपनाते हुए, वयस्क एफिड्स में कम प्रजनन के द्वारा इन प्रतिऑक्सीकारकों की भरपाई हुई; जबकि पंखयुक्त अवस्थाओं का उत्पन्न होना, एक “फाइट टु फाइट” रणनीति को दर्शाता है। प्रजनन में कमी के माध्यम से परभक्षण जोखिम प्रभाव सीधे एफिड आबादी में कर सकता है और अप्रत्यक्ष रूप से, नए स्थान पर प्रसार हेतु पंखयुक्त अवस्थाओं के प्रेरण हेतु यह ऐसा कर सकता है।

कीटमुक्त पत्ती (यूएल), एफिड सहित ग्रसित पत्ती (डब्ल्यूए), एफिड रहित ग्रसित पत्ती (डब्ल्यूओए) तथा केवल एफिड (एओ) से डीसीएम एवं एन-हेक्सेन के माध्यम से

कपास के पौधे से वाष्पशीलों का निष्कर्षण किया गया। वाई-ट्यूब ऑल्फेक्टोमीटर के साथ, विभिन्न निष्कर्षों की ओर *सी. सेक्समैक्यूलेटा* के उन्मुखीकरण व्यवहार का अध्ययन किया गया। जीसी-एमएस विश्लेषण द्वारा वाष्पशील कम्पाउंड की पहचान की गई। तुलना एवं आगे विवेचना करने के लिए, मिथायल सैलीसिलिक अम्ल (एमईएसए) के छिड़काव के पश्चात और वैक्यूम पम्प वोलेटाइल कलक्शन सिस्टम का उपयोग कर भी वाष्पशील एकत्रित किए गए। एफिड-मुक्त पत्ती (यूएल) के निष्कर्ष के जीसीएमएस विश्लेषण ने 27 कम्पाउंड की उपस्थिति दर्शाई जो एफिड सहित ग्रसित पत्ती (डब्ल्यूए) के निष्कर्ष के लिए बढ़कर 38 और एफिड रहित ग्रसित पत्ती (डब्ल्यूओए) के निष्कर्ष के लिए 34 थी। तथापि एफिड के शरीर (एओ) के निष्कर्ष ने केवल 22 वाष्पशील कम्पाउंड की उपस्थिति दर्शाई। ऑल्फेक्टोमीटर प्रयोग ने दर्शाया कि मादा बीटल का 5% डब्ल्यूए निष्कर्ष (डीसीएम) के प्रति 83.8 ± 3.2 प्रतिशत का अधिकतम आकर्षण था जिसके पश्चात 5% डब्ल्यूए निष्कर्ष (एन-हेक्सेन) के प्रति $72.5 \pm 2.5\%$ आकर्षण एवं 5% एओ निष्कर्ष (डीसीएम) के प्रति $70.0 \pm 2.7\%$ आकर्षण का स्थान रहा। तथापि, नर 5% डब्ल्यूए निष्कर्ष के प्रति सबसे अधिक ($72.5 \pm 3.7\%$) आकर्षित हुए जिसके पश्चात 10% डब्ल्यूए निष्कर्ष (डीसीएम) के प्रति $68.8 \pm 4.0\%$ आकर्षण तथा 10% डब्ल्यूए निष्कर्ष (एन-हेक्सेन) के प्रति $68.8 \pm 2.9\%$ आकर्षण का स्थान रहा।

एफिडग्रस्त पादप निष्कर्ष ने वाष्पशील कम्पाउंड की संख्या में बढ़ोतरी तथा आपेक्षिक रूप में अधिकता के साथ एक भिन्न प्रोफाइल दर्शाया है। *सी. सेक्समैक्यूलेटा* के वयस्क, 17 कम्पाउंड से बने इस शाकाहारी प्रेरित पादप वाष्पशील (एचआईपीवी) मिश्रण की ओर प्रमुख रूप से आकर्षित हुए जिनमें 3-कारेन, हेप्टेन, पेंटाडीकेन, 3-हेक्सेनल, एल्फा-

कोपीन एवं एल्फा-लिनोलेनिक अम्ल प्रमुख कम्पाउंड हैं। बड़े खतरे की स्थितियों में एसओडी, सीएटी एवं एमडीए की महत्वपूर्ण रूप से अधिक मात्राएं रिकार्ड की गईं। प्रमुख कम्पाउंड के रूप में हेक्साट्राइएकोन्टेन, 3-कारेन, पेंटाडीकेन, 3-हेक्सेनाल एवं टेट्राडेकानोइक अम्ल दर्शाने के द्वारा, वैक्यूम प्लान्ट वोलेटाइल कलेक्शन सिस्टम के माध्यम से एकत्रित वाष्पशील पदार्थ, डब्ल्यूए निष्कर्ष (डीसीएम) से सूचित पदार्थ के साथ समानता दर्शाते हैं। एमईएसए (0.1%) क छिड़काव के 2 एवं 4 घण्टे पश्चात एकत्रित निष्कर्षों ने अपने वाष्पशील कम्पाउंड में भिन्नताएं नहीं दर्शाईं किन्तु छिड़काव के 10 घण्टे पश्चात वाष्पशील कम्पाउंड की संख्या एवं अधिकता बढ़ गई। एमईएसए प्रेरित पादप वाष्पशीलों में कुछ प्रमुख एफिड प्रेरित कम्पाउंड भी रिकार्ड किए गए जैसे कि, 3-कारेन, टेट्राडेकानोइक अम्ल, 3-हेक्सेनोल एवं पेंटाडीकेन। प्रस्तुत अध्ययन के परिणाम, ए. गौंसिपाई से ग्रस्त कपास की फसल की ओर परभक्षी, सी. सेक्समैक्यूलेटा की ऑलफैक्ट्री अनुक्रियाओं के बारे में एक विस्तृत सूचना उपलब्ध कराते हैं। पहचान किए गए प्रभावी वाष्पशील कम्पाउंड का उपयोग सी. सेक्समैक्यूलेटा के लिए आकर्षणकारी का एक संभावित संश्लेषित मिश्रण विकसित करने के लिए किया जा सकता है ताकि संरक्षण तथा वृद्धिकारी जैविक नियंत्रण में इसका उपयोग किया जा सके।

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11. APPENDICES

