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Biology of pulse beetle [*Callosobruchus chinensis* (L.)] and its reaction against greengram [*Vigna radiata* (L) R. Wilczek] varieties

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ABSTRACT

A laboratory study was conducted to evaluate resistance/preference in six green gram varieties *viz*., RMG-62, RMG-268, RMG-344, RMG-492, SML-668 and IPM-02-3 against the *C. chinensis* (L.). Different biological parameters of life cycle like, egg laying, percent adult emergence alongwith growth index and economically important parameters like, percent grain damage, weight loss and germination were also observed. The biology of *C. chinensis* was studied on SML-668 variety of green gram. Female beetles laid on an average 90.0 eggs with mean incubation period of 4.8 days, larval pupal period took on average of 29.4 days. Males lived for 6.2 days while females for 8.6 days. The mean developmental period was 33.7 days and the growth index recorded was 2.79 on SML-668 variety of green gram. The varieties, SML-668 was most preferred host for egg laying and maximum percent adult emergence while least preferred variety was recorded RMG-62 with minimum percent adult emergence. Variety RMG-62 was the also least preferred food for the *C. chinensis* with the lowest growth index of 1.57. The maximum seed damage up to (80.21%) and weight loss (17.47%) was observed in SML-668, while minimum seed damage (40.99%) and weight loss (10.49%) occurred in RMG-62. Germination per cent was recorded highest in RMG-62 and RMG-344 with 85.37% in both and was lowest in SML-668 with 65.44%. Variety, RMG-62 was found relatively less damaged or less preferred and can be used in integrated pest management programmes against *C. chinensis* during storage.

Key words : Adult emergence, biology, grain damage, greengram, growth index, pulse beetle, weight loss.

INTRODUCTION

Pulses being is a vital source of protein form a major constituent of the vegetarian diet for a majority of the rural and urban population in India, where the consumption of animal protein except milk is still considered a religious and social taboo. Among the pulse crops, greengram [*Vigna radiata* (L.) R. Wilczek] is important as it is widely used as a whole grain or as a split pulse on account of its easy digestibility. It contains 24% protein, 56.7% carbohydrates, 3.5% fibre and 1.3% fat.

The area under pulse crops is approximately 25.23 m ha and the total production is 19.27 mt in the world. The productivity of pulse crops is hampered by abiotic and biotic stresses; among the biotic stresses, insects cause considerable damage to the crops in the field and storage. The pulse beetles (*Callosobruchus chinensis* and *C. maculatus*) are the key pests of stored pulses and are mainly responsible for causing significant damage. In severe cases, the entire quantity of stored grain gets reduced to a mass of empty shells and dust. It has been reported that *C. chinensis* also harbours certain pathogenic micro organisms that cause food poisoning and spoilage besides quantitative damage (Neelgund and Kumari, 1983).

In India, the present per capita availability of pulses is less than 40 g/day as against the recommendation of 80 g/day as per FAO and WHO standards (Asthana and Chaturvedi, 1999). The availability of pulses may go down considerably due to heavy losses caused by insects during storage. It is not enough to boost the pulse production, but a check on quantitative and qualitative losses caused by insect pests in the field and storage is equally necessary. The pulse beetle alone under storage conditions requires special attention, as in India

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about 8.5 per cent losses have been reported in post harvest handling of pulses (FAO, 1997; Agarwal *et al*., 1988). Post harvest losses of pulses other than gram are 5% due to insects, 2.5% due to rodents and 0.5% due to transportation, bird damage and moisture loss (Anonymous, 1996). Apart from quantity, the quality and nutritional value of pulses get deteriorated during storage.

The life cycle of pulse beetle is reportedly completed in 25.2 days (Varma and Anandhi, 2010), 33.5 days (Patel *et al.*, 2012) and 31 days (Thakur and Pathania, 2013). Use of insect resistant varieties is a safer and economic way of saving stored grains. Efforts should also be made to reduce the storage losses through eco-safe management techniques. Considering all the above facts, present study was conduct on, biology and evolution of green gram (*Vigna radiate*) varieties for resistance against *Callosobruchus chinensis* (L.).

MATERIALS AND METHODS

A laboratory investigation on "Study on the biology of pulse beetle [*Callosobruchus chinensis* (L.)] and their reaction against different greengram [*Vigna radiata* (L) R. Wilczek] varieties for resistance/ preference" was carried out at Department of Entomology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India during 2015-16. The details of materials used and methods adopted during the course of investigation for conducting the experiments are here.

Rearing of test Insect : The distinguishing features of the male and female of *C. chinensis* (Pandy and Singh, 1997) are given in Table 1.

Maintenance of nucleus culture : The nucleus culture of, *C. chinensis* (L.) was obtained from the laboratory of Post Harvest Technology, Collage of Technology and Agriculture Engineering, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India. This culture was maintained in the Department of Entomology, Bioagent laboratory at ambient conditions of temperature and relative humidity. Greengram was procured from the local market, cleaned, washed, shade-dried and sterilized in an oven at 50°C for 24 hours night to eliminate the hidden infestation, if any.

The sterilized greengram seeds were used to maintain the culture for experimental needs. Ten pairs of one-day old adults were released into plastic containers (250 ml capacity) containing 100 g greengram seeds on different dates and replicated thrice to obtain regular supply of the test insect of uniform age. The jars were covered with muslin cloth and fastened with rubber bands. The released adults were removed from the containers of the dated culture after 3 days. Utmost care was taken to pick or transfer the seeds or test insects with the help of a forceps, camel hair brush and an aspirator.

Study on biology of C. chinesis : The biology of, *C. chinensis* was studied on SML-668 variety of greengram, for which, three plastic containers (250 ml capacity) containing 100 g seeds were taken wherein freshly emerged single pairs of *C. chinensis* were released in each container. Identification of the sexes was made by employing the method suggested by Pandey and Singh (1997). Mouth of the containers were covered by muslin cloth and secured with rubber bands and later maintained at ambient conditions of temperature and relative humidity in the laboratory. Adults were removed from these containers after death. The number of eggs laid by a single female on host grains was recorded taking a sample of 100 grains. Eggs laid on each

Table 1. Distinguishing characters of male and female adults of *C. chinensis*.

Features	Male	Female
Size	Average 2.5 x 2.0 mm broad	3.5 x 2.5
Antennae	Pectinate	Serrate
Elytra	1. With deep vertical and closed stripes. 2. With light dark bands expanding laterally and tapering in the mid-dorsal line (usually two bands)	Stripe present No such transverse dark band present
Pygidium	Broad, shiny area spreading over the lateral margin of posterior mid-dorsum, giving shape of expanded inverted 'V'	Narrow, shiny area confined to the posterior mid dorsum, giving a shape of closed inverted 'V'

day were kept in separate containers covered with muslin cloth and observations were recorded for incubation, development (larval and pupal), total developmental period and the growth index. The incubation period, larval and pupal period within the grain was recorded by gently splitting-open the whole grain with the help of the needle and forceps to observe the stage of insect under a stereo-zoom binocular.

Varietal reaction against C. chinensis : Freshly harvested seeds of greengram varities *viz*., of RMG-62, RMG-268, RMG-344, RMG-492, SML-668 and IPM-02-3 were collected from Rajasthan Agriculture Research Institute, Durgapura (Jaipur), Rajasthan, India.

Experiment was laid out in controlled condition in which applied Completely Randomized Design with six varieties as treatment and four times replicated each variety. Sound seeds of each genotype were kept in hot air oven for six hours at 50°C in order to eliminate insect pest infestation, if any. Seeds of each variety weighing 100 g were kept in plastic containers of 250 ml capacity separately to evaluate the preference of variety by *C. chinensis*. Five pairs of healthy, one-day old emerged adult bruchids were introduced in each plastic jar and the top was covered with muslin cloth, tightened by rubber bands.

Observations were recorded from each plastic jar regularly for the following parameters:

Preference for egg laying : At 3, 7 and 10 days after release; average number of eggs per grain laid by *C. chinensis* was calculated to check the preference of varieties for its oviposition/ fecundity. Ten grains were randomly selected from each replication egg laid on those grains were counted. At the end, their average was calculated to determine number of eggs per grain in each jar.

Adult emergence (%) : Number of emerged adults in each jar was calculated to see the effect on adult emergence of *C. chinensis* by different varieties after 30, 60 and 90 days of storage. Per cent adult emergence was calculated using following formulae (Howe, 1971).

$$\text{Per cent adult emergence} = \frac{\text{No. of adult emerged}}{\text{No. of eggs laid}} \times 100$$

Grain damage (%) : Before estimating the damage grains, the grains were thoroughly mixed and samples of 100 grains were drawn for five times from each bottle, then damage grains in 100 seeds were counted for each sample after 90 days of storage. Grain/seed damage was computed as suggested by Adams and Schulten method (1978):

$$\text{Grain/seed damage (\%)} = \frac{\text{No. of holed seeds}}{\text{Total seeds}} \times 100$$

Weight loss (%) : The per cent weight loss was calculated at the end of experiment by using the following formula:

$$\text{Weight loss (\%)} = \frac{\text{Initial weight of seeds} - \text{Final weight of seeds}}{\text{Initial weight of seeds}} \times 100$$

Mean development period : Mean development period (MDP) is the time taken for 50% of adults to emerge. It was estimated using the following formulae (Howe, 1971)

$$\text{Mean development period} = \frac{D_1A_1 + D_2A_2 + D_3A_3 + ...D_nA_n}{\text{Total no. of adults emerged}}$$

Where,

D_1 = Day at which the adults started emerging (First day)

A_1 = Number of adults emerged on D_1 th day

Growth index : Growth index was calculated using the following formula (Howe, 1971)

Growth Index = LOG S/T

Where,

S = Per cent adult emergence

T = Mean development time (days)

Germination (%) : The germination tests were carried out according to International Rules of Seed Testing (Anonymous, 1976). After 90 days of storage; hundred seeds from each replication were placed in a petri dish (diameter 15 cm) having blotting paper moistened with distilled water. The petri dishes were kept at a temperature of 28 ± 2°C. The number of germinated seeds was counted and the percentage was calculated.

Statistical analysis : The data recorded were subjected to statistical analysis as Completely Randomized Design (CRD). The data in percentages were transformed in to angular and number values were transformed to square root transformations before statistical analysis.

RESULTS AND DISCUSSION

Biology of pulse beetle on SML-668 variety of greengram : Biology of *C. chinensis* was studied on SML-668 variety of greengram under laboratory conditions during November 2015 to January 2016. It was observed that the average incubation period was 4.8 days which varied from 3 to 7 days in which could be due to the impact of temperature and humidity. Earlier, Kumari *et al*. (1991) observed average incubation period ranging from 9-13 days on blackgram. Incubation period of 4-5 days by *C. chinensis* was also reported by Singh and Kumari (2000) on greengram and cowpea seeds at 28 ± 2ºC and 70 ± 5% RH. Variations in incubation period among different generations in different hosts have been reported by many workers, which were attributed to differences in temperature, relative humidity and host species (Table 2).

The larval pupal period varied from 23 to 33 days with an average of 29.4 days during the present studies. Kumari *et al*. (1991) reported the larval period of 17-20 days in blackgram. While working the biology of *C. chinensis* on greengram and cowpea, Singh (1962) reported the combined larval and pupal

Table 2. Biology of pulse beetle on greengram variety SML-668.

S. No.	Stage of the Insect	Average days ± S.D.
1.	Egg	4.8 ± 1.23
2.	Larval + Pupal	29.4 ± 3.33
3.	Total developmental period	33.7 ± 3.13
4.	Adult longevity	
	Male	6.2 ± 0.79
	Female	8.6 ± 1.07
5.	Pre-oviposition	5.8 ± 1.62
6.	Oviposition	4.9 ± 1.66
7.	Post- oviposition	3.6 ± 0.97
8.	Fecundity	90 ± 8.04

S.D. = Standard deviation

period to vary from 18.9 - 38.3 days on different pulses at room temperature of 28 ± 2ºC and 70 ± 5% RH; the pupal stage lasted 7.00 to 9.33 days during the six generations of *C. chinensis chinensis*, Kumari *et al*. (1991) and Singal and Borah (2001) reported pupal periods of 7-9 days on blackgram and 7.2 ± 0.18 days on pigeonpea, respectively.

The time taken by the beetle to complete total developmental period varied from 29 to 38 days (average: 33.7 days) during the present study. Earliar, Kumari *et al*. (1991) reported the total period of egg laying to adult emergence to be 36-39 days on blackgram and 28-40 days in greengram. Meghwal and Singh (2005) also observed the average period for development from egg laying to adult emergence to be 25.49 days on moth bean.

The fecundity of *C. chinensis* female varied from 78 to 102 eggs with an average of 90 eggs in the present study. Pandey and Singh (1997) reported the average egg laying per female by *C. chinensis* to be 110 eggs. The mean longevity of male was 6.2 days and 8.6 days for the female. Patel *et al*. (2005) reported the adult longevity of *C. chinensis* to vary between 11.37 to 14.83 days on different pulses. Singal and Borah (2001) reported lower mean longevity of female and male beetle as 6.2 ± 0.36 and 6.8 ± 0.25 days.

Screening different greengram varieties against pulse beetle

Preference for egg laying : The maximum average numbers of eggs were laid by the pulse beetle on SML-668 variety of greengram and minimum eggs were laid on variety RMG-62 after 3, 7 and 10 days of time intervals. The varieties SML-668, IPM-02-3, RMG- 492, and RMG-268 were more preferred for egg laying than RMG-62 and RMG-344 (Table 3).

Similar results were found by Chavan *et al*. (1997) for ovipositional preference of *C. chinensis* in cowpea lines. The beetle showed a definite varietal response for oviposition. The cowpea line with rough seed surface was less preferred for oviposition. Raina (1970) reported that *C. chinensis* female laid an average of 78 eggs, ranging from 63.90 over a period of 8 days and maximum number of eggs were laid on the first day of oviposition, Rajak and Pandey(1965) reported a range of 50-103 eggs and Takasugi (1924) between 70-80 eggs under natural conditions.

Adult emergence (%) : The result indicated that average maximum adult emergence was 87.09, 89.12 and 89.12% on SML-668 variety of greengram after 30, 60 and 90 days, while minimum adult emergence was 72.44% (after 30, 60 and 90 days) on RMG-62 and RMG-344. Gokhle (1973) reported that adult emergence of *C. chinensis* on cowpea was 96.62% and on pea it was 73.35%; whereas, Kumari *et al.* (1991) reported 46.77% adult emergence in blackgram.

Growth index : As per the growth index values RMG-62 was the least preferred host for pulse beetle with the least growth index (1.57), while SML-668 was most suitable host with a growth index of 2.79. Kumari *et al*. (1991) reported that the highest

Table 3. Comparative preference for egg laying on greengram varieties.

Variety	Number of eggs laid/100 grains		
	3 DAR	7 DAR	10 DAR
RMG-62	1.86 (72.44)	1.86 (72.44)	1.86 (72.44)
RMG-268	1.87 (74.13)	1.87 (74.13)	1.87 (74.13)
RMG-344	1.86 (72.44)	1.86 (72.44)	1.86 (72.44)
RMG-492	1.89 (77.62)	1.88 (75.85)	1.88 (75.85)
SML-668	1.94 (87.09)	1.95 (89.12)	1.95 (89.12)
IPM-02-3	1.93 (85.11)	1.93 (85.11)	1.93 (85.11)
SEm ±	0.005	0.003	0.003
CD at 5%	0.015	0.008	0.009

Figures in brackets () are retransformed log values
DAR = Days after release

Table 4. Comparative adult emergence and growth index of *Callosobruchus chinensis* (L.) on different greengram varieties.

Variety	Adult emergence(%) days after release			Mean development period (days)	Growth Index
	30 DAR	60 DAR	90 DAR		
RMG-62	45.57 (50.99)	46.72 (53.00)	47.88 (55.01)	40.5	1.57
RMG-268	49.03 (57.01)	50.21 (59.04)	53.17 (64.06)	36.5	1.79
RMG-344	46.15 (52.00)	48.46 (56.02)	50.21 (59.04)	38.5	1.64
RMG-492	57.51 (71.14)	58.81 (73.18)	60.14 (75.21)	34.5	2.23
SML-668	71.68 (90.12)	75.07 (93.36)	76.00 (94.14)	31.5	2.79
IPM-02-3	69.55 (87.79)	70.56 (88.92)	73.85 (92.26)	32.5	2.77
SEm ±	2.42	2.95	2.99	0.72	
CD at 5%	7.20	8.77	8.88	2.17	

Figures in parentheses are retransformed per cent values

growth index for *C. chinensis* was on pigeonpea (2.37) and minimum on urd (1.07) (Table 4).

Grain damage, weight loss and germination (%) : The results of grain damage showed that the mean maximum damage to seed was recorded on SML-668 and minimum on RMG-62 variety of greengram. Kalyan and Dadhich (1999) reported that the minimum per cent grain damage was recorded in the variety R-298 (27.51%) followed by K-851 (30.61%); whereas, the maximum was recorded in Pusa-105 (77.31%) and PS-16 (85.9%) by *C. maculatus*. Similar results of wide variation on grain damage was reported by Satyavir (1989) and Singal (1987) (Table 5).

Choudhary *et al*. (1982) reported that greengram with larger seed size and thinner testa was preferred more as compared to small seed with thicker testa by *C. chinensis*, Kopergaon was the most susceptible variety, while J-45 was least susceptible and least preferred host for the development and survival of *C. chinensis*.

Table 5. Comparative damage to different greengram varieties 90 days after storage due to pulse beetle.

Varieties	Grain damage (%)	Weight loss (%)	Germination (%)
RMG-62	39.81 (40.99)	3.24 (10.49)	9.24 (85.37)
RMG-268	47.88 (55.01)	3.67 (13.46)	8.97 (80.46)
RMG-344	41.55 (43.99)	3.39 (11.49)	9.24 (85.37)
RMG-492	53.78 (65.08)	3.81 (14.51)	8.68 (75.34)
SML-668	63.59 (80.21)	4.18 (17.47)	8.09 (65.44)
IPM-02-3	55 (67.10)	4.06 (16.48)	8.39 (70.39)
SEm ±	3.84	0.08	0.19
CD at 5%	11.42	0.23	0.55

Figures in parentheses are retransformed per cent values
Figures in square brackets are retransformed square values

The results of weight loss showed that the average maximum weight loss was in SML-668 and minimum in RMG-62 and the results of germination per cent showed that the maximum germination was in RMG-62. Our observations are supported by those of Sadozai *et al*. (2003) who reported maximum weight loss in cowpea and greengram.

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