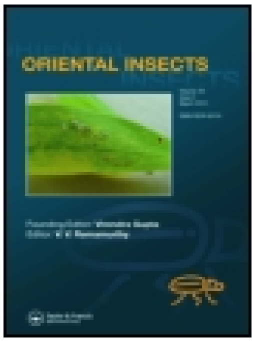

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Biology and seasonal incidence of *Physocephala* (Diptera: Conopidae) on Carpenter Bees (Hymenoptera: Apidae)

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ABSTRACT

Studies were conducted to explore the extent of conopid fly infestation in carpenter bee rearing. Conopid flies are larval parasitoids of carpenter bees. The samples were collected from fields, natural nesting sites and artificially domiciled nests. The seasonal incidences of conopid flies in *Xylocopa* were observed from January 2019 to June 2020. The biology of conopid flies revealed different stages of their life cycle. Two species of conopid fly, *Physocephala rufipes* and *Physocephala scutellata* were identified to infect carpenter bees in this region. The total development period of *P. rufipes* ranged between 130 and 185 days while it was 136–191 days in *P. scutellata*. Seasonal incidences showed higher infestation during June to August which ranged between 25 and 38% and the infested foragers died after 7–11 days of infestation. A comparative seasonal incidence of *P. rufipes* and *P. scutellata* revealed higher infestation in *X. tenuiscapa* (15.96% and 16.79%) over *X. fenestrata* 14.29% and 14.66% during 2019 and 2020. The incidence of *P. rufipes* was higher in both the carpenter bees (64–71.11%) as compared to *P. scutellata* (28.29–36.93%).The highest incidence of *P. rufipes* was recorded from Dimapur (29.68%) while *P. scutellata* infestation was higher in Peren.

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Carpenter bees; conopidae; *Physocephala rufipes*; *Physocephala scutellata*; developmental biology; seasonal incidence

Introduction

Pollination is an ecologically important service rendered by bees. With the increase in protected cultivation of crops, the need for pollination inside protected structures stimulated a search for potential pollinators. Bumble bees, stingless bees and carpenter bees are among the important pollinators that provide an opportunity to gratify the requirements of pollination under protected structures. Since bumble bees are yet to be commercialised in India, research has been shifted to explore the pollination potential of carpenter bees.

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Carpenter bees belong to the order Hymenoptera, with more than 500 species spread across the globe (RL 1998). They are important wild pollinators found in subtropical and tropical regions of the world. The colours vary with the species and range from metallic black to dark black, sometimes with yellowish pubescence on the thorax in some species and are classified under the genus *Xylocopa* Latreille (Michener 2007). The species diversity of carpenter bees has been studied in India by Bingham (1897) and recently by Gupta and Yanega (2003). About 40 species of carpenter bees are reported from India (Rahman and Taye 2016). These species are spread all over the country except the alpine regions. Among all the species found in India, *Xylocopa tenuiscapa* (Westwood, 1840) and *X. fenestrata* (Fabricius, 1798) are important species which are utilised in artificial domiciliation for pollination of tomato and cucurbits under protected conditions (Rahman and Taye 2016). The nesting of carpenter bees under natural conditions can be observed in the form of tunnels in pithy stems of various bushes, nesting galleries in solid wood and stumps, logs, dead branches of trees and bamboo culms (Hurd and Moure 1960; Hurd 1978). In human habitations, these bees make their nests on the timber used in roofs of thatched houses and bamboo/ wooden supports. These bees rear four to six larvae in the wooden or bamboo hives whereas some species are solitary. According to available literature, different aspects of carpenter bee rearing were undertaken by different researchers (Kapil and Daliwahi 1968; Borollosy and Ismail 1973; Maeta et al. 1985; Gerling et al. 1989; Rahman and Deka 2006; Raju and Rao 2006; Junqueira et al. 2012; Ali et al. 2016). In India, several attempts have been made to domesticate these pollinators (Rahman and Deka 2006, 2011; [AICRP HB&P] All India Coordinated Research Project on Honey Bees and Pollinators 2016, 2018). The different factors responsible for creating hurdles in rearing of *Xylocopa* bees are designing of artificial domiciles, nest architecture, crop preference, foraging activity and pests/diseases. Among all the stresses that hampers *Xylocopa* bee rearing, the pests and diseases are not yet studied in the country.

Conopid flies, tachnid flies and mites are important pests of large bees (Chauhan et al. 2016). Conopid flies impact the growth and development of colonies by acting as endoparasitoids of adults (Hurd 1978; Chauhan et al. 2016). The diversity of Conopidae revealed 51 species in India (Stuke 2017). Out of the total extant conopid fauna in the world, about 6.25% is explored from India and more species are still unexplored (Mitra et al. 2015).

All known conopid larvae are endoparasitoids. In most cases, parasitism leads to changes in host behaviour, and ultimately host death (Malfi et al. 2014; Chauhan et al. 2016). The World's conopid diversity was recently summarised by Stuke (2017). Gibson and Skevington (2013) proposed a new classification of conopid flies (all genera) by cladistic analysis of 117 morphological characters recorded from 154 species. Barahona-Segovia and

Barcelo (2020) described two new species of conopid flies, *Myopa nebulosa* and *Myopa bozinovici* from Chile. While, Melo et al. (2008) reported conopid flies attack in male and female orchid bees. In India, literature is not available on the incidence of conopid flies in *Xylocopa* bees and so, an attempt was made to disclose the biology and incidences of this pest on carpenter bees which is a constraint in Xylocopiculture in the country.

Material and methods

Study site

The study was carried out around southern region of Nagaland in North Eastern India having an elevation ranged from 110 m to 2100 m above mean sea level (MSL) with geographical location of 25°45′N latitude and 93°33′E longitude. Nagaland is a biodiversity hotspot, having a diverse range of flora and fauna spread in 16,579 km^2. This region has higher pollinator diversity as compared to other states in India because of the diversity of habitats and elevations (Chauhan and Singh 2020). Carpenter bees are among the important pollinators found in all districts of Nagaland and adjoining north eastern states. Agriculture is the major activity of the people of this region and about 70 per cent of the population depends on agriculture related activities. *Xylocopa* bees play an important role in fruit and seed production of different crops like tomato, cucurbits, papaya, pegion pea, cow-pea, citrus, guava, passion fruit, pomelo, mango, chillies etc. which are important crops of this region (Rahman and Taye 2016; Chauhan et al. 2019). Carpenter bees are reared as pollinators in traditional wooden blocks in some villages. Knowing the importance and potential of these pollinators, an attempt was made to study the Conopidae bio-stresses that hamper the development of carpenter bee colonies.

Collection of carpenter bees

Carpenter bees (*X. tenuiscapa* and *X. fenestrata*) were swept with caging nets while foraging on the flowers and also from their nesting sites. During the period of study, a total of 1747 (951 samples of *X. tenuiscapa* and 796 samples of *X. fenestrata*) samples were collected (dead and live) and brought to the Apiculture laboratory at School of Agricultural Sciences and Rural Development for dissection from different districts (Kiphire, Kohima, Phek, Dimapur, Mokokchung, Peren, Zunheboto, Tuensang and Mon) of Nagaland. The presence of eggs, larvae and pupae of conopid flies were recorded while dissecting the bees. The dead samples were also collected from the natural nesting sites and dissected for the presence of conopid flies.

In addition to the natural nesting sites, twelve colonies of *X. tenuiscapa* and eight colonies of *X. fenestrata* were established artificially and the dead adults below the nest entrances were also collected during different months and dissected.

Identification of carpenter bees and conopid flies

The representative collected samples of carpenter bees were studied for morphology and morphometrics (Rahman and Taye 2016). For conopid flies, characters like wing pattern, face, frons etc were recorded and the keys designed by KGV (1969); Stuke (2006b), Stuke (2016) were also consulted accordingly. The photographs of the conopid flies specimens were sent to Dr Jens-Herman Stuke, Germany for confirmation of identifications. The samples of carpenter bees and conopid flies (larvae, pupae and adults) were also sent to Division of Entomology, IARI, New Delhi and ICAR Laboratory, Umiam, Meghalaya for molecular barcoding and identification.

Rearing of *X. tenuiscapa* and *X. fenestrata*

Ten colonies of *X. tenuiscapa* and eight colonies of *X. fenestrata* were selected out of total reared colonies. Thirty wooden blocks of 1.52 metres in length and 15 cm thickness of *Gmelina* sp. and *Artocarpus* sp. timbers were drilled with holes of 2.5 cm diameter (seven holes on both sides) and hung at a height of 2.74 metres from the ground tied with binding wires and ropes in the month of November, 2018. Similarly, bamboo poles with 2 cm holes (four holes on opposite sides) were also hung and tied in the field at 1.82 metres height. By January 2019, twelve blocks had been accepted by carpenter bee, *X. tenuiscapa* and eight bamboo poles by *X. fenestrata* for their nesting sites (Figure 1A-B). The nests were perennial and the samples were collected until June, 2020 to know the seasonal incidence of conopid flies on the foragers.

Attacking behaviour

The attacking behaviour of conopid fly on carpenter bees was studied in the field when bees visit the flowers for pollen and nectar as reported by Chauhan et al. (2016). The female conopid flies attacks the foraging bee and inserts their egg in the terminal part of abdomen of *Xylocopa*.

Seasonal incidence

The seasonal incidence of conopid fly was studied by collecting the foragers from natural nesting sites and flowers while they were foraging in the field

Figure 1. (**a and b)** Nesting sites of *Xylocopa*. A, *X. tenuiscapa*; B, *X. fenestrata.*

and from the ground below the artificially domiciled nests during period under investigation (January 2019–June 2020) and dissected them under the stereomicroscope as per Chauhan et al. (2016).

Developmental biology

The development biology of conopid fly was studied inside the incubator while egg, larval and pupal period with adult emergence as total developmental period was worked out correspondingly.

Duration of egg stage and hatchability

Freshly parasitised carpenter bee samples were collected from the field after the female conopid fly injected the egg in the abdomen of the foragers. The foraging workers were also collected both from the natural and the established field nests. These workers were dissected and the different developmental stages of conopid fly were extracted and observed for different parameters (size, colour). The egg period was recorded/ established by subtracting one day (24 h) from the first instar larva.

Duration of larval period

The neonate larvae (12–24 hour old) were transferred to the petri plates containing the freshly dissected healthy carpenter bee with the help of camel's hair brush. The Petri plates were then kept inside incubator at 28.0 ± 1°C. The dissected bees were changed daily (when we were not changing the dissected host bee, the mortality of larvae was high) and observations on duration of different instars were recorded daily. This experiment was replicated five times with 5 larvae/replication (total 25 larvae).

Duration of pupal period

The pupa formed in each replication was kept separately in the plastic vial inside incubator at 28.0 ± 1°C without light and also at room temperature in dark. The number of days for which the insect remained in the pupal stage were counted when adult emerged.

Results

Attacking behaviour

The attacking behaviour of conopids on foraging carpenter bees was witnessed many times (four to five times) in the field. Both male and female carpenter bees were attacked by conopid flies. It was recorded that females of conopid fly attack the foraging or resting bee and insert an egg in the terminal part of abdomen. The infected carpenter bee was immobilised for 35–138 seconds after insertion of egg before flying away. Parasitised bees die within 9–11 days after insertion of the egg. The dead foragers were found lying on their dorsums on the ground.

The conopid flies infesting carpenter bees collected from all locations were identified as belonging to the genus *Physocephala*. Two species of conopid flies, *Physocephala rufipes* (Fabricius, 1781) and *Physocephala scutellata* (Krober, 1915) were identified infesting carpenter bees in this region. Both species of carpenter bees, *X. tenuiscapa* and *X. fenestrata* were infected with both conopid flies. The *X. fenestrata* bees were less infected as compared to *X. tenuiscapa* which may be due to their small size. The dominant endoparasitoid conopid fly was *P. rufipes*.

Developmental biology

Conopid flies are larval endoparasitoids of carpenter bees which parasitise foraging carpenter bees. During the studies, the different life stages were observed in conopid flies (Figure 2A-I). The egg was oval shaped with micropyles on one side (1.45 mm and 1.61 mm) and off-white in colour both in *P. rufipes* and *P. scutellata*. Initially, the larvae were very small and did not have posterior spiracles. After development from first larval instar to third larval instar, the full grown larvae (maggot) were white in colour, heart shaped with swollen constricted body measuring average 11.78 mm x

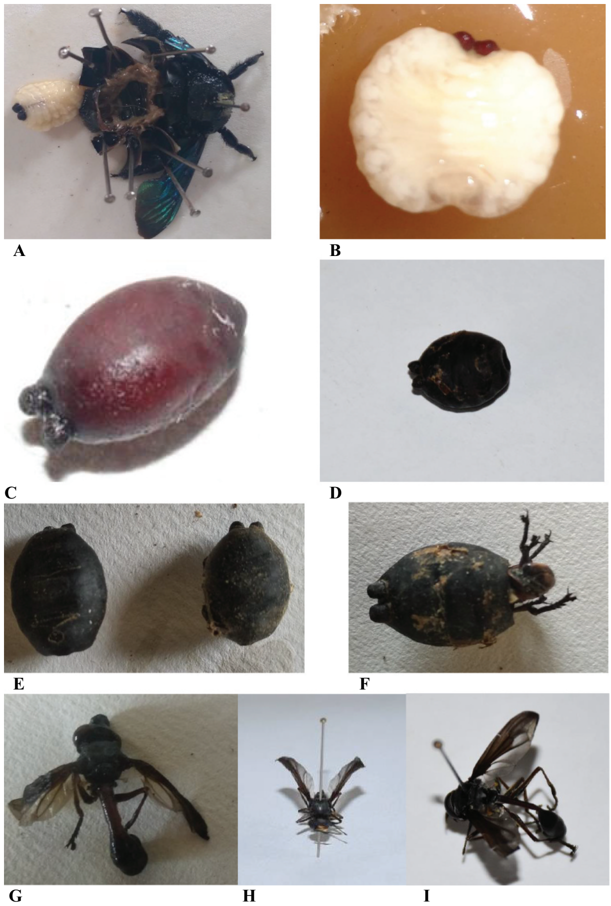

Figure 2. **(a–i)** Different developmental life stages of conopid fly, *Physocephala rufipes* and *Physocephala scutellata*. A, larva of *P. rufipes*; B, larva of *P. scutellata*; C, freshly pupated *P. rufipes*; D, freshly pupated *P. scutellata*; E, pupa of *P. rufipes* (left) and *P. scutellata* (right); F, emergence of *P. rufipes*; G, *P. rufipes* adult; H-I, *P. scutellata* adult (front and dorsal view).

8.54 mm in *P. rufipes* and 11.49 mm x 7.55 mm in *P. scutellata*, respectively. The larva in both species had a pointed head with extended Buccopharyngeal apparatus oriented towards the head of carpenter bees. Development was completed within 9–12 days after oviposition in both species when the host was killed and the parasite pupated inside the body of carpenter bee. The size of pupa varied from 10 to 12 mm x 8.5–9.5 mm and was elongated in shape with reddish brown colour in both the species of conopid fly. The bees died after 8–10 days of infestation with symptoms of open ovipositor due to conopid parasitism. The adult flies emerged after average 148 days with range of 130–185 days in *P. rufipes* and 160 days and range of 136–191 days in *P. scutellata* after pupation (Table 1).

Table 1. Developmental biology of conopid fly, *P. rufipes* and *P. scutellata*.

		Duration (days)			
		P. rufipes		*P. scutellata*	
Developmental Stages[a]		Mean± SE	Range	Mean± SE	Range
Incubation period		1.06 ± 0.11	1–1.5	1.14 ± 0.08	1–1.5
Larval instars	I	2.15	2–3	2.19	2–2.5
	II	3.31	3-4	3.42	3–4.5
	III	4.28	4–5	4.32	4–5
Total larval period		9.74 ± 0.11	9–12	9.93 ± 0.18	9–12
Pupal period		138.24 ± 7.55	121–173	149.81 ± 9.22	127–179
Total developmental period		147.98 ± 13.15	130–185	159.74 ± 9.66	136–191

[a]*n* = 25 where *n* is size of sample

Seasonal incidence

Seasonal incidence of *P. rufipes* and *P. scutellata* is presented in Table 2, revealing that infestation in *X. tenuiscapa* was 15.96% and 16.79%. However, in *X. fenestrata* it was lower, 14.29% and 14.66% during 2019 and 2020, respectively. The maximum infestation was recorded from June to August (27.42–38.10% and 25.49–38.89) and the minimum from December and January (0–3.12%) in *X. tenuiscapa* during both experimental years 2019 and 2020. Similarly, in *X. fenestrata*, maximum incidence was recorded from June to August (22.95–25.00%) in 2019 and 27.08% in June 2020. The studies thus revealed that the infestation started in February- March, peaked during June to August and then declined from September to January. The incidence of *P. rufipes* was higher in both the carpenter bees (64–71.11%) as compared to *P. scutellata*. (28.29–36.93%) during the period of studies (Table 2). The highest incidence of *P. rufipes* was recorded from Dimapur (29.68%) followed by Peren (25%), Kiphire (18.75), Zunheboto (14.06%) and Mokokchung (12.50%) districts while the highest infestation of *P. scutellata* was recorded from Peren (42.30%) followed by Zunheboto (23.07%), Dimapur (15.38%), Tuensang (11.53%) and Mon (7.69%). Kohima and Phek district did not report conopid infestation in collected samples. The infested carpenter bee colonies (*X. tenuiscapa* and *X. fenestrata*) collapsed more rapidly (within 4.37 months of activity) than the healthy colonies (13.54 months). The healthy colonies survived the winter and again started foraging in the next season. The infestation of conopid flies in carpenter bees increased with the increasing temperature and humidity during summer and rainy seasons while it decreased with the decrease in temperature and humidity in early autumn season.

Discussion

Conopid flies, *P. rufipes* and *P. scutellata* are the larval endoparasites of *Xylocopa* parasitising both male and female bees. The larval endoparasites of *X. augusti* (Lepeletier, 1841) were reported by Lucia et al. (2020) as *P. wulpi*

Table 2. Seasonal Incidence (%) of conopid fly, *P. rufipes* and *P. scutellata* on carpenter bee, *X. tenuiscapa* and *X. fenestrata*.

X. tenuiscapa									X. fenestrata			
Month	2019			2020			2019			2020		
	Collected bees	Infected bees	Infestation (%)	Collected bees	Infected bees	Infestation (%)	Collected bees	Infected bees	Infestation (%)	Collected bees	Infected bees	Infestation (%)
January	37.00	0.00	0.00	24.00	0.00	0.00	24	0	0	21	0	0
February	59.00	4.00	6.78	67.00	3.00	4.47	42	3	7.14	54	2	3.70
March	62.00	7.00	11.29	72.00	5.00	6.94	53	4	7.55	46	6	13.04
April	68.00	9.00	13.24	57.00	7.00	12.28	61	9	14.75	52	7	13.46
May	64.00	9.00	14.06	51.00	13.00	25.49	51	6	11.76	61	11	18.03
June	62.00	17.00	27.42	54.00	21.00	38.89	47	14	23.40	48	13	27.08
July	42.00	16.00	38.10	62.00	16.00	25.80	61	11	22.95	59	11	18.64
August	48.00	17.00	35.42	-	-	-	44	14	25.00	-	-	-
September	52.00	6.00	11.54	-	-	-	32	4	12.50	-	-	-
October	39.00	2.00	5.13	-	-	-	31	1	3.23	-	-	-
November	36.00	2.00	5.56	-	-	-	19	2	10.53	-	-	-
December	32.00	1.00	3.12	-	-	-	14	0	0	-	-	-
Sum	564.00	90.00	15.96	387.00	65.00	16.79	455	65	14.29	341	50	14.66
P.sp1 (%)		71.11			66.15			63.07			64.00	
P. sp2 (%)		28.89			33.85			36.93			36.00	

**P*. sp1 = *Physocephala rufipes; P*. sp2 = *Physocephala scutellata*

(Camras, 1996) in Argentina. Similarly, the parasitic associations between conopid flies and carpenter bees were also described (Lucia et al. 2010). Wasps of genus *Leucospis* were observed as the ectoparasites of carpenter bees (Lucia et al. 2019). However, contrary, we did not observe wasps in the nests of carpenter bees. Feinstein (2011) described *Xenox tigrinus* (Evenhius, 1984) as the parasite of *X. virginica* (Linnaeus, 1771). Vickruck et al. (2010) found *Pyemotes* sp. (Pyemotidae) and *Baryscapus americana* (Eulophidae) as the most common natural enemies of small carpenter bees. Carpenter bee nests are susceptible to several types of natural enemies, including parasitoid wasps and flies (Gerling et al. 1983). Several researchers also observed conopid flies in commercial and wild bumble bees (Hempel and Hempel 1996a, 1996b; Clements 1997; Chauhan et al. 2016). The larvae of reported conopid flies were white in colour, heart shaped and developed inside the abdomen to full size and gradually developed into pupa. Smith and Van-Soleiren (1968) revealed the biology and larval development of *P. bimarginipennis* Karsch. Similarly, Abdalla et al. (2014) described the larval development of conopid fly, *P. rufipes* and observed that the larvae developed both in length and width, reaching the full developed stage with 10 mm in length and 7 mm in width. The size of pupa varied from 10 to 12 mm x 8.5 to 9.5 mm and is smaller in *P. scutellata*. Stuke et al. (2011) observed the pupa of *P. wulpi* and recorded the size of pupa. They described that orange-brown pupa had length of (excluding dorsal flap and posterior spiracles) 7.5–10.3 mm with maximum width 5.2–7.1 mm and the posterior spiracles were black in colour. The variation in width may be due to species variation. Adult flies had thick heads with large compound eyes and dark brown bodies. The ovipositor was modified for opening of tergal plates and insertion of egg into abdomen of carpenter bees. The adult flies emerged after 147.98 ± 13.15 days in *P. rufipes* and 159.74 ± 9.66 days in *P. scutellata* after pupation, respectively. Similar results were recorded by different workers (Howell 1967; Askew 1971; Hempel and Hempel 1988). According to them, conopid flies attacked foraging bees. A single egg is either attached to or inserted into the abdomen, where it hatches into larva and feeds on haemolymph and internal organs (Cumber 1949). Further, Pouvreau (1973) had also reported its three larval instars in 6–10 days after which it undergoes pupation inside the bee body. The adult emerges in the next summer (Postner 1951). Similarly, Chauhan et al. (2016) have also reported larva of conopid fly feeding inside the abdomen of *Bombus haemorrhoidalis* (Smith, 1852) which caused mortality of infected queens and workers in 8–10 days. The adults emerged between 140 and 205 days after infection. These arguments help in understanding the presence of Conopidae as parasitoids of carpenter bees. The maximum infestation of conopid flies in *Xylocopa* bees was reported during June to August and minimum during December and January in both species. Similarly, Chauhan et al. (2016) reported maximum infestation during June to August months of the year in *B. haemorrhoidalis*. Chauhan et al. (2013) have recorded its infestation in 20.58% of bees. Likewise, Hempel and Hempel (1988); Shykoff and Hempel (1991) also recorded 20.20–21.70% conopid parasitism in *B. terrestris*. Hempel and Durrer (1991) reported frequency of conopid infestation in worker bees which ranged between 19.70 and 62.50% depending on environmental variations.

Conopid flies, *P. rufipes* and *P. scutellata* attack foraging carpenter bees (males and females) which affects the colony strength. When females become infested and die, it becomes a threat to the developing brood due to risk of colony collapse in the early seasons of development (April-September). These flies have short larval period which kill the foragers in 9–12 days of infestation. Incidences showed higher infestation during June–August. It is concluded from the experimental results that conopid flies of the genus *Physocephala* hampers the development of *Xylocopa* rearing. This study may help in establishment of conopid as an important pest in Xylocopiculture which is a new field of pollinator development for integrated agriculture.

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Disclosure of potential conflicts of interest

No potential conflict of interest was reported by the author(s).

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