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Studies on various factors affecting female sex pheromone release in *Xanthopimpla predator* - A pupalparasitoid of tasar silkworm

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Abstract

Xanthopimpla pedator is a serious pupal parasitoid on tasar cocoons and the infestation has become an increasing problem to sericulture industry. Insecticides are costly, build up large amount of chemicals in the environment and finally disrupt the balance of the ecosystem. Extraction of female sex pheromones and application in mating disruption is an alternative approach to insecticide treatment. The virgin female *Xanthopimpla pedator* pheromone gland extract activity and factors influencing sex pheromone production were studied under laboratory conditions. Present wind tunnel experiment show that most of the females started calling from thestarting of photo phase (13ng/h). Maximum calling (825ng/h) occurred between the second and fourth hours of the photo phase period (10am to 12 pm).Volatiles were collected in U tube immersed in liquid nitrogen. Gas chromatographic–electroantennogram detection (GC–EAD) analysis of these female extracts indicated the presence of five peaks or five volatile compounds (A,B,C,D,E) to which the male antenna responded. Active response was noted with B volatile. Pheromone production started in one day old female *Xanthopimpla* (0.6µg/day), reached maximum on 7th day (9.6µg/day) and decreased later as the female *Xanthopimpla* increased in age. The density of female *Xanthopimpla* has no impact on total pheromone production. *Xanthopimpla* held in one, two and five in number had released same quantity of pheromones. *Xanthopimpla* fed with white sugar syrup has released maximum pheromones(12.5µg/ female/day)followed by honey fed(10.2µg/ female/day) and normal water fed(3.6µg/ female/day).An efficient female pheromone isolation is of importance in mating disruption which helps to develop new control methods for prevention of the infestation in tasar cocoons.

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Introduction

Rearing of tasar silkworm, *Anthereae mylittadrury* on forest grown plantation like *Terminalia arjuna, Terminalia tomentosa and Shorea robusta* results in 80-90% crop loss due to parasites, predators and vagaries of nature (Mathur and Shukla,1998). Ichneumon fly, Canthecona bug, reduvid bug, *Hicrodulla bipapilla* (Praying mantis) etc., are natural enemies in the rearing field which cause maximum crop loss (Singh *et al*., 1992).*Ichneumons* like *Xanthopimpla (Hymenoptera*), *Blepharipa (Diptera*) are important endoparasitoids of insect hosts mainly larvae and pupae of order *Lepidoptera* (Singh *et al*., 2010). It was also recorded that *Xanthopimpla pedators* have sexual preference for male cocoons in parasitism (Lakshmi and Bhagavanulu, 2012).Because of the concealed feeding behaviour of *pedator*, application of insecticides for its management is restricted. Furthermore, quite often the indiscriminate and unscientific use of pesticides has led to many problems, such as pests developing resistance, resurgence of once minor pest into a major problem besides environmental and food safety hazard(Cork*et al*., 2005). Hence, eco-friendly pest management would be the best strategy for managing this key *predator* of Tasar cocoons.

Sex pheromones can provide a means of monitoring and controlling insects which is non-toxic to animals and plants and specific for the target crop pest (Salma *et al*., 2011; Srivastavaand Dhaliwal, 2012). Female sex pheromones can be involved in mate location from a distance when mating takes place after dispersal, as it does in most parasitoidspecies(Robert Holdcraft *et al*., 2016).In numerous species females attract males by emitting volatile sex pheromones that are detectable from a distance at specific times (Fauvergue *et al*., 2007;Foster and Anderson, 2011).This is occasionally associated with calling behaviour such as wing fanning while the ovipositor is exposed to the atmosphere (Jurenka, 2003). Mate finding in parasitoids is mainly based on pheromones released by females, although on some occasions, the roles are reversed, with the males also releasing sex pheromones (Ruther, 2003).

Numerous studies proved that pheromone titre is known to vary temporally, with age, time of photoperiod, density and feed (Allison and Carde, 2006; Symonds and Elgar, 2008). Pheromone quantity varies with age and reaching maximum levels with sexual maturity and later declines(Foster and Johnson, 2010).Several insects shift between carbohydrates and fats according to starvation levels for general metabolism and pheromone biosynthesis (Blomquist *et al.,* 2011). The requirements of sugar sources for sustained reproduction were observed in several lepidopterans (Tisdale and Sappington, 2001). In *Xanthopimpla pedator* female sex releases volatiles to attract the males (Lakshmi and Benarjee, 2017). There is an urgent need for a sensitive means of monitoring and control of *Xanthopimpla* infestation using mating disruption. It is necessary to maximize the collection of the pheromone to perform the chemical analysis and to improve attractiveness of synthetic lures used to manage *Xanthopimpla pedator* infestation. The objective of the present study was to investigate several factors which could affect pheromone production in female *Xanthopimpla pedator*.

Materials and methods

Pheromone collection is an effort to optimize pheromone production for identification purposes and to investigate potential effects of several environmental factors. The factors studied include effect of female age, effect of time of day, effect of density and effect of diet.

Rearing o fxanthopimpla pedator

Xanthopimpla pedators were collected from the *Terminalia arjuna* field immediately after emerging from the infested cocoons. They were reared in the laboratory under a photoperiod of 12 h L: 12 h D, at 28 ± 2°C temperature and 75–80% humidity. Adult *Xanthopimpla* were kept in mica boxes and fed with sugar syrup.

Pheromone collection

24 – 48 hr old female and male *Xanthopimpla* were released in the wind tunnel. Males started flying towards wing fanning females. Both male and female

Xanthopimpla were removed from the wind tunnel. Female *Xanthopimpla* were transferred to aeration chamber (10cm X 10 cm) containing mesh stands. Air was passed in to the aeration chamber constantly at a flow rate of 200ml/min. Volatiles released from the fanning females were collected in U tube immersed in liquid nitrogen. The condensed volatiles collected in the glass wool filled in tube was washed with 2ml pentane and stored in glass vials at 4°C for further analysis. The above process was repeated resulting in ten samples for gas chromatographic analysis.

Gas chromatography-electroantennogram detection (GC-EAD)

Gland extracts (solvent hexane) of female *Xanthopimpla pedator* was analyzed in a gas chromatograph (GC, Hewlett-Packard 5890 Series II) equipped with flame ionization detector (FID) set at 320 °C with Helium as carrier gas. The temperature conditions were 2 min at 80 °C, increased at a rate of 5 °C/min to 280 °C, and held at this temperature for 18 min. The column used was fused-silica capillary column (50-m length, 0.32ram, 0.25pm film thickness, Chrompack, flow velocity 26cm/s). The injector and detector temperatures were set at 190 °C and 275 °C and operated in split less mode throughout the analyses.

The carrier gas was Helium (purity 99.96%) with a flow rate of 1.5 mL/min and at a constant pressure of 100 KPa. Approximately 2 µL of each extract was injected into the GC for analysis. Ten antennae of unmated male *Xanthopimpla* responded to wing fanning in the wind tunnel were excised, held between two glass electrodes filled with a 10% KCl solution and their responses to the volatiles produced by the females were investigated using a coupled GC-EAD system, in which the effluent from the GC capillary column was delivered simultaneously to the antennal preparation and the GC detector (Wadhams, 1990). Computer was used to record the responses from both FID and EAD. Signals from the antenna were passed through an amplifier and data storage and processing were carried out with a PC-based interface.

Effect of age

Volatiles were collected daily from female *Xanthopimpla* from age 1 to 11 days old. Volatiles were collected from three replications of *Xanthopimpla*.

Effect of time of day

The volatile collections were done six times per day. The lights came on at 8 am. The first collection was made between 8 am–10 am, second collection between 10 am–12noon, third collection between12noon–2 pm, fourth collection between 2 pm–4 pm and fifth collection between 5 pm–7pm. All these collections were done in presence of light. Sixth collection was made after the lights were off between 8pm-10pm. Volatiles were collected from 4 individual *Xanthopimpla* starting from 3days old and ending at 10 days old.

Effect of female density

The three densities of *Xanthopimpla pedator* tested were 1, 4 and 6 per volatile collector. Volatiles were collected from female *Xanthopimpla* for 5 consecutive days beginning at 3 days old and ending at 7 days old.

Effect of diet

The effect of diet on pheromone production was investigated. Food quality and quantity has strong effect on longevity and productivity of parasitoids. *Xanthopimpla* fed with water, honey and white sugar syrup. *Xanthopimlpla* preferred white sugar syrup as food source. Volatiles were collected from female *Xanthopimpla* over 6 days beginning at first day old and ending at 10 days old.

Statistical analysis

Each experiment was repeated three times and the results mentioned are an average of three replications. Values were expressed as mean ± SE of replication and Student's *t*-test was applied to locate significant differences ($P < 0.05$). The data collected was analysed by SPSS-19 Statistical Software.

Results

Wind tunnel experiment and *Gas chromatography-electroantennogram detection*

Female *Xanthopimpla* settled on height in wind tunnel and started fanning the wings. It also rotated the abdomen. The time taken for the male to reach the fanning female was 2min 15 sec. Wing fanning ceased upon arrival of a male *Xanthopimpla pedator*. Females would only wing-fan at 28 ± 2°C temperature and 75–80% humidity during the hours between 8am and 8pm. Wing-fanning was observed only when the air flow was 200ml/min. Wing fanning was continued in the absence of male *Xanthopimpla* also. GC –EAD result show that five volatile compounds are released (A,B,C,D,E) to which the male antenna responded by eliciting a response to the female volatile compounds emitted(Fig. 1). Active response was noted with B volatile.

Table 1. Effect of time on pheromone production in *Xanthopimpla pedator*.

S.No	Time of Day	Pheromone quantity collected (ng/hr)
1	8am-10am	610±25
2	10am-12 noon	825±52
3	12noon-2pm	550±38
4	2pm-4pm	425±65
5	5pm-7pm	312±42
6	8pm-10pm	13±2

Effect of age

The effects of age on total pheromone production are shown in Fig. 2. Pheromone production started in one day old female *Xanthopimpla* but was very low (0.6µg/day) and then increased steadily till 7th day with a maximum production of 9.6µg/day. Afterward, pheromone production decreased as the female *Xanthopimpla* increased in age.

Effect of time of day

Table 1 shows the effect of time of day on pheromone production. During the scotophase (between8pm- 10 pm) female *Xanthopimpla* produced very little pheromone (i.e., ca. 13ng/h). But during photophase between 8am-8pm pheromone productions increased dramatically. The period between 10am and 12 noon gave the highest pheromone production rate of ca. 825ng/h. After 2 pm, pheromone production gradually decreased to 312ng/h.

Table 2. Effect of density on pheromone production in *Xanthopimpla pedator*.

S. No	Age	Single	Four	Six
1	Third	2.4±0.6	2.5±0.5	2.4±0.5
2	Fourth	4.6±0.8	4.5±0.6	4.5±0.8
3	Fifth	5.7±0.6	5.8±0.6	5.6±0.6
4	Sixth	6.9±0.8	6.9±0.8	6.8±0.6
5	Seventh	7.8±0.4	7.6±0.5	7.6±0.5

Effect of female density

The female *Xanthopimpla* density has no impact on total pheromone production (Table 2). The individually held female *Xanthopimpla pedator* showed an increase in pheromone production with the age till 7th day. When five female *Xanthopimpla* were held together, the total pheromone release rate on per female was similar with that of individually held together. The total pheromone release rate on per female basis for the two *Xanthopimpla* held together was overall similar to that seen for the individual female.

Effect of diet

The effect of diet on pheromone production by female *Xanthopimpla* is shown in Table 3. Female *Xanthopimpla* started on white sugar syrup increased pheromone production with age and were producing ca. 12.5µg/ female for day .

When they were switched to normal water their pheromone production immediately decreased to 3.6µg/female for day. On the other hand, the female *Xanthopimpla* on honey for continuous four days produced more pheromone (10.2µg/ female for day) than water fed *Xanthopimpla* of identically aged female's.

Once these females fed on water and honey were switched to white sugar syrup, they began producing pheromone immediately and after 2 days, they were producing over 10µg/female for day.

Table 3. Effect of diet on pheromone production in *Xanthopipla pedator*.

S.No	Age (Day)	Water (µg/female/day)	Honey (µg/female/day)	White sugar syrup (µg/female/day)
1	First	0.6±0.04	1.8±0.4	2.5±0.4
2	Second	1.4±0.4	2.5±0.5	3.4±0.6
3	Third	2.4±0.6	3.5±0.8	4.6±0.8
4	Fourth	4.5±0.8	5.6±0.5	10.8±0.4
5	Fifth	5.7±0.6	6.8±0.6	12.0±0.5
6	Sixth	7.8±0.8	8.6±0.4	12.2±0.8
7	Seventh	9.6±0.4	10.2±0.8	12.5±0.4
8	Eighth	8.4±0.5	9.4±0.5	12.2±0.6
9	Ninth	7.2±0.6	8.2±0.6	12.1±0.8
10	Tenth	5.8±0.6	7.8±0.8	11.2±0.5

Discussion

Wind tunnel experiment and *Gas chromatography-electroantennogram detection* Present GC-EAD results suggested that the volatile compounds released by female *Xanthopimpla* when wing-fanning, may be a sex pheromone used to attract males prior to copulation. Confirmation of whether the compound is used as a sex pheromone can be done only after further analysis using gas chromatography-mass spectral analysis (GC-MS).

Effect of age

The highest level of pheromone production by females was reached when the female was 7days old and then decreased when the females became old. This appears to help the insect to continue mating and producing fertile eggs during its whole life span. The decrease in sex pheromone production during the last few days of female life is probably attributed to old age which is usually accompanied with much physiological disturbance and decrease or inhibition of many biological processes (Sallam *et al.*, 2000). Rafaeli *et al.* (2003)observed that maximum levels of pheromone production in the American bollworm, *Heliothis armigera* (Hb.), were extracted from middle aged females.

The amount of pheromone produced by the weevils increased each day until weevils were about 9 days old .This is because pheromone production were associated with well-developed accessory glands(Spurgeon,2003).

Effect of time of day

Female *Xanthopimpla* had the highest amount of pheromone production during the early hours of photophase till noon and recorded very low before the lights were off. Highest pheromonotropic activity is observed during photophase in *Helicoverpa armigera* females (Ada and Carina,1995).

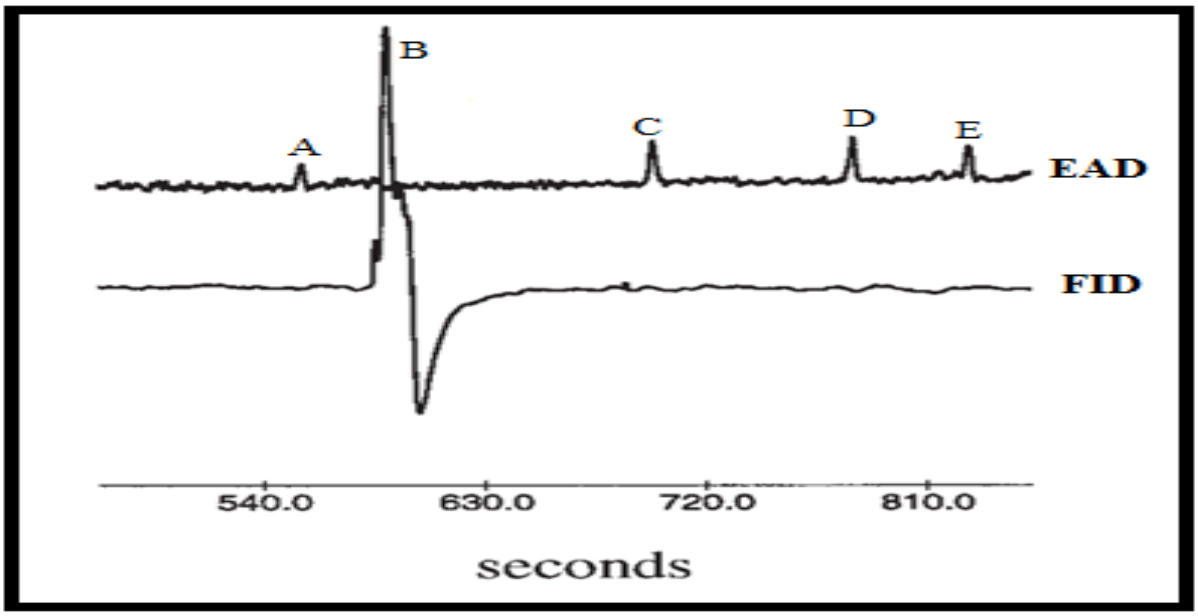

Fig. 1. Coupled Gas chromatograph and electroantennogram (GC-EAD) of male *Xanthopimpla pedator* antenna responding to female *Xanthopimpla* pheromone gland extracts.

In *S. acupuncatus* maximum pheromone was released during the photophase (Ceasar *et al.,* 2009). Increase in dilute acetate pool from B-oxidation fatty acid pathway increases the pheromone precursor levels during this phase(Foster and Anderson, 2012).

Effect of female density

Female *Xanthopimpla pedator* held either in single or in groups had released same quantity of pheromone showing that density will not impact the pheromone levels.

In case of wood lice density did not show any impact on pheromone production in turn did not show any influence on aggregation pattern(Pierre Broly *et al.*, 2012). Social interactions in woodlice will not is not influence aggregation pheromones (Devigne *et al.,* 2011).

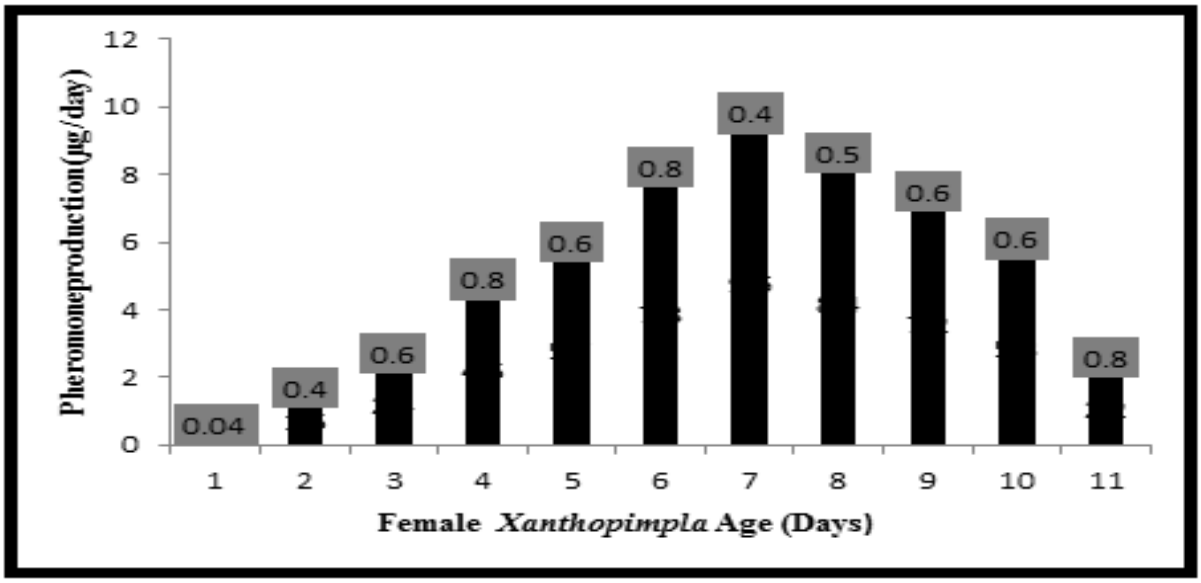

Fig. 2. Effect of age on pheromone production in Female *Xanthopimpla pedator*. Black bars denotes mean values and grey bars denotes standard deviation.

Effect of diet

The pheromone production in *Xanthopimpla* is almost doubled with sugar syrup feeding in comparison to normal water feed. Sugar feeding increases the pheromone production in *Heliothis* moths than water fed(Stephen Foster, 2009). The increase in pheromone production in sugar-fed, relative to water-fed or starved females is seen in *Helicoverpa* sp. (Casimero *et al.,* 2001). Food is an important factor as weevils deprived of plant material did not release any pheromone (Ceasar *et al*., 2009). In *Epiphyus postvittana* the age related decline in pheromone production is due to decline in fatty acids production, possibly due to shortage of pheromone precursor, and to a decline in fatty acid reductase activity(Foster and Johnson,2010;Harari*et al*.,2011). The large amounts of pheromone produced by *H. virescens* females after feeding on sugar reports an increase in pheromone precursor fluxes(Stephen Foster,2009).Reproductive process generally influences by both larval diet and adult sugar feeding (Kassim *et al*., 2012). Feeding in larvae provides most of the nutrients for the development and reproductive activities of holometabolousinsects (Brien *et al*., 2004). Feeding is important for increasing female fitness (StephenFoster,2009).Carbohydrate feeding especially sugar and honey feeding increases the reproductive potential in *Phthorimaea operculella* (Alexandre Jordao *et al*., 2010). In *Xanthopimpla* sugar syrup feeding increased the pheromone production followed by honey and water feed. Sugar feeding in *Heliothis* increases the trehalose and fatty acids concentration in haemolymph which rapidly glycolysed and converted to acetyl Co-A for biosynthesis of pheromone titres (Walter Eanes, 2006; Tsfadia *et al*., 2008).

Conclusion

Finally, it could be concluded that adult females of *Xanthopimpla pedator* emit maximum quantity of sex pheromone during photophase of seventh day. The density of female *Xanthopimpla* has no impact on total pheromone production. *Xanthopimpla* fed with white sugar syrup has released maximum pheromones followed by honey fed and normal water.

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