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Identification and Behavioral Evaluation of Sex Pheromone in *Xanthopimpla pedator* (Fabricius)—A Serious Pupal Parasitoid of Tropical Tasar Silkworm *Anthereae mylitta* Drury

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ABSTRACT

Xanthopimpla is a major parasitoid of silk worm cocoons. The female *Xanthopimpla pedator* (Fabricius) lays the eggs in male cocoons. Control of this infestation with pesticides is not recommended because of its concealed behavior. Various control methods were found to be inefficient. Ecofriendly management is the best strategy that can be applied. We have studied the sex communication in *Xanthopimpla pedator* (Fabricius), which helps to develop management strategy. Bioassays were done in the laboratory by using olfactometer and pheromone extraction chambers. It was found that female *Xanthopimpla* produces sex pheromones. The results show a strong attraction of male by female *Xanthopimpla*. Present results with male and female volatiles also show that female volatiles attract male *Xanthopimpla*. Fractionation of female volatiles by column chromatography has proven that 20% fraction has highest attraction of males by females.

1. Introduction

The tasar silk is produced by *Anthereae mylitta* Drury (*Lepidoptera: Saturnidae*), a wild polyphagous tropical sericigenous insect distributed over central India. The species has wide distribution over diverse ecological niche as high as 44 ecoraces, but only a few are semidomesticated and applied commercially for seed (egg) and silk production (Suryanarayana and Srivastava 2005). Rearing of tasar silkworm, *Anthereae myliia* Drury, on forest grown plantation like *Terminalia arjuna*, *Terminalia tomentosa*, *and Shorea robusta* results in 80–90% crop loss because of parasites, pedators, and vagaries of nature (Mathur and Shukla 1998). It has been estimated that in hibernating stock about 20–30% loss of seed cocoons was because of pupal mortality and unseasonal emergence, which in turn reduces the multiplication rate of tasar cocoons. *Ichneumon fly*, *Canthecona bug*, *reduviid bug*, *Hicrodulla bipapilla* (*praying mantis*), and others are natural enemies in the rearing field, which cause maximum crop loss (Singh *et al.* 1992). *Ichneumons* like *Xanthopimpla* (*Hymenoptera*) and *Blepharipa* (*Diptera*) are important endoparasitoids of insect hosts, mainly larvae and pupae of order *Lepidoptera* (Singh *et al.* 2010). The cumulative effect of these pathogens results in 30–40% of Tasar crop loss. A pupal parasitoid, *Xanthopimpla stemmator*, was recorded from Maharashtra and Andhra Pradesh (Duale and Nwanze 1999). It was also recorded that *Xanthopimpla pedator* has sexual preference for male cocoons in parasitism (Lakshmi and Bhagavanulu 2012).

Xanthopimpla is one of the largest genera of Ichneumonidae, and the species of the genus are endoparasitoids of the Lepidopterans (Gomez *et al.* 2009), which show greater degree of biological adaptations (Gauld and Bolton 1988). The Pimplinae subfamily has become taxonomically one of the best-known ichneumonid taxa (Gauld 1991). Many *Xanthopimpla* species are abundant in tropical areas and are lemon yellow in coloration and has very stout bodies. *X pedator* begins its life cycle in 5th instar spinning larva of *Anthereae mylitta* by the time of hammock formation to early cocooning stage and uses its long ovipositor to drill through silky envelope of spinning larva and deposits single egg in the abdominal segment (Aruna *et al.* 2014). In the course of development, silkworm transforms into pupa, and after hatching, ichneumon larva feeds the entire content of pupa. Parasitoid's larva pupates there and transforms into adult and pierces its way out by making a circular characteristic hole at the anterior region of the cocoon near peduncle (Singh *et al.* 2010). Pimpla instigator detects host-mediated vibrational echoes for locating their hosts in microhabitats (Henaut and Guerdoux 1982).

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Wackers *et al.* (1998) also established that ichneumon wasps transmit their self-produced vibrations via the antennae onto the substrate covering the host and analyze the reflected signals to detect the position of host for accurate oviposition.

Because of the concealed feeding behavior of pedator, application of insecticides for its management is restricted. However, applied insecticides also kill nontarget arthropods, typically insects involved in pollination and pedators. Insecticide residues find their way into water courses and affect the water we drink and food we eat. Furthermore, quite often the indiscriminate and unscientific use of pesticides has led to many problems, such as pests developing resistance and resurgence of once minor pest into a major problem besides environmental and food safety hazard (Cork *et al.* 2005). Hence, ecofriendly pest management would be the best strategy for managing this key pedator of tasar cocoons. Pheromones can provide a means of monitoring and controlling insects that are nontoxic to animals and plants and specific for the target pest (Cork and Hall 1998).

In parasitoid insects, most of the studies on mate finding have focused on the role of sex pheromone. Sex pheromones in like manner are widely and successfully used against several crop pests, particularly forests (Srivastava and 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 parasitoid species (Hardy 1994). In parasitic wasps, learning of odors in association with oviposition in their victims has also been shown to be highly important (Vinson 1984). In numerous species, females attract males by emitting volatile sex pheromones that are detectable from a distance at specific times (Fauvergue *et al.* 2007). This is occasionally associated with calling behavior 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 (Quike 1997; Ruther 2013). After mating, females switch from releasing pheromones or searching for males to search for hosts (Jang 1995; Kugimiya *et al.* 2010).

There is an urgent need for a sensitive means of monitoring and control of *Xanthopimpla* infestation using mating disruption. The existing literature on pheromone communication in this pedator revealed that no published work is available on this aspect. To provide this needed tool, the present research was carried out to know the sexual communication in this species so as to develop a management strategy (Table 1).

2. Materials and Methods

2.1. Collection and rearing of *X pedator* (Fabricius)

X pedators were collected from the *Terminalia arjuna* field immediately after emerging from the infested cocoons (Figure 1).

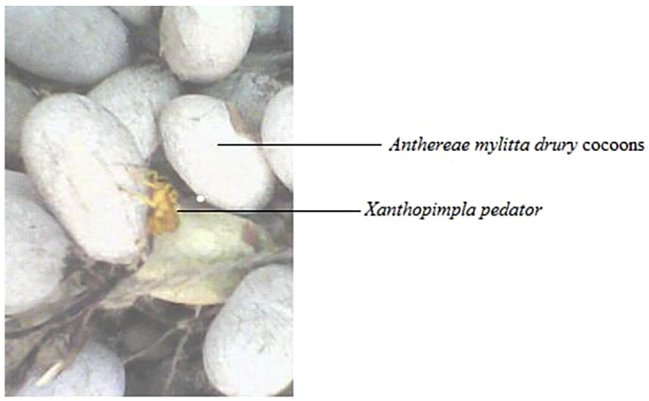

Figure 1. *Xanthopimpla pedator* attacking *Anthereae mylitta* Drury cocoons.

They were reared in the laboratory under a photoperiod of 12 hours light/12 hours dark cycle, at 28 ± 2°C temperature, and 75–80% humidity.

2.2. Behavioral assay

2.2.1. Olfactometer

Olfactometer is used to identify the sex having pheromonal attraction. Behavioral assay was carried by the olfactometer according to the method of Willem *et al.* (1999) (Figure 2). It consists of a central tube (13.5 cm long and 24 mm diameter) and two lateral arms (5.75 cm long and 24 mm diameter) (14.5 cm long and 19 mm diameter) that are fitted to broad tubes serving as a test chamber (B) in which the materials to be tested and compared were kept. The middle portion of the y-tube is fitted with a broad conical chamber called as release chamber (C), where the insects to be tested for responsiveness were released. There is a sieve inlayed in the extending glass tube 5.25 cm away from the connection to prevent escape of insects. Humidified and purified air was passed from an end of y-tube (A) at a rate of 200 mL/min. Airflow was regulated by a valve situated in the release chamber. Using the olfactometer, selecting the arm containing either the live adults or their volatile extract is possible. The entire olfactometer was washed thoroughly using soap solution and oven dried before each experiment.

2.2.2. Volatile-collecting apparatus

Pheromone collection was done in the dark room during early hours of scotophase or calling behavior according to Golub and Weatherson (1984). After confirmation of the sex that releases pheromone, it is confined to volatile-collecting apparatus for collection of volatiles (Figure 3). It consists of an air-loading chamber (B) of size (46 and 32 cm) through which air was blown

Table 1. Behavioral assay of live males, females, and volatiles

Experiment	Adults in release chamber			Percentage attracted	Test chamber live sex/volatile	Combination	χ^2	Notes
	Sex	Number	Number responded					
1	Female	30	01	3.3	40 M	M vs. F	22.0 NS	Females are not attracted by males
2	Male	30	30	100	40 F	F vs. M	0.0*	Males are attracted by females
3	Female	25	—	—	FV	FV vs. F	22.0 NS	Females are not attracted by female volatiles
4	Male	25	25	100	FV	FV vs. M	0.0*	Males are attracted by female volatiles
5	Female	25	—	—	MV	MV vs. F	22.0 NS	Females are not attracted by male volatiles
6	Female	25	—	—	FV, MV	FV vs. F	22.0 NS	Females are not attracted by females
						MV vs. F	22.0 NS	Females are not attracted by male volatiles
7	Male	25	25	100	FV, MV	FV vs. M MV vs. F	0.0*	Males are attracted by female volatiles

M = male; F = female; NS = nonsignificant; FV = female volatile; MV = male volatile.
* Significant.

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