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EVALUATION OF FIELD AND LABORATORY RESPONSES OF MALE XANTHOPIMPLA PREDATOR TO SYNTHETIC PHEROMONE LURES

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Keywords:

Wind tunnel, Synthetic pheromone components, Infestation rate, Mating disruption, Xanthopimpla predator

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ABSTRACT: Mating disruption by an artificial release of synthetic female pheromone components like Tetradecyl acetate (A), Ethyl (Z)-9-hexadecenoate (B) and a mixture of these two at different concentrations is a feasible method to control Xanthopimpla predator. Male Xanthopimpla predator was evaluated for responses to these synthetic pheromone components in a laboratory wind tunnel and in *Terminalia arjuna* field. Septa loaded with different concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 mg) of Tetradecyl acetate (A), Ethyl (Z)-9-hexadecenoate (B), A and B blend in 1:1 ratio (C) has disrupted the male Xanthopimpla predator orientation in the wind tunnel and blend with 1.0 mg had shown more attraction towards Xanthopimpla predator. Mating disruption efficacy in tasar field was evaluated in first, second and third crops with 16 traps in each crop. In all crops, 5 traps baited with pheromone loads of 0.2, 0.4, 0.6, 0.8 and 1.0 mg of Tetradecyl acetate, 5 traps with Ethyl (Z)-9-hexadecenoate and 5traps with a blend of both in 1:1 ratio. One blank control trap without any type of solvent is used in all three crops. Traps baited with a blend of 1.0 mg concentration caught more males than Tetradecyl acetate (A) and Ethyl (Z)-9-hexadecenoate (B). The infestation rate is least in the cocoons reared on Terminalia plantation with traps baited with A and B blend (1.0 mg) followed by Ethyl (Z)-9-hexadecenoate (B) traps, Tetradecyl acetate (A) traps and control traps.

INTRODUCTION: Tropical tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera: *Saturniidae*) is a polyphagous silk-producing insect of commercial importance, widely distributed in moist deciduous, semi-evergreen, dry deciduous and tropical dry deciduous forests in India [1]. The insect feeds primarily on, *Terminalia tomentosa*, *Shorea robusta* and *Terminalia arjuna* in addition to secondary and tertiary food plants [2].

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The species has a wide distribution over diverse ecological niche as forty-four ecoraces but only a few are semi-domesticated and applied commercially for seed (egg) and silk production [3].

The physiological potential of life performance of the insect is always challenged by the abundance of food and its quality, various abiotic factors, presence of predators, parasites and diseases which affect the cocoon yield. In outdoor forest rearing, pest and predators attack the larvae and pupae of *A. mylitta* causing 40-45% loss [4]. It has been estimated that in hibernating stock about 20-30% loss of seed cocoons due to pupal mortality and unseasonal emergence which in turn reduces the multiplication rate of tasar cocoons.

Ichneumons are important endoparasitoids of insect hosts mainly larvae and pupae of Lepidoptera. Among that, Xanthopimpla (*Hymenoptera*), Blepharipa (*Diptera*) are pupal and larval parasites of silkworm [5]. The pupal parasitoid, Xanthopimpla stemmator, was recorded from Maharashtra and Andhra Pradesh [6]. Ichneumonidae was also the dominant pupal parasitoid of the painted apple moth [7]. Xanthopimpla predator is a serious pupal parasitoid on tasar cocoons and the infestation has become an increasing problem to sericulture industry [8]. Extensive parasitism by Xanthopimpla predator results in pupal mortality and broken cocoons which declines the wild silk production.

Agricultural insect pest management is largely dependent on the use of chemical insecticides. However, the problems associated with insecticide usage are environmental pollution, development of resistance in the pests, *etc.* Pheromone trapping can provide tools for monitoring the activity of target insects, providing information that will assist in insect pest management. Pheromones are efficient at low pest population densities, with no adverse effect on natural enemies, and their use leads to a long-term reduction in insect pest populations that cannot be accomplished with conventional insecticides [9]. Traps baited with pheromone lures will be helpful in early detection of pest, mating disruption and timing of management procedures based on threshold [10]. Mass trapping of cocoa mirids has been efficiently done by pheromone trapping [11].

Synthetic pheromone is used to permeate the atmosphere so that an insect will be unsuccessful in finding a mate [12]. The pheromone Ethyl (Z)-9-hexadecenoate of female Syndipnus, a sawfly parasitoid of class Ichneumonidae and Pteromalus cerealellae have been identified and are used for management of these pests by attracting the male Syndipnus [13, 14]. The acetates of pentadecanol are used for mating disruption in pine sawfly *Neodiprion sertifer* has reduced the male saw fly by trapping [15]. Mating disruption by an artificial release of the sex pheromone components like Tetradecyl acetate and Ethyl (Z)-9-hexadecenoate may be a feasible method to control Xanthopimpla predator. The present study focuses on the identification of suitable synthetic pheromones of different concentrations for mating disruption of Xanthopimpla predator by laboratory and field studies, to reduce infestation and to increase the silk yield.

MATERIALS AND METHODS:

Rearing of Xanthopimpla Predator: Xanthopimpla predator was 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.

Wind-tunnel Bio-assays: A wind tunnel of 1 m wide × 1 m high × 3 m long was used [16]. Two-speed fans (D8025B12M, Elan Vital, Taiwan) one at the tunnel input and the other on the exhaust with a wind speed of 40-50 cm/s were used and speed was verified with an anemometer (Mastech MS 6250). Experiments were conducted at 24-26 °C with a relative humidity of 50-60%. 25-W lights, on each side of the room including the room ceiling and tunnel wall, were set with a rheostat to provide a diffuse light of 8–12 lux in the wind tunnel. THIEYE 160+4K Ultra HD camera (2 Inch screen, 170-degree wide lens, 12 MP photos, shutter speed 1/100 sec) mounted in the wind tunnel towards the downwind end to record the male Xanthopimpla behavior to the source [17].

All experiments were conducted during the last 3 h of scotophase, the period when female Xanthopimpla call and mate. Rubber septa loaded with synthetic pheromones placed inside a hair roller which consists of a tubular plastic frame covered with plastic mesh (6 cm length, 1.5 diameters) that was closed on each end with a Styrofoam lid. Septa were loaded with different concentrations of Tetradecyl acetate (A), Ethyl (Z)-9-hexadecenoate (B), A and B blend in 1:1 ratio (C) and control septa. Test males were brought into the assay room and transferred into small wire mesh (4 cm diam × 6 cm high) cage, with one end open and the other closed. Male Xanthopimpla of 24-48 h old were released in the wind tunnel. Males were acclimated to the conditions of the assay room for 1-4 h before being tested. The closed-end of the cage was placed facing upwind, on a mesh stand placed 20 cm above the tunnel floor and 160 cm downwind of the pheromone source.

Once the male Xanthopimpla began wing fanning, the cage was rotated 180°, thereby allowing male Xanthopimpla to initiate an upwind flight. Males started flying towards the source. Test males were used once are discarded.

Rearing of Anthereae Mylitta Drury: The cocoons harvested from first crop, second crop, and the third crop was subjected for selection for second, third crop and first crops. First, second and third crops cocoons were preserved in the rooms under a temperature of 29 ± 1 ºC and humidity 70 ± 1%. The emerged moths were tested for pebrine disease by a method derived from that used in sericulture [18]. The eggs laid by healthy moths were collected and incubated for hatching. Hatched larvae were reared on Terminalia plantation in all three crops. The effective rate or rearing in the first, second and third crop was recorded as follows:

ERR% = (Total number of cocoons produced / Total number of larvae brushed) × 100

Field Tests: Tests were conducted in Terminalia arjuna plantation (Host plant of *Anthereae mylitta* drury) at Chenoor, Adilabad District, Telangana during the year 2018 in all the three crops. Terminalia field of 2700 m^2 (45 m × 60 m) where host plants were separated by 2 feet × 2 feet in rows and columns. Each plant with five traps containing different concentrations of each synthetic pheromone and one control trap were suspended in tree crowns at 20 m apart. The trapped Xanthopimpla males were counted every week and traps were rotated two positions after each census. Three field tests were conducted comparing the attractiveness of Tetradecyl acetate (A), Ethyl (Z)-9-hexadecenoate (B) and a blend of both (C) in 1:1 ratio in different concentrations like 0.2, 04, 0.6, 0.8 and 1 mg.

Dose-Response: The lures and dispensers used infield were prepared and supplied by Agri phero solution z, (Hyderabad, Telangana, India). Lures were collected from the same batch and stored at 4 °C until use. Commercial sex pheromone lures composed of these major components attract the male Xanthopimpla. The male response to synthetic pheromone was evaluated in first, second and third crops. In the first crop traps (n = 16) were employed in Terminalia field from June 3 to July 30, 2018, in the second crop traps (n = 16) were employed from August 8 to September 30, 2018 and in the third crop traps (n = 16) were employed from October 6 to December 20, 2018. In all the trials 5 traps baited with the pheromone loads of 0.2, 0.4, 0.6 ,0.8 and 1.0 mg of Tetradecyl acetate, 5 traps with Ethyl (Z)-9-hexadecenoate and 5 traps with a blend of both in 1:1 ratio. One blank control trap without any type of solvent is used in all the three crops. Lures attracted more males.

Statistical Analysis: Captures are displayed as mean values of males/week/trap. The effect of mating disruption was evaluated as mean number of males trapped per week for each period.

RESULTS AND DISCUSSION: Fig. 1 shows that male Xanthopimpla predator behaviour towards various synthetic lures in wind tunnel was observed. Most of the males (95%) that took for flight oriented towards the lures and only 5% of male Xanthopimpla directed towards the control. Out of 95% males oriented towards lures 20% of male Xanthopimpla have took flight towards synthetic lures of Tetradecyl acetate (A) and 25% towards Ethyl (Z)-9-hexadecenoate (B). However a blend of A and B in 1:1 ratio (C) was used, male Xanthopimpla orientation towards that has increased significantly by 50%.

As different concentrations like 0.2, 0.4, 0.6, 0.8 and 1.0 mg of these synthetic lures were used, blend with 1.0 mg had shown more attraction towards Xanthopimpla predator. The use of this blend as a disruption chemical will solve the problem of Xanthopimpla infestation. Optimizing pheromone deployment for effective mating disruption is vital [19].

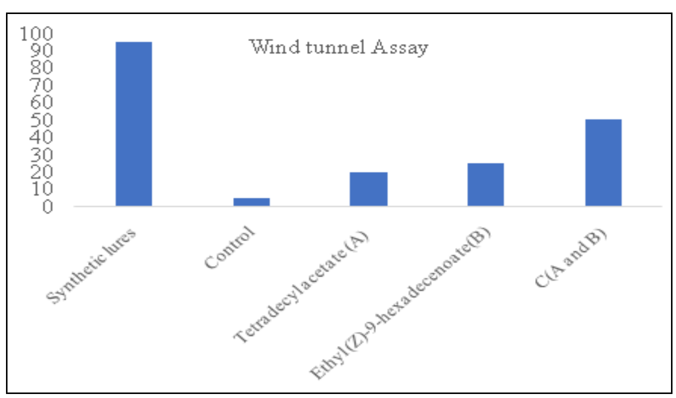

FIG. 1: GRAPH SHOWING BEHAVIOUR OF MALE XANTHOPIMPLA PEDATOR TOWARDS SYNTHETIC PHEROMONES (1.0 mg CONCENTRATION) AND CONTROL

Table 1 shows the field data of a number of male Xanthopimpla trapped in the first crop with different doses of synthetic pheromone lures and control. In first crop Tetradecyl acetate (A) has not shown any effect in male Xanthopimpla predator capture with 0.2 and 0.4 mg dosage whereas traps baited with 0.6, 0.8 and 1 mg Tetradecyl acetate (A) has increased the male trap. Traps baited with 0.4, 0.6, 0.8 and 1.0 mg of Ethyl (Z)-9-hexadecenoate (B) trapped male Xanthopimpla except for the trap with 0.2 mg. It is also observed that the male trapping rate of Xanthopimpla with Ethyl (Z)-9-hexadecenoate (B) was 75% more than with Tetradecyl acetate (A). A more pronounced increase was observed in males caught by traps baited with 0.2, 0.4, 0.6, 0.8 and 1 mg dose of A and B blend in 1:1 ratio (C). In comparison with Tetradecyl acetate (A) and Ethyl (Z)-9-hexadecenoate (B), traps baited with the blend caught 175% and 64% more male Xanthopimpla respectively. It is also observed that an increase in pheromone concentration increased the male trapping. Mating disruption in *Pandemis pyrusana*, observed that traps baited with high concentration (0.8%) of synthetic pheromones captured more males than low concentrations (0.16%) [20]. Pheromone trapping and aerial spraying increased the ease of controlling bagworm, Metisa plana walker (Lepidoptera: *Psychidae*) [21].

TABLE 1: FIELD DATA OF NUMBER OF MALE XANTHOPIMPLA PEDATOR TRAPPED WITH DIFFERENT SYNTHETIC LURES AND DIFFERENT CONCENTRATIONS IN FIRST CROP

Crop	Pheromone load (mg)	Number of male *Xanthopimpla* captured per trap for a week			
		Tetradecyl acetate (A)	Ethyl (Z)-9-hexa decanoate (B)	Blend of A and B in 1:1 ratio (C)	Control (D)
First	0.2	-	-	1	-
	0.4	--	1	3	-
	0.6	1	2	4	-
	0.8	2	4	8	-
	1.0	4	7	11	-

Present results in **Table 2** shows that in second crop Tetradecyl acetate (A) has not shown any effect in male Xanthopimpla predator capture with 0.2 and 0.4 mg dosage whereas traps baited with 0.6, 0.8 and 1 mg has shown male Xanthopimpla trap. Traps baited with Ethyl (Z)-9-hexadecenoate (B) trapped male Xanthopimpla at all concentrations. It is also observed that Ethyl (Z)-9-hexadecenoate (B) traps captured 75% more male Xanthopimpla predator than the traps baited with Tetradecyl acetate (A). A significant increase in male capture was observed in traps baited with A and B blends in 1:1 ratio (C). It is also observed that as the concentration increased, males trapped were also increased. In comparison with Tetradecyl acetate (A) and Ethyl (Z)-9-hexadecenoate (B) traps baited with the blend caught 150% and 87.5% more male Xanthopimpla respectively. An increase in concentration of synthetic sex pheromone concentration increases the trapping of white grub beetle Dasylepidaishigakiensis (Coleoptera: *Scarabaeidae*) in the laboratory and sugarcane fields [22].

TABLE 2: FIELD DATA OF NUMBER OF MALE XANTHOPIMPLA PEDATOR TRAPPED WITH DIFFERENT SYNTHETIC LURES AND DIFFERENT CONCENTRATIONS IN SECOND CROP

Crop	Pheromone load (mg)	Number of male *Xanthopimpla* captured per trap for week			
		Tetradecyl acetate (A)	Ethyl(Z)-9-hexa decanoate (B)	Blend of A and B in 1:1 ratio (C)	Control (D)
Second	0.2	-	1	1	-
	0.4	-	2	3	-
	0.6	1	2	4	-
	0.8	2	3	8	-
	1.0	6	8	15	-

Table 3 explains the effect of various synthetic pheromones in capturing the male Xanthopimpla predator in the third crop. In third crop Tetradecyl acetate (A) has not shown any effect in male Xanthopimpla predator capture with 0.2 and 0.4 mg dosage whereas traps baited with 0.6, 0.8 and 1 mg Tetradecyl acetate (A) has shown male Xanthopimpla trap. Acetate extracts of females of

parasitoid *Campoletis sonorensis* (Ichneumonidae) are active in eliciting the courtship behavior of males [23]. Similar results were observed in a study on courtship behavior in *Lysiphlebus testaceipes* males [24]. Traps baited with Ethyl (Z)-9-hexadecenoate (B) trapped male Xanthopimpla at all concentrations [25]. It is also observed that Ethyl (Z)-9-hexadecenoate (B) traps captured 71.5% more male Xanthopimpla predator than the traps baited with Tetradecyl acetate (A).

TABLE 3: FIELD DATA OF NUMBER OF MALE XANTHOPIMPLA PEDATOR TRAPPED WITH DIFFERENT SYNTHETIC LURES AND DIFFERENT CONCENTRATIONS IN THIRD CROP

Crop	Pheromone load (mg)	Number of male *Xanthopimpla* captured per trap for week			
		Tetradecyl acetate (A)	Ethyl (Z)-9-hexadecenoate (B)	Blend of A and B in 1:1 ratio (C)	Control (D)
Third	0.2	-	2	3	-
	0.4	-	2	3	-
	0.6	2	4	6	-
	0.8	2	7	11	-
	1.0	7	12	18	-

A significant increase in male capture was observed in traps baited with A and B blends in 1:1 ratio (C). It is also observed that as the concentration increased, males trapped were also increased. In comparison with Tetradecyl acetate (A) and Ethyl (Z)-9-hexadecenoate (B) traps baited with the blend caught 157% and 50% more male Xanthopimpla respectively. Present studies are in correlation with the studies of Trissolcus a hymenopteran insect [26]. The studies on this insect have shown that virgin male Trissolcus have shown a positive response to Tetradecyl acetate, Ethyl (Z)-9-hexadecenoate GC-EAD components on exposure to EAG machine [27]. Present results also show that the number of Xanthopimpla trapped was more in the third crop than first and second crops. This may be because of the prevalence of Xanthopimpla predator during this period. Ichneumon fly is a serious and prevalent fly in the fields of *Terminalia arjuna* during the months of November and December which is the third crop of *Anthereae mylitta* drury [28]. In association with different abiotic factors pests and predators also plays a major role in survivability of tasar silkworm in outdoor rearing fields [29].

TABLE 4: REARING PERFORMANCE OF *ANTHEREAE MYLITTA* DRURY DURING FIRST CROP

Crop	Treatment	Number of larvae Brushed	Larval Duration (days)	Number of cocoons produced	ERR% (Effective Rate of Rearing)	No.of Cocoons infested with *Xanthopimpla*
First	Tetradecyl acetate (A)	500	34	388	76.6	8
	Ethyl (Z)-9-hexadecenoate (B)	500	33	392	78.4	7
	Blend of A and B in 1:1 ratio (C)	500	34	458	91.6	4
	Control (D)	500	34	312	62.4	16

Table 4, 5, 6 explains the rearing performance of Anthereae mylitta drury. Among 500 larvae brushed on *Terminalia arjuna* plantation during first, second and third crops, with Tetradecyl acetate (A) traps, Ethyl (Z)-9-hexadecenoate(B) traps and traps baited with A and B blend in 1:1 ratio (C) of different concentrations like 0.2, 0.4, 0.6, 0.8 and 1.0 mg. In all the three crops larvae brushed on *Terminalia arjuna* with pheromone traps baited with A and B blend has shown more cocoon yield and ERR% compared to Tetradecyl acetate (A) traps, Ethyl (Z)-9-hexadecenoate(B) traps control traps. It is also observed that the infestation rate is least in the cocoons reared on Terminalia plantation with traps baited with A and B blend followed by Ethyl (Z)-9-hexadecenoate (B) traps, Tetradecyl acetate (A) traps and control traps. In the members of lepidoptera successful application of Tetradecyl acetate (A) and dodecenyl acetate has reduced the infestation and loss by 91% [30]. These results are the first demonstration of the efficacy of synthetic pheromone components for Xanthopimpla predator in field conditions. The present study suggests the feasibility of pheromone-based monitoring as a simple and low-cost technique for management of the Xanthopimpla predator infestation on tasar cocoons.

TABLE 5: REARING PERFORMANCE OF *ANTHEREAE MYLITTA* DRURY DURING SECOND CROP

Crop	Treatment	Number of larvae brushed	Larval duration (days)	Number of cocoons produced	ERR% (Effective rate of rearing)	No. of Cocoons infested with *Xanthopimpla*
Second	Tetradecyl acetate (A)	500	37	395	79	9
	Ethyl (Z)-9-hexadecenoate (B)	500	37	410	82	9
	Blend of A and B in 1:1 ratio (C)	500	36	462	92.4	3
	Control (D)	500	37	304	60.8	72

TABLE 6: REARING PERFORMANCE OF *ANTHEREAE MYLITTA* DRURY DURING THIRD CROP

Crop	Treatment	Number of larvae brushed	Larval duration (days)	Number of cocoons produced	ERR% (Effective Rate of Rearing)	No. of Cocoons infested with *Xanthopimpla*
Third	Tetradecyl acetate (A)	500	41	414	82.8	10
	Ethyl (Z)-9-hexadecenoate (B)	500	42	428	85.6	9
	Blend of A and B in 1:1 ratio (C))	500	42	462	92.4	4
	Control (D)	500	42	310	62	148

CONCLUSION: Thus, in conclusion, Xanthopimpla predator infestation can be controlled by using a blend of tetradecyl acetate and ethyl (Z)-9-hexadecenoate in 1:1 ratio with 1mg concentration and is an efficient mating disruption synthetic pheromone.

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