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Kairomonal Effect of Acetone Extracts of *Pennisetum glaucum* (L.) and Their Influence on the Parasitic/Predatory Potential of *Trichogramma chilonis* Ishii and *Chrysoperla zastrowi sillemi* (Esben-Peterson) against Eggs of *Spodoptera litura* (Fab.)

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Efficacy of acetone extraction (1%) of various parts of bajra plant was tested on parasitization by *Trichogramma chilonis* Ishii and predation by *Chrysoperla zastrowi sillemi* (Esben-Peterson). Laboratory bioassay with acetone extracts of young leaves, old leaves, flowers and stems of bajra plant with *T. chilonis* and *C. zastrowi sillemi* revealed their kairomonal activities under *in vitro* condition. Treating irradiated eggs of *Spodoptera litura* (Fab.) with acetone extract of flowers of bajra recorded the parasitization of 16.54 per cent by *T. chilonis* on third day after inoculation which increased from 52.31 to 70.12 per cent on fifth and seventh day after inoculation and they were 7.31, 18.46 and 26.12 per cent when the eggs were treated with acetone alone on third, fifth and seventh days after inoculation, respectively. Maximum emergence (69.21%) was observed with acetone extract of flowers followed by acetone extract of young leaves (53.23%). The highest predation by *C. zastrowi sillemi* on acetone extract of flowers treated eggs of *S. litura* was recorded (70.18%) whereas it was 29.87 per cent in acetone treated eggs.

Key words: *Chrysoperla zastrowi sillemi*, *Corcyra cephalonica*, Kairomone, *Pennisetum glaucum, Spodoptera litura*, *Trichogramma chilonis*

In nature, tritrophic interactions of host plant, host insects and their natural enemies are mediated by a complex array of stimuli, of which the role of semiochemicals is commendable (Paul *et al.,* 2008). Many types of stimuli influence the habit location and host selection behaviour of parasitoids and predators among which the semiochemicals play a major role (Joachim and Weisser, 2015). Parasitoids exhibit varied foraging behavior in response to sensitization by semiochemical stimulus. The ability of infochemicals to enhance the orientation efficiency of parasitoids, culminating in control of the pest infestation forms the basis of IPM strategies. Due to heavy dependency of natural enemies on infochemicals for optimal performance as biological control agents in IPM strategies identification of the chemical nature of these cues is vital. Various studies have proved these cues are mainly hydrocarbons in nature (Mathur *et al.,* 2012). These saturated long chain hydrocarbons that are present on the surface of host plants and host insects have been reported to elicit synomonal and kairomonal responses in *Trichogramma* spp.

Similarly, plant surfaces are coated with a thin layer of waxy material that has a myriad of functions. This layer is microcrystalline in structure and form the outer boundary of the cuticular membrane. It serves many purposes, for example to limit diffusion of water and solutes while permitting controlled release of volatiles that may deter the pests or attract natural enemies and pollinators (Eigenbrode, 1996). The surface of the insect is also covered by a layer of wax and the nature of this lipid is dependent on species and in general a high proportion tends to be saturated alkanes (C_{21} to C_{31}). It is becoming increasingly clear that a major function of cuticular hydrocarbons (CHCs) in arthropods is to serve as recognition signal between individuals. In parasitoid wasps, non-volatile host cuticular lipids are used as very short range signals, while in specialist parasitoids these lipids serve as chemical recognition signals to identify host species (Seenivasagan and Paul, 2011) to discriminate suitable individuals for oviposition. Higher alkanes are known to be one of the main components of cuticular waxes of plant leaves and extracts. In order to evaluate the role of kairomones released by plant on parasitism and predation by *T. chilonis* and *C. zastrowi sillemi* laboratory bioassay were conducted with the acetone extracts (1%) of various plant parts of bajra (young leaves, old leaves, flowers and stems) to explain the kairomonal interaction between the parasitoid, predator and the host.

Materials and Methods

Chemical cues or kairomones present in various parts of bajra, selected as best intercrop in a field during 2014 – 2015. Laboratory studies were carried out at Bio-control laboratory, Agricultural College and Research Institute, Madurai during 2014 – 2015 to

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study the kairomonal effect of acetone extracts of bajra to natural enemies.

Insect cultures

The field collected *S. litura* egg masses, *C. cephalonica* and *T. chilonis* were cultured as per method suggested by Parthiban *et al.* (2014). Mass rearing of *C. zastrowi sillemi* was done with *C. cephalonica* eggs as feed followed the method described by Swamiappan (1996).

Extraction of kairomone

Various parts of bajra (young leaves, old leaves, flowers and stem) were collected separately and shade dried for 12 h. About 20 g of each plant sample was chopped into small pieces and transferred to 250 ml conical flask. A known volume (100 ml) of acetone (HPLC grade) was poured into the individual conical flask containing chopped plant materials. The mouths of the flasks were closed with non-absorbent cotton and were incubated for 72 h and shaked in water bath (Genuine model) at 28°C for two hours followed with 20 min. at 50°C. The plant materials were filter through Whatman number 1 filter paper. The acetone fraction was subsequently concentrated by vacuum evaporation at 40° C (LARK model). The extracts were stored at –20°C in deep freezer (REMI model) till further use for bioassay studies. A concentration of 1 per cent (10000 ppm) of the extracts of various parts of bajra was prepared after dilution with acetone and used throughout the experiment (Shankarganesh and Khan, 2006).

Bioassay

Bioassay studies of acetone extracts (1%) of various plant parts of bajra (young leaves, old leaves, flowers and stem) were carried out at 26 ± 2°C and 75 ± 5% R.H. with the photoperiod 16:8 h scoto/photo regime. Clean, healthy, 0-24 h old eggs of *S. litura* sterilized under UV light for 45 min. were washed twice in acetone to remove any trace of scales or kairomones present on the surface of eggs. These eggs were pasted with pure white gum on dull coloured cardboard, measuring 7 x 2 cm at the rate of average of 80 - 100 eggs per piece (egg card). Kairomone extracts (10000 ppm) of various parts of bajra (young leaves, old leaves, flowers and stem) was used to treat the acetone washed eggs, separately and shade dried. Each egg card was considered as one replication and each treatment was replicated six times. Control was maintained with acetone alone.

The egg card taken in a glass tube (7.5 x 2.5 cm) was introduced with freshly emerged *T. chilonis* adults (6:1). The per cent parasitization was observed on 3rd, 5th and 7th day after introduction. Similarly, one second instar of *C. zastrowi sillemi* was released in a vial with acetone washed *S. litura* eggs (80-100 nos.) and per cent predation was calculated 24 h after release (Murali baskaran, 2013).

Statistical analysis

Data obtained from the bioassay of acetone extracts of various plant parts of bajra were subjected to ANOVA (Analysis of Variance). By subjecting the data on per cent parasitism to arcsine transformation. In order to know the interaction between treatments, data from laboratory bioassay were subjected to factorial CRD (Completely Randomized Design) analysis and the means obtained were separated by LSD (Least Significant Difference) (Gomez and Gomez, 1984).

Results and Discussion

The efficacy of acetone extracts of various plant parts of bajra on parasitism revealed that the highest mean percentage parasitism of 46.32 by *T. chilonis* (Table 1) was recorded in acetone extract of flower of bajra (10000 ppm) followed by 39.48 percentage in acetone extract of young leaves. Among the plant extracts stems and old leaves extract resulted in the lowest mean percentage parasitism of 26.33 and 24.82, respectively, whereas the control (acetone) had the least mean parasitism (17.30%). When the interaction between the different plant parts were analysed, it was found that the acetone extract of flower of bajra aided in the highest mean parasitization level of *T. chilonis* on eggs of *S. litura*, recording 16.54, 52.31 and 70.12 per cent on 3rd, 5th and 7th day after introduction of parasitoids, respectively which was significantly different from acetone extract of young leaves (11.97, 44.32 and 62.14%), stems (10.65, 28.79 and 39.54%) and old leaves (10.11, 25.67 and 38.69%), while it was 7.31, 18.46 and 26.12 per cent parasitization in acetone alone treated eggs for the same period.

Table 1. Acetone extracts of bajra plant parts influencing parasitism by *Trichogramma chilonis* on eggs of *Spodoptera litura*

Plant parts	% parasitization by *T. chilonis* after*			Mean
	3rd day	5th day	7th day	
Young leaves	11.97 (20.24)[b]	44.32 (41.74)[b]	62.14 (52.03)[b]	39.48 (38.93)[b]
Old leaves	10.11 (18.54)[c]	25.67 (30.44)[d]	38.69 (38.46)[c]	24.82 (29.88)[d]
Flowers	16.54 (24.00)[a]	52.31 (46.33)[a]	70.12 (56.87)[a]	46.32 (42.89)[a]
Stems	10.65 (19.05)[c]	28.79 (32.45)[c]	39.54 (38.96)[c]	26.33 (30.87)[c]
Control (Acetone)	7.31 (15.68)[d]	18.46 (25.45)[e]	26.12 (30.74)[d]	17.30 (24.58)[e]
SEd	0.3780	0.2581	0.2495	0.2623
CD (P=0.05)	0.8423	0.5750	0.5559	0.5845

*Mean of eight replications

Figures in parentheses are arcsine transformed values

In a column, means followed by the same letter(s) are not significantly different by LSD (P=0.05)

Similarly, the highest mean per cent emergence (69.21%) was recorded in acetone extract of flower of bajra followed by acetone extract of young leaves (52.23%) (Table 2) while the lowest mean emergence was recorded in acetone extract of old leaves (39.44%) among the different washes followed by acetone extract of stems (40.05%) and the lowest mean per cent emergence was recorded in control (28.15%).

Table 2. Acetone extracts of bajra plant parts influencing adult emergence of *T. chilonis* on eggs of *S. litura*

Plant parts	% emergence of *T. chilonis**
Young leaves	53.23 (46.85)[b]
Old leaves	39.44 (38.90)[c]
Flowers	69.21 (56.30)[a]
Stems	40.05 (39.26)[c]
Control (Acetone)	28.15 (32.04)[d]
Mean	46.01 (42.71)
SEd	0.2458
CD (P=0.05)	0.5476

*Mean of eight replications

Figures in parentheses are arcsine transformed values

In a column, means followed by the same letter(s) are not significantly different by LSD (P=0.05)

Predatory activity by *C. zastrowi sillemi* was enhanced from 29.87 (acetone treated eggs of *S. litura*) to 70.18 per cent (Table 3), 24 h after treatment when treated with acetone extract of flower of bajra, followed by acetone extract of young leaves (55.54%), stems (45.05%) and acetone extract of old leaves (43.69%), while it was 29.87 per cent predation in acetone alone treated eggs for the same period.

The outcomes of the present study indicate that kairomonal compounds from acetone extract of flower of bajra increased parasitization when applied over target sites. This findings is in agreement with the earlier reports in this direction in a number of host-parasitoid systems (Mathur *et al*., 2012; Murali baskaran, 2013a). Hendry *et al*. (1976) analyzed five host plants of *Heliothis zea* Boddie to determine the presence of hydrocarbons acting as synomones for *Trichogramma evanescens* Westwood and identified series of hydrocarbons present in host plants ranging from C_{21} to C_{25} in varying quantities. Rani *et al*. (2008) brought out the variation in the quantity and concentration of saturated hydrocarbons influenced the parasitization efficiency of Trichogrammatids. Yadav *et al.* (2001) also reported the presence of pentacosane in potato *(Solanum tuberosum)* and soybean *(Glycine max)* and classified pentacosane as favourable saturated hydrocarbon for *T. exiguum.*

The present result was endorsed with the findings of Kumar *et al*. (2012) reporting highest per cent mean parasitisation was found in hexane extracts of cabbage and khol-khol. Murali baskaran (2013b) explicated that acetone extracts of flowers of clusterbean and okra performing very high parasitism and predation for *T. chilonis* and *C. zastrowi sillemi* indicating the presence of high kairomonal activity. The significance of these kairomonal substances in behavioural manipulation of entomophagous insects was earlier emphasized and reviewed by Lewis *et al*. (1976).

Table 3. Acetone extracts of bajra plant parts on predation by *Chrysoperla zastrowi sillemi* on eggs of *S. litura*

Plant parts	% predation by *C. zastrowi sillemi* after 24 h*
Young leaves	55.54 (48.18)[b]
Old leaves	43.69 (41.38)[d]
Flowers	70.18 (56.90)[a]
Stems	45.05 (42.16)[c]
Control (Acetone)	29.87 (33.13)[e]
Mean	48.86 (44.34)
SEd	0.2446
CD (P=0.05)	0.5450

*Mean of six replications

Figures in parentheses are arcsine transformed values

In a column, means followed by the same letter(s) are not significantly different by LSD (P=0.05)

Elanchezhyan *et al*. (2009) found that kairomones from acetone extracts of flowers of brinjal and acetone extract of onion leaves favoured very high parasitism and predation for *T. chilonis* and *C. zastrowi sillemi* indicating high kairomonal activity. Presence of single chain hydrocarbons like dotriacontane and nonadecane would have been responsible for the enhanced predatory activity of *C. carnea*, as suggested by Singh and Paul (2002). Sulphur containing amino acids present in the leaf extract of onion were found to evoke response in many entomophagous insects including *T. chilonis* and *C. zastrowi sillemi* (Yadav *et al*., 2009). Spraying of aqueous leaf extract of bajra on groundnut had reduced the oviposition by adults of leafminer and settling and feeding rate of larvae and attracted more numbers of *Goniozus* sp. Similar results were reported by Ohri *et al*. (2007) on broissinosteroids. Semi *et al*. (2011) observed synomonal response of *H. armigera* females to flowers extract of African marigold, cotton, okra and pumpkin to varying degree which was due to the presence of favourable hydrocarbons thus confirming the role of activity released plant volatiles for enhancing the host seeking beahaviour of parasitoids.

Behaviour of a natural enemy can be manipulated by selecting appropriate plant variety through breeding for certain characters which could potentially enhance the foraging ability of a parasitoid in an eco-system against the target pest (Bottrell *et al*., 1998). Under natural situations, the interface where tri-trophic interaction takes place is often the cuticle of a plant (Muller and Riederer, 2005). The epicuticular wax layer of plants has been shown to influence the foraging success of natural enemies (Eigenbrode, 2004). cuticular hydrocarbons are also known from other herbivore-parasitoid associations to serve as kairomones (Colazza *et al*., 2007). Larvae of the generalist predator *C. zastrowi sillemi* have specific preference to certain hydrocarbons and other chemicals at a particular concentration. Such preferential behaviour of the larvae may be utilized for their activity of manipulation in the release programmes to enhance their host searching activity.

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