Bioassay of *Bemisia Tabaci* (Gennadius) Nymphs, Adults and Exuviae Extracts as Kairomonal Sources for Its Parasitoid *Eretmocerus* sp. Near *furuhashii* Rose & Zolenerowich

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Abstract: *The response of* Eretmocerus sp. near furuhashii *Rose & Zolenerowich female to its host,* Bemisia tabaci *(Gennadius) nymph, exuviae or adult extracts was bioassayed in Petri dish arena. The tested materials were extracted by hexane, ethanol and distilled water. Data showed that the parasitoid used exuviae, nymphs or adult of* B. tabaci *as kairomonal sources to locate its host. The parasitoids intensified their search in the treated patches and exhibited both orthokinetic and klinotaxic responses. The highest mean of the retention time in the contaminated patches with the tested materials, was found in case of nymph water extract (132.72 seconds). Moreover, parasitoid searching time was concentration-independent in all tested extracts. Such findings might help in enhancing the parasitoid searching efficiency and guide it directly to the patch that contains its suitable host.*

Key **word**: *Eretmocerus, Bemisia tabaci,* Kairomone, Searching behavior

INTRODUCTION

Bemisia tabaci (Gennadius) is increasingly becoming a threat to crop growers worldwide not only in the open fields but also in the greenhouses. It is a very aggressive polyphagous pest that attacks more than 600 host plants and there may be many additional hosts not yet formally documented (Oliveira *et al.*, 2001). *B. tabaci* can seriously injure plants by sucking juices causing wilting, stunting, irregular ripening of fruits or even death (Byrne & Bellows, 1991). The adult can also transmit plant viruses from infected to healthy plants (Byrne and Bellows, 1991; Oliveira *et al.*, 2001).

Bemisia tabaci is attacked by an array of insect parasitoids mostly belonging to genera *Encarsia* and *Eretmocerus* (Gerling *et al.*, 2001). *Eretmocerus* sp. near *furuhashii* Rose & Zolenerowich is found to be the dominant parasitoid species of *B. tabaci* in Guangdong, China with 45.5% of the total parasitism (Qiu, 2002).There is no available data on the searching behavior of this parasitoid or how it finds its host.

Kairomones have been reported in many studies to play very important roles in habitat and host location and in enhancing the percent of parasitism/predation of large number of parasitoids/predators (Vet and Dicke, 1992). Honeydew, exuviae and the host/prey itself have also been reported as kairomones for attracting and/or arresting natural enemies and may enhance the searching behavior and parasitization/predation of several insect parasitoids/predators (Shonouda, 1996; Singh and Srivastava, 1989).

Therefore, this study aimed to bioassay extracts from *B. tabaci* exuviae, nymphs and adult sages as kairomonal sources for the parasitoid *Eretmocerus* sp. nr. *furuhashii*; using Petri dish as an observation arena.

MATERIALS AND METHODS

***Insect material*:**

A colony of *Eretmocerus* sp. nr. *furuhashii* was established on poinsettia plants (*Euphorbia* sp.) infested with *B. tabaci* in an air conditioned room with a photoperiod of 16L:8D h; T=25±2 °C and 60 ±10% RH in Lab of Biological Control, SCAU, Guangzhou, China.

Collecting and preparing the tested kairomonal materials

To extract the kairomone from *B. tabaci* nymphs (1st-4th instars), adults and exuviae, insect materials were collected and transferred to a 2-ml appendorf tube containing 1 ml of distilled water, 90% ethanol or n- hexane, crushed and kept in room temperature. Sex hours later, these extracts were centrifuged at 2000-5000 rpm/min, filtered and stored in vials in a refrigerator at -4 °C until needed. Two concentrations were tested; 100 and 200 nymphs, exuviae or adults /ml for the 3 previously mentioned solvents.

The bioassay

The parasitoid females used in the current study were 24-48 h old. Newly emerged parasitoid individuals were collected from the colony and kept for 24 h in cohort in a 10×2cm glass tube for mating.

These wasps were provided with droplets of honey solution on the inner wall as food. Parasitoids could therefore be assumed to be food satiated. Before the experiments, the females were separated and confined individually in 0.5×2 cm glass tubes with a drop of honey solution; each parasitoid was

tested only once.

The effect of these extracts on the searching time of *Eretmocerus* sp. nr. *furuhashii* was bioassayed using the same methodology described by Zabroski *et al.* (1987) and Mandour *et al.* (2003). The experiment was conducted at 26±2 °C and 60±10% RH. The parasitoid searching behavior was observed in a 12 cm Petri dish arena containing a filter paper disc with a diameter of 11 cm. A treatment and a surrounding area were marked with pencil as two concentric circles (4 and 7 cm diameter) (Mandour *et al.*, 2003, 2005). 100 µl of the tested extracts were added to the inner circle as a circular spot (4 cm diameter) and was allowed to dry for 30 minutes. Tests were started by releasing a single parasitoid female in the center of the treated patch and the searching time in the treated and surrounding areas was observed for 10 minutes. Parasitoids showed a tendency to search near the edges of the treated area that led to short visits to the neighboring area. Therefore, having left the treated patch, a female is expected to return to the treated patch in 30 seconds, otherwise it is considered to have left the patch and the number of returns is counted. The observation was terminated prematurely when the parasitoid left the outer boarder of the surrounding area or when it started to fly to the sides or the top of the Petri dish. Having testing five parasitoid females, the treated paper discs were replaced with new treated ones (Zaborski *et al.*, 1987; Mandour *et al.*, 2003, 2005). Unless otherwise stated, 30 parasitoid females were tested for each extract.

Statistical Analysis: Different concentrations within the same treatment were compared using analysis of variance (ANOVA) and means were separated using Duncan Multiple Comparison Test (DMRT) (SAS Institute, 1999).

RESULTS

Data in Table 1 indicated that *Eretmocerus* sp. nr. *furuhashii* used *B. tabaci* nymphs as contact kairomone to locate its hosts in close vicinity. The time spent by wasps in the patches impregnated with nymph-extracts did not increase with increasing concentration. The arrestment responses of parasitoids in these patches peaked in nymphs extracted with water at 132.72 seconds in 200-nymphs/ml.

Statistical analysis showed that in nymph water extract, there are significant differences in time spent in the treated patches ($P< 0.0022$), neighboring areas ($P< 0.0606$) or in number of returns ($P<0.0180$). In case of hexane extract, there is no significant difference in the three investigated parameters. With ethanol extract, significant differences were detected to that of the control only in case of time spent in the treated patches ($P< 0.0056$).

B. tabaci adult extracts that arrested *Eretmocerus* sp. nr. *furuhashii* were descendingly arranged as water, hexane and ethanol. The longest average time spent in the treated areas was recorded at 84.56 seconds in 200-adults/ml water (Table 2). Statistical analysis indicated that there were significant differences in materials extracted with water ($P< 0.0020$), hexane ($P< 0.0165$) and ethanol ($P< 0.0404$). The time spent by parasitoid females searching in the neighboring area was less pronounced, but significant difference was observed in materials extracted with water ($P< 0.0820$) and ethanol ($P< 0.0075$). With number of returns to the treated patches, significant difference to that of the control was recorded only with material extracted with water ($P< 0.0404$).

Eretmocerus sp. was arrested to extracts of *B. tabaci* exuviae and the arrestment response in water extracts of exuviae was likely longer (Table 3). The parasitoid response to all exuviae extracts was concentration-independent and peaked at 113.23 seconds in exuvial-extracts with water. Statistical analysis indicated that significant differences were found in water extract of exuviae ($P< 0.0001$), hexane ($P< 0.0136$) and ethanol ($P< 0.0062$). Pertaining to the time spent in the neighboring area, there was significant increase in the residence time with an increase in the concentration of the extracted materials in water ($P< 0.0001$) and hexane ($P< 0.0057$). Number of returns to treated patches showed significant difference to that of the control only in case of exuviae extracted with water ($P< 0.0308$).

DISCUSSION

It is more reliable that *Eretmocerus* sp. nr. *furuhashii* responded strongly to extracts from their sessile host (nymphs or its exuviae) than the mobile stage (adults). This is due to the fact that the chance of the female parasitoids to have contact or antennate *B. tabaci* adults in the natural environment is somewhat weak. These data are in accordance with those recorded by Mandour *et al.* (2003) for *Encarsia bimaculata*. However, the response reported for *Eretmocerus* here is significantly higher than that for *E. bimaculata*.

Buckner *et al.* (1999) found that over 85% of the surface lipids of *B. argentifolii* nymphs, exuviae and emerged adults consisted of long-chain wax esters, with lesser amounts of long-chain aldehydes, alcohols, hydrocarbons and trace amounts of free fatty acids. They further suggested that the cuticular components of exuviae and nymphs might play a crucial role in host acceptance and suitability or use as prey-recognition factor.

Other studies also reported the attraction (long range response) of the natural enemies to their host or their host's kairomones. For example, Crade and Lee (1989) found that *Brachymeria intermedia* walked upwind to odor from its host, *Lymantria dispar* and its pupal extracts. Analogously, a contact kairomone from pupae of European spruce sawfly, *Gilpinia hercyniae* elicited frequent antennal drumming for the

parasitoid *Dahlbominus fuscipennis* and the kairomone was likely existed in the cocoon and the outer layer of the pupae (Rostas *et al.*, 1998).

The manipulation of natural enemies' environment using host derived kairomones enhanced the performance of natural enemies in the natural environment. For example, the application of the aqueous extract of *Aphis fabae* Scop. on clean *Vicia fabae* leaves stimulated females of the syrphid *Metasyrphus corollae* Fabr. to intensify their search on the sprayed plants than in control under all densities of aphids. Subsequently, females laid eggs at all aphid densities and the number of eggs was directly proportional to aphid concentration (Shonouda, 1996). Also, the spraying of cucumber plants with aqueous extract of *B. tabaci* nymphs increased rate of parasitism by *Eretmocerus* sp. nr. *furuhashii* (Mandour, unpublished).

Table 1. Average times spent and number of returns (±SE) of *Eretmocerus* sp. nr. *furuhashii* on a filter paper treated with different *B. tabaci* nymph-extracts

Treatments	n	Searching time (Second ± SE)		Number of returns (Mean ±SE)
		Treated area	Neighboring area	
Water				
Control	30	21.70±2.71a	2.87±1.14 a	0.17±0.08a
100nymphs/ml	25	126.88±27.75b	8.08±2.47ab	0.40±0.15ab
200nymphs/ml	25	132.72±36.76b	9.48±2.65b	0.80±0.22b
n-Hexane				
Control	30	20.50±2.74a	3.20±1.16a	0.17±0.08a
100nymphs/ml	25	27.32±4.12a	2.68±0.85a	0.08±0.08a
200nymphs/ml	25	31.28±3.99a	3.16±0.94a	0.12±0.07a
Ethanol				
Control	30	20.37±4.23a	3.43±1.00a	0.13±0.10a
100nymphs/ml	25	47.24±7.99b	3.60±1.20a	0.20±0.10a
200nymphs/ml	25	44.68±7.50b	4.80±1.39a	0.60±0.40a

Means with the same letters are not significantly different to control (DMRT, P>0.05)

Table 2. Average searching time and number of returns (±SE) of *Eretmocerus* sp. nr. *furuhashii* on filter paper treated with *B. tabaci* adult-extracts.

Treatments	n	Searching time (Second ± SE)		Number of returns (Mean ± SE)
		Treated area	Neighboring area	
Water				
Control	30	21.70±2.71a	2.86±0.98a	0.17±0.08a
100adults/ml	30	79.93±11.72b	14.27±5.83b	1.00±0.37b
200adults/ml	30	84.56±20.20b	11.03±2.27ab	0.93±0.22b
n-Hexane				
Control	30	20.50±2.74a	3.20±1.16a	0.17±0.08a
100adults/ml	30	51.70±11.99b	5.57±1.15a	0.07±0.05a
200adults/ml	30	55.20±11.19b	9.67±1.20a	0.30±0.13a
Ethanol				
Control	30	20.37±4.23a	3.43±1.00a	0.13±0.10a
100adults/ml	30	52.60±14.67ab	12.13±2.18b	0.30±0.11a
200adults/ml	30	77.80±22.65b	8.03±2.27ab	0.40±0.13a

Means with the same letter are not significantly different to control (DMRT, P> 0.05)

Table 3. Average searching time and number of returns (±SE) of *Eretmocerus* sp. nr. *furuhashii* on filter paper disc treated with different *B. tabaci* exuviae-extracts.

Treatments	n	Searching time (Second ± SE)		Number of returns (Mean ±SE)
		Treated area	Neighboring area	
Water				
Control	30	21.70±2.71a	3.20±1.16a	0.17±0.08a
100adults/ml	30	111.50±17.40b	27.83±5.12b	1.53±0.51b
200adults/ml	30	113.23±15.50b	14.53±4.07c	0.66±0.35ab
n-Hexane				
Control	30	20.50±2.74a	3.20±1.16a	0.17±0.08a
100adults/ml	30	52.77±11.90b	6.23±1.16ab	0.53±0.21a
200adults/ml	30	51.17±8.38b	9.33±1.56b	0.43±0.14a
Ethanol				
Control	30	20.37±4.23a	3.43±1.01a	0.13±0.10a
100adults/ml	30	59.40±11.78b	5.03±1.02a	0.20±0.10a
200adults/ml	30	63.80±12.71b	7.43±1.93a	0.37±0.15a

Means followed by different letters indicate significant difference (DMRT, $P< 0.05$)

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