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EFFECTS OF DIFFERENT KAIROMONAL SOURCES ON THE PERFORMANCE OF *ERETMOCERUS* SP. NEAR *FURUHASHII* AGAINST *BEMISIA TABACI* ON CUCUMBER: II – IN GREENHOUSE

Nasser Said Mandour[1], Shun-xiang Ren[2], Bao-li Qiu[2], Felix Wäckers[3]*

[1] Department of Plant Protection, Faculty of Agriculture, Suez Canal University 41522, Ismailia, Egypt
[2] Department of Entomology, South China Agricultural University 510642, Guangzhou, China
[3] Centre for Sustainable Agriculture, Lancaster Environment Centre, Lancaster University, United Kingdom

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Abstract: The effects of application of an artificial honeydew mixture of glucose, fructose and trehalose (GFT), honey and *Bemisia tabaci* nymph-extract as kairomonal sources in enhancing the foraging efficiency and performance of *Eretmocerus* sp. near *furuhashii* on cucumber plants were studied. Experiments were conducted in small greenhouses (4×3×3 m) using life table methods. Life table data indicated that the total mortality in *B. tabaci* immature cohorts in all treatments was in the order of fourth instar > first instar > second = third > egg > pupa cohorts. The tested kairomonal materials had a significant effect on the rate of parasitism ($p > 0.0415$) with 13.23, 9.04 and 10.54% higher than that of control in artificial honeydew of GFT, nymph-extract and honey treatments, respectively. *B. tabaci* egg/adult survival ratio was also significantly affected ($p > 0.0001$) by the tested kairomonal sources being lowest (22.91%) in nymph-extract treatment. Moreover, the tested kairomonal materials arrested significantly more parasitoids to colonize the treated plants comparing to control. Apparently, the tested materials were significantly effective in attracting the parasitoids up to 3 days after applications then significant difference was not found between treatments.

Keywords: *Eretmocerus* sp., whitefly, kairomone, parasitism, colonization, life table

INTRODUCTION

Bemisia tabaci (Gennadius**)** (*Homoptera: Aleyrodidae*) is a polyphagous pest of Asian or oriental origin (Commonwealth Institute of Biological Control 1981). Now, it oc-

*Corresponding author:
rensxcn@yahoo.com.cn

curs widely in both tropical and subtropical regions and has become a limiting factor for the production of several vegetables, field crops, ornamentals and fruit trees (Oliveira *et al.* 2001). *B. tabaci* undergoes parasitization by large numbers of insect parasitoids belonging to genera *Encarsia*, *Eretmocerus* and *Amitus* (Gerling *et al.* 2001). The parasitic wasps related to the genus *Eretmocerus* are the proper candidates to control *B. tabaci* population under greenhouse/open field conditions (Hoddle *et al*. 1999).

Eretmocerus sp. near *furuhashii* Rose & Zolenerowich (*Hymenoptera: Aphelinidae*) contributes by as high as 45.5% of the total parasitism of *B. tabaci* in Guangdong, China (Qiu *et al.* 2005). This parasitoid was arrested to *B. tabaci* natural honeydew and its sugar components *i.e.* trehalose, trehalulose, fructose and glucose, but it did not respond to melezitose and sucrose (Mandour *et al.* 2005a). Moreover, *E.* sp. nr. *furuhashii* was also arrested to nymph and exuviae extracts of *B. tabaci* (Mandour *et al.* 2005b). In earlier study, we studied the effect of 9 kairomonal/food sources on *E.* sp. nr. *furuhashii* in cages. Data indicated that an artificial honeydew mixture of glucose + fructose + trehalose (GFT), *B. tabaci*-nymph extract and honey solution were the most effective treatments in enhancing the foraging efficiency of the parasitoid *E.* sp. nr. *furuhashii* as indicated by *B. tabaci* total cohort mortality, egg to adult survival and rates of parasitization (Mandour *et al.* 2006).

In the last three decades, many studies have shown that through the manipulation of natural enemies' environment using host derived kairomones or food supplement, it might enhance the performance of natural enemies in the natural environment. This undoubtedly could lead to more successful biological control programs (Singh and Srivastava 1989; Lewis *et al.* 1998; Wäckers 2003; Mandour *et al.* 2006). For instance, the application of honeydews in the natural ecosystem could play a dual function: food and/or kairomonal source. For parasitoids to maintain high reproductive success, it is important that disruption of their food foraging process is minimal so that most of their adult lifetime energy can be allocated to finding hosts (Staple *et al.* 1997; Lewis *et al.* 1998).

With these views in mind, the present work therefore is focusing on studying the effectiveness of aqueous sprays of artificial honeydew of GFT, *B. tabaci* nymph-extract and honey solution as kairomonal sources for enhancing the performance of *E.* sp. nr. *furuhashii* in the greenhouses. Life table was constructed to quantify the mortality rates in *B. tabaci* immature cohorts. Moreover, the effect of different investigated kairomonal materials on the rate of plant colonization by *E.* sp. nr. *furuhashii* was studied under greenhouse conditions.

MATERIALS AND METHODS

Insect materials

A colony of *B. tabaci* was reared on potted cucumber plants in a laboratory of 16l: 8 D h; 25±2°C and 60±10% R.H. Cucumber plants infested with *B. tabaci* 2nd–3rd instar-nymphs were subjected to parasitization by *E.* sp. nr. *furuhashii*.

Plant materials

Seeds of cucumber, *Cucumis sativus* (variety Jin Mantian) were soaked in clean Petri dishes with a fine film of water. These Petri dishes were covered with moistened piece of cotton to facilitate and accelerate sprouting. Sprouted seeds were then care-

fully separated using fine forceps and moved to 18 cm diameter plastic pots and kept at 25°C. To withstand high population of *B. tabaci*, plants were regularly watered, and provided with fertilizers and supplementary lighting. Plants were kept free of *B. tabaci* in small greenhouse (4×3×3m) till they produced 5–6 leaves, and then used for experiments.

Source of chemicals

Glucose, fructose and trehalose were purchased from Sigma Co. (Guangzhou office, China). Honey was obtained from Guangdong Entomological Institute, Guangzhou, China.

Preparation of the test kairomonal materials

The test kairomonal materials were artificial honeydew of GFT, *B. tabaci* – nymph extract and honey. The artificial honeydew was prepared at 15g/100 ml water at a ratio of 1:1:1 of its component sugars. Honey solution was diluted in distilled water at a rate of 15g/100 ml water. For nymph-extract treatment, *B. tabaci* nymphs (2nd–3rd instars) were collected under binocular microscope using fine entomological pine. The collected nymphs were put in plastic vials at the rate 100 nymps/ml water, crashed well and centrifuged at 3000 rpm for 10 minutes. The contents of all vials were filtered, mixed and sealed. Nymph extract was mostly prepared 6–12 hours before application.

Experimental set up

The experiments were carried out in small greenhouses (4×3×3 m) covered with a fine plastic net. This cover with small mesh was impenetrable by both *Eretmocerus* sp. and *B. tabaci*. To establish a good colony of *B. tabaci* and ensure the even deposition of *B. tabaci* eggs all over the experimental plants, every 5 cucumber plants (40–50 cm high) were placed in small cages (70×70×70 cm). Then adults of *B. tabaci* were collected from the stock colony using an aspirator and introduced in the center of the cage at the rate of 50 adults/plant. Cages were kept under laboratory conditions of 25±2°C; 60±10% RH. and 14L: 10D. Caged plants were supplied with water and/or fertilizer when needed. Seven days later, all cages were moved to the greenhouses. Plant were then taken out and distributed randomly in the greenhouses. Plants were lined up in the greenhouse 0.5 m apart in 4 rows of 10 plants and given numbers from 1 to 40.

After distributing cucumber plants in the assigned greenhouses, 10 cucumber plants were chosen randomly as the sample plants and one leaf from each sample plant was selected randomly as a sample leaf. Selected leaves were tagged and labeled. Having selected the sample leaves, data were taken repeatedly in 3 day-interval with the aid of magnifying lens of 17X. Data were recorded in the form of numbers of alive, dead, disappeared, and parasitized nymphs in all instars and stages until the emergence of all adult whiteflies and/or parasitoids.

Once a mixed cohort of *B. tabaci* eggs, 1st, 2nd and 3rd instars were established on the majority of the sample leaves, plants were sprayed with distilled water using clean small handle sprayer to remove the natural honeydew. Then plants were left for 2–3 hours to dry up. After that, plants were sprayed with 3ml/plant of the tested kairomonal materials (GFT, honey, nymph extract, or distilled water as control). When the treated plants dried up (2–3 hours later), adult parasitoids were introduced between

the plants at the rate of 3 females +3 males/plant. Newly emerged adult parasitoids were collected, sexed and confined in glass tubes (10 × 2 cm). Tubes were distributed between the rows inside the greenhouses. After that, plants were observed continuously at 3-day interval. The experiments lasted 8 weeks.

Each kairomonal treatment besides control II, which sprayed with distilled water and received parasitoids, was replicated twice. Moreover, to compare the population status of *B. tabaci* in the presence and absence of *E.* sp. nr. *furuhashii*, a control trial without parasitoids (Control I) was conducted using the same protocol but repeated only once.

Effect of different kairomonal sources on plant colonization by *Eretmocerus* sp.

Having sprayed the test kairomonal materials and released *E.* sp. nr. *furuhashii*, 10 random cucumber plants (5 plants × 2 greenhouses) were selected to count the number of adult *Eretmocerus* sp. (males and/or females) per plant. Data were recorded at an interval of 2 hour, 6 hours, 1, 2, 3, 4 and 5 days after parasitoid introduction in the greenhouses. The selected plants were checked carefully leaf by leaf to avoid disturbing the searching parasitoids. Counts were mostly done in the early morning between 9:00 and 11:00 a.m. With each reading, 10 new plants were chosen randomly for parasitoid counting.

Statistical analyses

Stage-specific life table of *B. tabaci* was constructed as described by Southwood (1978). Data of the 20-cucumber sample leaves across the 2 greenhouses for each treatment were used to calculate percents of mortality ($100q_x$) and survivorship of *B. tabaci* immatures. In comparing rates of parasitism and egg to adult survival, data from each plant were used as replicate with values subjected to ANOVA. When F values were significant, separation of means was achieved using DMRT. Similarly, data for colonization of cucumber plants by *E.* sp. nr. *furuhashii* were separated using AVOVA and DMRT as well (SAS Institute 1999).

RESULTS

The loss during the egg stage was mostly due to unknown cause (non-viability of eggs) and was lower than 10% in all treatments. The major mortality factor that caused death in *B. tabaci* first, second and third instar cohorts was primarily unknown. This unknown cause included host feeding by parasitoids, unsuccessful parasitism and normal mortality. The mortality rates due to the unknown cause were 14.24, 16.20, 17.51, 17.83 and 17.53% for first instar; 6.72, 7.90, 6.78, 7.72 and 6.87% for second instar; 6.98, 11.16, 10.71, 11.49 and 9.72% for third instar in control I, control II, GFT, nymph extract and honey treatments, respectively (Table 1). In the fourth instar cohorts, the rates of mortality were the greatest in all *B. tabaci* immature cohorts. This could be mainly due to the higher mortality rate due to successful parasitism (Table 1). For pupal cohorts, the loss due to the unknown cause was less pronounced and never exceeded 7% in all treatments.

Data in Table 2 indicated that the tested kairomonal treatments had a significant effect ($p > 0.0415$) in increasing the parasitization of *B. tabaci* by *E.* sp. nr. *furuhashii* being 20.78, 34.11, 29.82 and 31.32% in control II, artificial honeydew of GFT, nymph-

Table 1. Life table of *B. tabaci* on cucumber plants treated with aqueous sprays of artificial honeydew of GFT, its nymph extract and honey sprays in the presence or absence of its parasitoid *E.* sp. nr. *furuhashii* in the greenhouse (Guangzhou, China, 2003)

Stage [x]	No. entering stage [l_x]					Mortality factor [fd_{xj}]	Mortality percent [100qx]				
	CK I	CK II	GFT	nymph-extract	honey		CK I	CK II	GFT	nymph-extract	honey
Egg	1806	3110	2754	2786	3551	unknown	7.48	5.72	7.73	8.22	9.57
1st instar	1671	2932	2541	2557	3211	unknown	14.24	16.20	17.51	17.83	17.53
						disappearance	1.20	1.77	1.85	1.60	0.84
						total	15.44	17.97	19.36	19.43	18.37
2nd instar	1413	2405	2049	2060	2621	unknown	6.72	7.90	6.78	7.72	6.87
						disappearance	1.06	0.79	0.29	0.58	0.53
						total	7.78	8.69	7.07	8.30	7.40
3rd instar	1303	2196	1904	1889	2427	unknown	6.98	11.16	10.71	11.49	9.72
						disappearance	0.54	0.55	0.53	1.27	0.54
						total	7.52	11.71	1.24	12.76	10.26
4th instar	1205	1939	1690	1648	2178	unknown	6.56	9.90	10.24	10.13	8.49
						disappearance	0.66	0.67	0.59	0.91	0.46
						parasitized	–	29.65	44.50	42.17	42.19
						total	7.22	40.22	55.33	53.21	51.14
Pupa	1118	1159	755	771	1064	unknown	5.10	5.69	6.62	6.74	5.26
						disappearance	0.45	0.52	0.66	0.65	0.47
						total	5.55	6.21	7.28	7.39	5.73
Adult	1056	1087	700	714	1003						

Ck I (control I) did not receive parasitoids, Ck II (control II) did receive parasitoids

extract and honey treatments, respectively. Regarding *B. tabaci* egg to adult survival, the average egg-adult survival rates of *B. tabaci* in control I differed significantly ($p > 0.001$) from all treatments that received parasitoids including control II. Kairomonal treatments also differed significantly from control II indeed (Table 2).

Table 2. Average percent of parasitism and egg to adult survival of *B. tabaci* on cucumber sprayed with artificial honeydew of GFT, nymph-extract and honey in the presence and absence of *E.* sp. nr. *furuhashii* in greenhouses (Guangzhou, China, 2003). Data expressed as mean ±SE

Treatments	Parasitism [%]*			Egg-adult survivorship [%]		
	n	mean ±SE	range	n	mean ±SE	range
Control I+	–	–	–	10	58.31±3.58a	42.2–57.8
Control II++	20	20.78±3.15a	6.2–61.7	20	33.05±2.69b	8.7–57.8
GFT	20	34.11±3.30b	9.6–58.9	20	24.10±1.73c	12.2–38.3
Nymph-extract	20	29.82±4.01ab	9.9–66.6	20	22.91±2.58c	4.5–49.6
Honey	20	31.32±3.06b	7.9–56.4	20	26.30±2.54bc	6.9–42.3
F and P values	df, 3, 76; F = 2.88; p = 0.0415			df, 4, 85; F = 21.60; p = 0.0001		

Means followed with the same letters (column wise) are not significantly different (DMRT; $p > 0.05$)
+Control I did not receive parasitoids, ++Control II did receive parasitoids
*percentage of parasitism was calculated as number of parasitized individuals/number of settled first instar × 100 (Hoddle *et al.* 1999)

For plant colonization by *E.* sp. nr. *furuhashii*, the number of adult parasitoids on plants sprayed with artificial honeydew of GFT was consistently higher than those treated with other treatments throughout the experimental periods. When counted 2 and 6 hours later, there was no significant difference among the different treatments

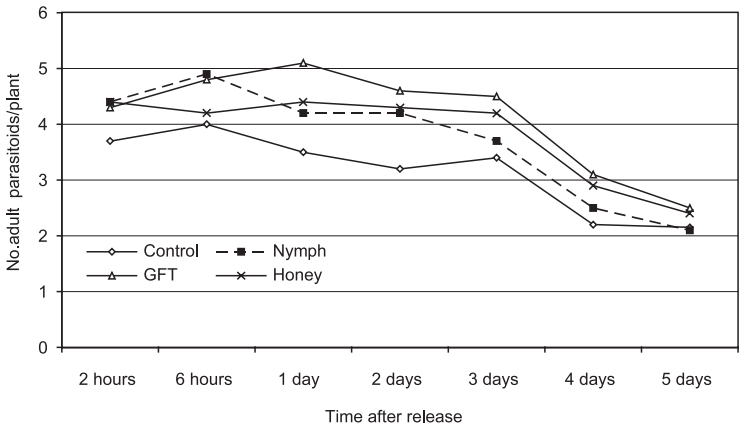

Fig. 1. Mean number of adults of *E.* sp. nr. *furuhashii* aggregated on cucumber plants treated with artificial honeydew of GFT, *B. tabaci* nymph-extract or honey solution in greenhouse (Guangzhou, China, 2003)

($p = 0.6822$ for 2 hours; $p > 0.3188$ for 6 hours). The counted numbers of the resident parasitoids on cucumber plants increased significantly in the artificial honeydew of GFT treatment a day ($p > 0.0471$) and 2 days ($p > 0.0852$) after release, but there was no significant difference among nymph-extract, honey and control treatments. Three days after release, the application of artificial honeydew of GFT and honey arrested more parasitoids and hence, elicited significant differences as compared to the control ($p > 0.0168$). In the last 2 investigated intervals (4 and 5 days after parasitoid-release), the average number of adult parasitoids per plants decreased gradually in all conducted treatments and no significant difference was observed between treatments (Fig. 1).

DISCUSSION

In this study, data of life table proved that the highest loss due to the unknown cause was recorded in the first instar cohort. This is mainly due to the failure to find the proper feeding position. These findings are in the same trends of those reported for *B. tabaci* in the presence of *E. eremicus* (Hoddle *et al.* 1999) or *E.* sp. nr. *furuhashii* (Mandour *et al.* 2006) or even in absence of the parasitoids (Qiu *et al.* 2003). Clearly, host feeding by female parasitoids was responsible for elevating the mortality rates by the unknown cause in parasitoid-released treatments. This inference goes with that reported by Hoddle *et al.* (1999) for the parasitoid *E. eremicus* Rose & Zolenerowich on poinsettia plants in the greenhouse.

The application of artificial honeydew (GFT and honey) or nymph-extarct enhanced the performance of *Eretmocerus* sp. in forms of higher rates of parasitism. Similarly, Staple *et al.* (1997) studied the influence of extrafloral nectar, sucrose, or whitefly honeydew on host- and food-searching behavior of *Microplitis croceipes* (Cresson). In their experiments, parasitoids that were starved for 2 days before releasing in plots with either sucrose or extrafloral nectar showed longer residence times and higher rates of parasitism than those in patches without food supplement. In contrary, Budenberg *et al.* (1992) found that the parasitoid *Aphidius rhopalosiphi* De Stephani-Perez increased their residence times on plants in the presence of artificial honeydew, but the rate of parasitism of *Sitobion avenae* (F.) did not elevate significantly over that of control. The failure in the latter study is probably due to the fact that the quantity and quality of the natural honeydew as food/arrestant source are relatively low compared to the artificial honeydew used in our study.

The presence of non-host kairomone or food like honey could unambiguously play a profound role in the parasitoid-host dynamics. In this study, the supplement of honey as an arrestant as well as food source enhanced the parasitization efficiency of *E.* sp. nr. *furuhashii*. These findings are in accordance to those of Wäckers (2003) who reported that the supplement of honey as food source increased the parasitoid *Anisopteromalus calandrae* Calandrae offspring in relative to its host *Callosobruchus chinensis* (L.) population.

For *B. tabaci* nymphs extract, the obtained data are encouraging and in the same trend as in earlier studies. For example, the aqueous extracts of *Aphis craccivora* significantly enhanced the rate of parasitism, reproduction, and the searching area by parasitoid (Singh and Srivastava 1989). Similarly, the application of the aqueous extract of *Aphis fabae* (Scope.) on clean *Vicia fabae* L. (Fab.) leaves stimulated females of

Metasyrphus corollae Fabr. to intensify their search on the sprayed plants when compared to control. Subsequently, females laid eggs at all aphid densities and number of eggs was proportional to aphid-extract concentration (Shonouda 1996).

In this study, the aggregation of *E.* sp. nr. *furuhashii* on plants sprayed with nymph-extracts was inferior to artificial honeydew and honey treatments, but still higher than that of control. These findings are in harmony with those reported by Ben Saad and Bishop (1976) who used artificial honeydew consisted of molasses, honey and tryptophan alone or combined with Feed Wheast®. The treated potato plants with the sprays attracted some natural enemies *viz*., *Chrysopa carnea* Stephens, *Hippodamia* spp., *Coccinella transversoguttata* Brown, *Scymnus postpinctus* Casey, and *Geocoris pallens* Stal. Analogously, the colonization of *Ephedrus cerasicola* on plant contaminated with natural honeydew in cage or greenhouse experiments was higher than uncontaminated plants (Hagvar and Hofsvang 1989). Artificially applied honeydew of GFT as well as honey solution was effective in attracting/retaining *E.* sp. nr. *furuhashii* on cucumber plants up to 3 days after application. These findings are in the same trend with those reported by Shaltiel and Ayal (1998). In their study, honeydew also lost its kairomonal activity after 3 days of application.

In conclusion, the present study has shown that performance of *E.* sp. nr. *furuhasii* was strongly affected by the applied kairomonal sources. Also, the application of these materials elevated the rates of parasitism over that of control and subsequently yielded lower rates of *B. tabaci* survival. These findings, could open new avenues for the use of specific or even non-specific artificial honeydews in *B. tabaci* – agroecosystem and be tailored to increase the effectiveness of *B. tabaci* parasitoids by enhancing their nutritional state, host location, and reproduction. However, the effect of these materials, particularly artificial honeydew, on the reproductive biology of both *B. tabaci* and its natural enemies should be conducted first before any ultimate conclusion can be drawn.

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POLISH SUMMARY

WPŁYW RÓŻNYCH ŹRÓDEŁ KAIROMONÓW NA AKTYWNOŚĆ GATUNKU *ERETHMOCERUS* NEAR *FURUHASHI* PRZECIW *BEMISIA TABACI* NA OGÓRKU: II – WARUNKI SZKLARNIOWE

Badano skutki zastosowania sztucznej mieszaniny glukozy, fruktozy i trehalozy (GFT) imitującej spadź oraz miodu i ekstraktu z nimf *Bemisia tabaci* na ogórku jako źródła kairomonów dla zwiększenia poszukiwania pożywienia i występowania gatunku *Erethmocerus* near *furuhashi*. Eksperymenty przeprowadzono w małych szklarniach (4x3x3 m) posługując się metodą 'life table'. Dane uzyskane za pomocą tej metody wykazały, że ogólna śmiertelność w niedojrzałych kohortach *B. tabaci* malała następująco: czwarte stadium > pierwsze stadium > drugie = trzecie > jajo > poczwarka. Testowane preparaty kairomonowe miały istotny wpływ na stopień pasożytowania ($p > 0.0415$), który był wyższy niż w przypadku kontroli odpowiednio o 13,23%, 9,04% i 10,54% dla sztucznej spadzi, ekstraktu z nimf i miodu. Znaczny także był wpływ źródeł kairomonów na stosunek przetrwania jajo/dorosły osobnik *B. tabaci* ($p > 0,0001$), a najniższy (22,91%) zaobserwowano w przypadku ekstraktu z nimf. Ponadto, preparaty kairomonowe przyciągnęły znacznie więcej parazytoidów do roślin traktowanych niż do kontrolnych. Testowane preparaty działały efektywnie do trzech dni po ich zastosowaniu.