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RECOGNITION OF CERTAIN KAIROMONES BY THE PARASITOID *TRICHOGRAMMA* SPP. IN EGGS OF IT'S HOST *CYDIA POMONELLA*

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ABSTRACT

A kairomone from adult *Cydia pomonella* L. scales is an important factor in the host recognition and searching behaviour process of *Trichogramma cacoeciae* Marchal and *Trichogramma dendrolimi* Matsumura. The *Trichogramma* spp. used the chemicals from the host eggs for orientation to host and increased their parasitism. The females of *T. cacoeciae**, *T. dendrolimi*** and *T. dendrolimi**** contacted the unwashed host eggs significantly longer than the washed eggs. Washing the codling moth eggs with hexan significantly reduced the parasitism of all the tested *Trichogramma* strains compared to unwashed eggs. The treated apple trees with wing scales of *C. pomonella* had significant influence on the parasitism compared to untreated trees. The percentage parasitism of codling moth eggs on the treated trees (31.6%) was significantly higher than on the untreated trees (19.3%).

Keywords: *Trichogramma cacoeciae*, *T. dendrolimi*, *Cydia pomonella*, Kairomone, Wing scales, Recognition, Searching behaviour, Parasitism

INTRODUCTION

Kairomones are used by parasitoid as cues for orientation to their host habitat, location and recognition during host selection (**Noldus, 1989**). The stage of host selection at which a female parasitoid uses kairomones depends upon the degree of her host specialisation (**Vet and Dicke, 1992**).The sources of kairomones used by *Trichogramma* spp. for host and host habitat location include sex pheromones and wing scales of host moths (**Noldus, 1989; Noldus *et al* 1990; Ananthakrishnan *et al* 1991**).

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* A strain collected from Germany 1990 and reared in Institute for Biological control, Darmstadt, Germany.

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Several studies demonstrated that the wing scales of host moths play an important role in the searching locomotion of *Trichogramma* females. **Noldus and van Lenteren (1983 and 1985)** reported that the wing scale of *Pieris brassicae* L., *P. rapae* L., and *Mamestra brassicae* L. contained a contact kairomone leading to arrestment of *T. evanescens* Westwood. Females of *T. evanescens* sought significantly longer on cabbage leaves treated with the wing scale of two hosts, *P. brassicae* and *P. rapae*. Also, the egg washes of *P. brassicae* had a contact-kairomonal effect on the parasites.

Recognition of specific kairomones was also acquired by learning, through the association of the host and its kairomones during oviposition **(Alphen and Vet, 1986). Huang and Gordh (1998)** tested the oviposition behaviour of female *T. australicum* Girault on host eggs and glass beads and found that females did not depend on chemical cues to recognize their host eggs.

The effect of kairomones on the searching capacity of three *Trichogramma* spp. *T. cacoeciae**, *T. dendrolimi*** and *T. dendrolimi**** to parasitize host eggs of *Cydia pomonella* L. was studied by a laboratory host preference test with washed and unwashed host eggs. Also, a semi-field test was conducted, using small apple trees planted in plots, which were treated with *C. pomonella* wing scales.

MATERIAL AND METHODS

Cydia pomonella as a host insect and three strains of *Trichogramma* parasitoids, *T. cacoeciae**, *T. dendrolimi*** and *T. dendrolimi**** were successfully mass reared according to **(Wührer and Hassan, 1993)** at the Department of Plant Protection, Faculty of Agriculture, Ain Shams University, Egypt.

The following two experiments were conducted under laboratory conditions of 27 ± 1 °C, 60 to 80% relative humidity.

Laboratory experiment

Approximately one day old eggs laid on plastic sheet, were collected from the established *C. pomonella* culture. The eggs (on their plastic sheet) were washed by soaking them in hexan for 10 minutes and then rinsed five times in the same solvent, **(Huang and Gordh, 1998)**. The eggs were then rinsed five times with water and then air-dried for an hour before their use in the experiment. Unwashed *C. pomonella* eggs served as the control.

The host preference and acceptance test used in this study was slightly modified from that developed by **Hassan (1989)**. Individual *Trichogramma cacoeciae**, *T. dendrolimi*** and *T. dendrolimi**** females aged approximately 24 hours, were selected from the stock culture and isolated individually in small glass tubes. To accomplish this, newly emerged adults were scattered from the rearing tubes on a white sheet of paper, they were individually captured by placing a small glass tube (50 mm long and 9 mm in diameter), on each insect. As the parasite walked up in the tube towards a source of light, their sex could be easily determined under a binocular. The captured female was transferred into a larger experimental glass tube (100 mm long, 26 mm in diameter) that included 50 washed and another 50 unwashed eggs of *C. pomonella*, which were placed in two

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groups on opposite corners of a piece of paper measuring 25 x 20 mm. A droplet of honey-agar was added in the centre of the searching arena, the glass tube was closed with cloth material. The opening of the tube was sealed with black paper to prevent the insects escape and the tube was placed horizontally in a tray. The test was monitored every thirty minutes for a total period of 4 hours and the location of the female in the test tube was recorded.

After 5 days, the washed and unwashed *C. pomonella* eggs were separated and each transferred into new tubes. After emergence of *Trichogramma* adults and following their death they were counted as well as the number of black eggs, specifying their parasitism.

The experiment was repeated 25 times for each of the three *Trichogramma* strains considered.

Semi-field test

The semi-field experiment was conducted only on *T. cacoeciae**. A wooden-frame cage measuring 120 high, 48 wide and 60 cm long was prepared, three sides of the cage was covered with muslin, the fourth side as well as it's roof was covered with plastic. Small apple trees grown in pots each measuring 100 cm in height, was placed in each cage. *C. pomonella* wing scales were collected from their moths and applied, by means of a camel hair brush, on the tree stem and up to the branches and leaves bearing the host eggs. Five newly deposited codling moth egg masses, with an average of 10 eggs each, were placed on the apple tree leaves upper surface.

T. cacoeciae adults were released by placing a glass tube (100 × 26 mm) containing an average of 1500 females on the floor of the cage. The host eggs were collected and removed after 2, 4 and 6 days from exposure to the *Trichogramma* adults and replaced with the same number of *C. pomonella* newly laid egg masses. The collected eggs were incubated at 25°C and 70 – 80% relative humidity until they turned black and rate of parasitism determined. The experiment lasted six days and was replicated three times. The same experiment was conducted but without the application of *C. pomonella* wing scales, to represent the control.

Data analysis

The data of laboratory glass tube test on parasitism and emergence were analysed using generalised Model (PROC GLM), **SAS Institute, (1996).** The contact time of the parasitoids with host eggs was analysed using Chi- Square test **(SAS, 1996).**

The semi-field data were subjected to analysis of variance (ANOVA) using the Linear Models (GLM) procedure **(SAS Institute, 1996).** Data of percentage parasitism was transformed (arcsine) for the ANOVA **(Sokal and Rohlf, 1981 and Scheiner, 1993).**

RESULTS AND DISCUSSION

Laboratory experiment

The results of the glass tube test to determine the preference of *Trichogramma* to hexan washed or unwashed *C. pomonella* eggs are shown in **Figures 1, 2 and 3.**

The females of the three species *T. dendrolimi***, *T. cacoeciae** or *T. dendrolimi****, contacted the washed host eggs of *C. pomonella* significantly less by 9.5, 6.5 and 4.5% respectively than on

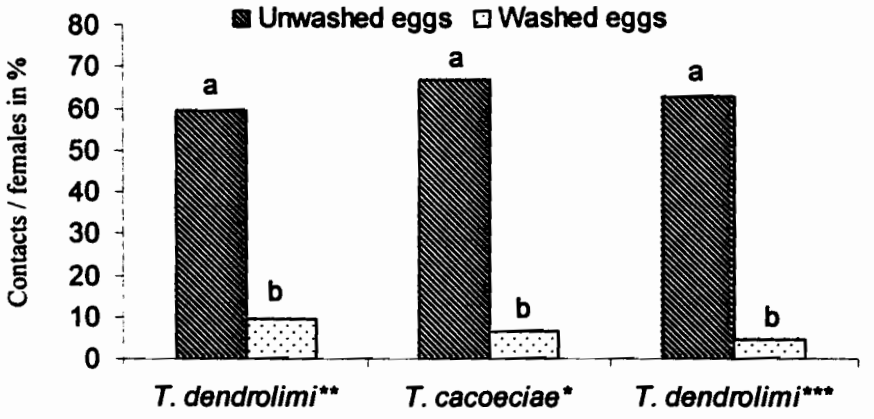

Figure 1. Frequency of contacts in 8 observations of three *Trichogramma* strains when offered the choice between hexan washed and unwashed eggs of *Cydia pomonella*

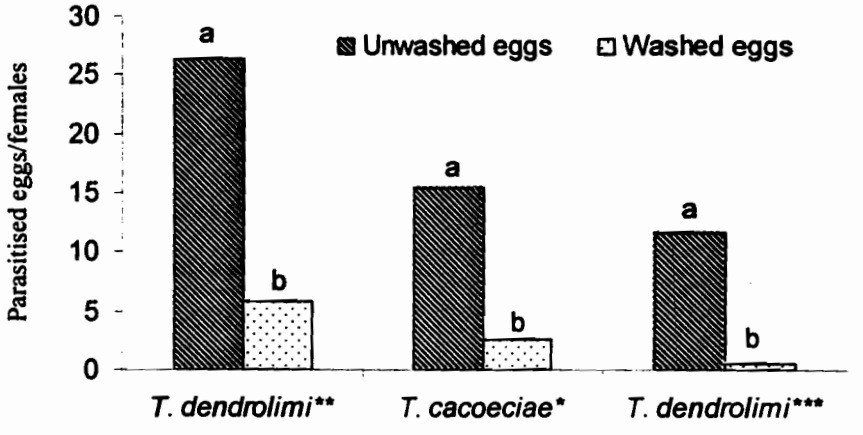

Figure 2. Frequency of parasitism of three *Trcichogramma* strains when offered the choice between hexan washed and unwashed eggs of *Cydia pomonella*

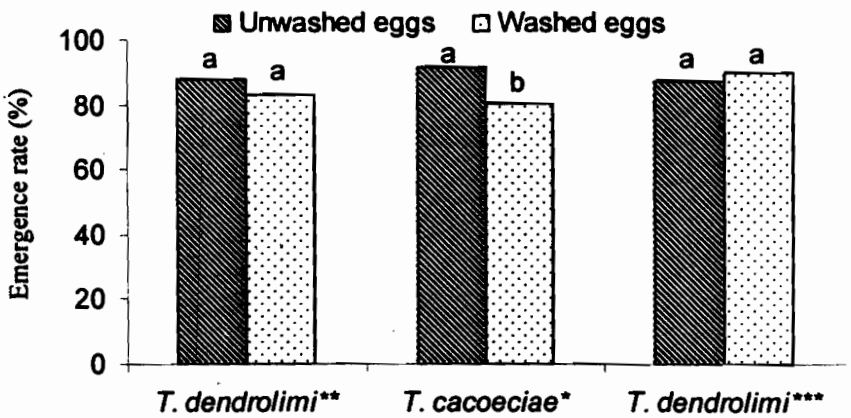

Figure 3. Emergence rate of three *Trichogramma* strains out of hexan washed and unwashed eggs of *C. pomonella*

unwashed host eggs *i.e.* 59.5, 67.0 and 62.5 % respectively, (F= 442.73) (**Figure 1**).

Washing *C. pomonella* eggs with hexan also significantly reduced parasitism by all three *Trichogramma* species. The rate of parasitism was found to be 5.8, 2.7 and 0.6 by *T. dendrolimi***, *T. dendrolimi**** and *T. cacoeciae**, respectively as compared to parasitism on unwashed eggs, *i.e.* (26.4, 15.4 and 11.7 respectively) to the three respective mentioned *Trichogramma* species (F = 224) (**Figure 2**).

No significant differences were found in the rate of adult emergence of the three considered strains of *Trichogramma* in hexan washed and unwashed of *C. pomonella* eggs (F = 1.32) (**Figure 3**).

Semi-field experiments

Small pillar apple trees treated with the wing scales of the host moth *C. pomonella* significantly increased parasitism by *T. cacoeciae** on codling moth eggs, reaching 31.6% as compared to 19.3% parasitism on eggs placed on trees not treated with moth scales, [**Table 1** (F = 6.36) and **Figure 4**].

Table 1. Statistical analysis of searching capacity on *T. cacoeciae** to parasitize *C. pomonella* eggs on apple trees treated and untreated with *C. pomonella* wing scales

Source of variation	df	F value	P
Wing scales	1	6.36	.0136
Time	2	56.16	.0001
Wing scales *time	2	4.22	.0180

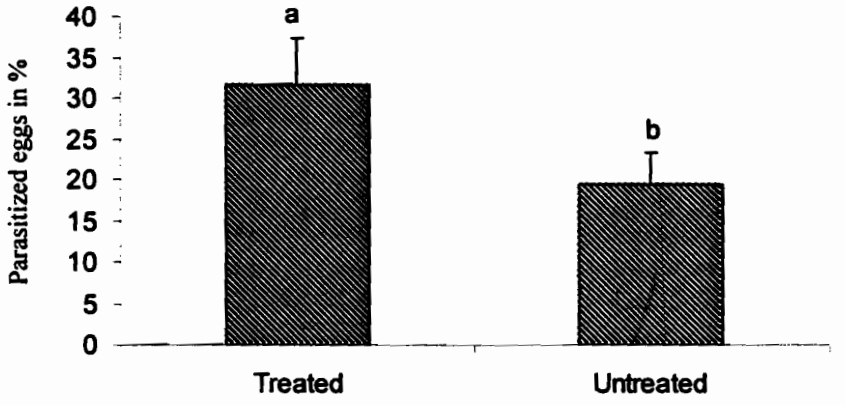

Figure 4. Parasitism (%) by *Trichogramma cacoeciae** using codling moth *Cydia pomonella* eggs on single potted apple trees treated and untreated with wing scales in cage.

As exhibited in **Figure 5**, percentage of parasitism was highest during the first two days following release reaching 75.3 ± 8.0 % and 44.0 ± 5.6 % on *C. pomonella* eggs placed on trees treated with wing scales and untreated trees, respectively. Egg parasitism gradually decreased in the following days to reach 16.7 and 2.7 % on the fourth and sixth day, respectively, following *T. cacoeciae* release on treated trees. This percentage was 14.0 and 0.0 % to the respective mentioned days for *C. pomonella* eggs placed on trees not treated with the moth wing scales.

Although at the three inspection periods rate of parasitism was always higher in *C. pomonella* eggs in trees treated with codling moth wing scales, this difference was only significant at the first inspection *i.e.* two days following release. At the following two inspections the difference was non significant.

DISCUSION AND CONCLUSIONS

In general, *Trichogramma* spp. are polyphagous parasitoids and appear to recognise their hosts by using physical features rather than host specific recognition kairomones (**Schmidt, 1994**), which are used by other genera of oligophagous parasitoid wasps (**Strand & Vinson, 1982, 1983; Bin *et al* 1993**). Few studies suggest that chemical cues might promote or inhibit host acceptance by some *Trichogramma* spp. (**Nordlund *et al* 1987**).

The chemical factors that *Trichogramma* spp. depend on to recognise their host vary among species in the genus. About 47% of the female *T. australicum*

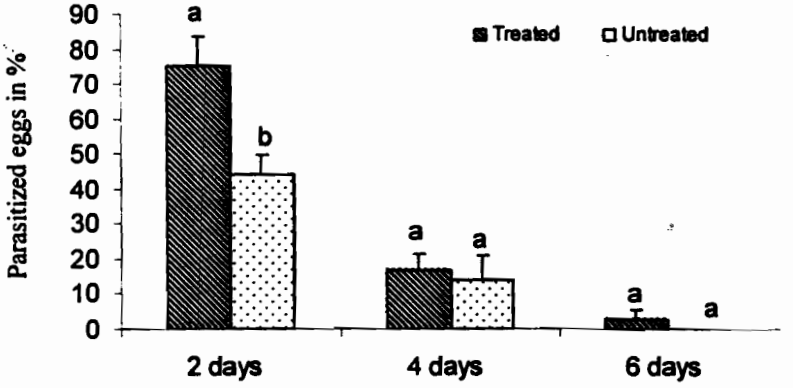

Figure 5. Percentage of parasitism by *T. cacoeciae** on *C. pomonella* eggs placed on treated with wing scales of the moth and untreated trees after different intervals from release

females attempted to settle on clean glass beads (**Huang & Gordh, 1998**), only 18% of *Trichogramma pretiosum* Riley females accepted clean glass beads lacking an accessory gland secretion recognition. The acceptance of the wasps increased to 60% when the beads were treated with accessory gland secretion (**Nordlund *et al* 1987**).

Results of the present work showed that three *Trichogramma* strains *T. dendrolimi****, *T. dendrolimi*****, and *T. cacoeciae** were able to recognise chemicals from their host *C. pomonella* eggs as depicited by an increased rate of parasitism. Females of these three *Trichogramma* strains contacted unwashed host eggs significantly longer than hexan washed eggs. Washing the codling moth eggs with hexan significantly reduced parasitism. In a similar study, **Noldus and van Lenteren (1983 and 1985)** showed that *T. evanescens* females sought significantly longer on cabbage leaves sprayed with a methanol or water wash of *P. brassicae* eggs than on leaves treated with solvent only. However, **Huang and Gordh (1998)** indicated that washing *H. armigera* eggs with various organic solvents or immersing eggs for 1 h in deionised water had no effect on the acceptability of *T. australicum* females.

Among the kairomones that *Trichogramma* wasps use as cues for host location are wing scales of the host moth. The results of this study showed the positive effect of the wing scales of the codling moth on the parasitism of *T. cacoeciae**, on this moth eggs. **Noldus and van Lenteren (1983 and 1985)**, reported the contact-kairomonal effects of wing scales of *Mamestra brassicae* for *T. evanescens* as a significant higher number of landings occurred on treated leaves. **Lewis *et al* (1975)** indicated the ability of field-applied tricosane, a compound identified from the scales of moth, to increase rates of parasitization by *Trichogramma* spp.

It could be concluded that the chemical host recognition cues were beneficial to *T. cacoeciae** females. The kairomone from the egg surface and the wing scales of codling moth improved the reaction of the three selected *Trichogramma* strains wasp to eggs of it's host *C. pomonella.*

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مجلة اتحاد الجامعات العربية للدراسات والبحوث الزراعية ، جامعة عين شمس ، القاهرة ،١٤(٢) ، ٨٣٥-٨٤٤ ، ٢٠٠٦

دور كيرومونات فراشة دودة ثمار التفاح في التعرف على العائل والسلوك البحثي لطفيل انترايكوجراما

[٥٤]

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يعتبر الكيرومون الموجود بحراشيف الحشرة الكاملة لدودة ثمار التفاح عاملا هاما في تعرف نوعي طفيل البيض *Trichogramma cacoeciae* and *T. dendrolimi* على عائلهما بالإضافة إلى قدرتهما على سلوك البحث . وجد أن كلا النوعين من الطفيليات يستخدمان رائحة بيض العائل في التعرف عليه مما يزيد من نسبة التطفل . وقد حدث اتصال من إناث الطفيليات *T. dendrolimi* 1984, *T. dendrolimi* 1990 and *T. cacoeciae* 1990 إلى بيض العائل غير المغسول بالهكسان بدرجة عالية بالمقارنة بنظيره الذي سبق غسلة بالهكسان. وقد أدت عملية الغسيل إلى إنخفاض معنوي في نسبة التطفل مقارنة بالبيض العادي (غير المغسول) في جميع أنواع وسلالات الترايكوجراما المختبرة.

وقد زادت نسبة التطفل بدرجة معنوية على أشجار التفاح المعاملة بحراشيف دودة ثمار التفاح بالمقارنة بالأشجار غير المعاملة. حيث وصلت نسبة التطفل على بيض هذه الآفة إلى ٣١,٦% على الأشجار المعاملة بينما كانت ١٩,٣% فقط على الأشجار غير المعاملة.

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