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# Kairomones of *Heliothis armigera* and *Corcyra cephalonica* and their influence on the parasitic potential of *Trichogramma chilonis* (Trichogrammatidae: Hymenoptera)

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**Abstract.** Kairomones from moth scales tend to influence the parasitic potential by *Trichogramma chilonis* Ishii. Hexatriacontane, pentacosane, heptadecane, docosane and 2, 6, l0-dodecatrienal-3, 7, 11-trimethyl were identified from the active moth scale extract of *Heliothis armigera* Hubner (its natural host) and *Corcyra cephalonica* Stainton (a laboratory host). The significance of an array of compounds from moth scales with kairomonal activity for manipulating entomophagous insects in biological control programmes is discussed.

**Keywords.** Kairomones; *Heliothis armigera; Corcyra cephalonica; Trichogramma chilonis;* biological control of insects.

## 1. Introduction

*Trichogramma chilonis* is widely distributed in the Indian subcontinent and is responsible for large-scale mortality of the American boll worm *Heliothis armigera* in several crops and efficiently controls other lepidopteran and heteropteran insect pests (Manjunath *et al* 1985). Successful establishment, retention in target sites and manipulated behaviour of natural enemies are important components of a successful biological control programme (Gross *et al* 1975). Use of chemicals emanating from the host and its by-products which enhance the behavioural dynamics of entomophages increasing their effectiveness were advocated by Brown *et al* (1970), Whittaker and Feeny (1971) and Lewis *et al* (1975a). Lewis *et al* (1975b) showed that the efficiency of *Trichogramma* sp. released into the field was significantly improved by the application of the kairomone, tricosane. Studies by Gross *et al* (1975), Nordlund *et al* (1976, 1984), Lewis *et al* (1982), Elzen *et al* (1984) and Nordlund (1987) clearly suggest that kairomones originating from both the hosts and their food sources influence the searching, attacking and retention of entomophagous insects. This is an aspect of great interest in biological control programmes.

Shu and Jones (1989) observed the klinokinetic and orthokinetic behaviour of *Trichogramma nubilale* Ertle and Davis in response to kairomones from the scales of its host, *Ostrinia nubilalis* (Hubner), while Shu *et al* (1990), identified, isolated and synthesized 11, 15, 13, 7 and 15, 10-dimethyl nonatriacontanes as kairomonal substances that influenced the host-seeking behaviour and parasitism of *T. nubilale.* The pattern of kairomonal application both in laboratory and field is also an

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Abbreviation used: SES, Stimulated egg surfaces.

important factor in the response elicited from *Trichogramma,* as evident from the investigations of Lewis *et al* (1975b).

In view of their potential importance to biological control programmes, an attempt was made in the present study to determine the nature and role of kairomones emanating from *H. armigera* scales on the parasitic potential of *T. chilonis.* This study also includes the evaluation of kairomones from *Corcyra cephalonica,* the factitious laboratory host of *T. chilonis.*

## 2. Materials and methods

### 2.1 *Insect cultures*

The eggs of *H. armigera* were obtained from the culture facility unit at the Entomology Research Institute, the alternate generations maintained on a standard semisynthetic diet and on a preferred host plant *Gossypium hirsutum* bolls (MCU 11). Adults were kept in mating cages (10 ×10") with *Cicer arietinum* L. plants for oviposition. Freshly laid eggs from the plants (mostly laid during the previous night) were removed every morning for experimental purposes. Eggs were washed in 0·1 % sodium hypochloride to avoid possible viral contaminations. The larvae and adults were reared at 29°C ± 2°C under 12D: 12L photoperiodic regimes. The eggs of *C. cephalonica* were obtained from the parasitoid culture unit of the Institute, especially for parasitoid colony maintenance. *C. cephalonica* larval cultures were maintained on a diet comprising pearlmillet and groundnut powder mixed in the ratio of 4: 1.

### 2.2 *Moth scale collection and extraction*

Scales of *H. armigera* and *C. cephalonica* were collected from freshly emerged laboratory reared moths by immobilizing them at 0–2°C. The wings were removed from about 50 moths. Abdominal scales were obtained by placing immobilized moths in large test tubes and shaking them. The collected wings and abdominal scales were extracted by vigorous shaking for 2 h in 200 ml analytical grade hexane and heating for 20 min at 50°C. The hexane fraction was subsequently concentrated by vacuum evaporation at 40° C.

### 2.3 *Isolation and identification*

The hexane extracts of the moth scales are injected into a coupled gas Chromatograph (Hewlett Packard 5890) with mass spectral detector, GC/MSD chemstation and a mass spectral library containing more than 40,000 compounds. Fused silica capillary column (10 × 0·2 m) with a cross-linked methyl silicon phase was used. Helium was used as the carrier gas. The temperature programme was 40° to 250° C at 5°C rise per min with a two min solvent delay. Injector transfer line and ion sources were set at 230° and 220° C respectively. Mass spectral data obtained during the assay were compared with the mass spectra of compounds available in the chemstation NBS 49K library.

2.4 *Bioassay of scale extracts in Petri dishes*

Comparative assessments of parasitism rates of *H. armigera* eggs by *T. chilonis* in Petri dishes in response to different treatments of *H. armigera* and *C. cephalonica* kairomones were made. The bottom of the 20 cm diameter Petri dish was covered with a filter paper (Whatman No. 1). In kairomone the treatments were restricted to egg sites herein referred to as stimulated egg surfaces (SES). UV-irradiated *H. armigera* eggs were arranged equidistant from one another in a circular ring. Hexane extract was applied at each egg site at the rate of 2 $\mu$l (total = 24 $\mu$l) using a syringe. In addition to spraying at random, in whole dish treatment, the entire surface was sprayed with 24 $\mu$l scale extracts. The bioassay of intact scales was performed by collecting from fresh immobilized moths and spraying over Petri dish surfaces. A control was maintained where only hexane was used. Eggs were glued to the respective egg site using a synthetic Fevibond adhesive. Two freshly emerged mated females of *T. chilonis* were introduced into each Petri dish and allowed to search for 4 h and the observations made during subsequent days for dark-coloured parasitized eggs. For each treatment, 10 observations were made and all the treatments conducted simultaneously in laboratory conditions.

2.5 *Bioassay of scale extracts in potted plants*

Twelve irradiated *H. armigera* eggs were placed on greenhouse grown cotton plants (approx. 1·5 feet in height). The three-scale extract treatment pattern includes (i) SES, (ii) random application and (iii) whole plant application. For random and whole plant application, scale extracts were sprayed using a chromatographic sprayer. Scale extract (15 ml) was used in various treatments in potted plant bioassay. To assess the moth scales, fresh scales were spread uniformly over the plant surfaces. Five mated *T. chilonis* females were introduced after covering the plant with a polythene sheet. The eggs were collected after a 6 h exposure to parasitoids and kept in small vials. Parasitized eggs were identified by their black colouration. Each treatment comprised ten replicates and all treatments conducted simultaneously. The data obtained in various treatments both in Petri dishes and potted plants were analysed by one-way ANOVA test (Zar 1974).

2.6 *Bioassay of scale extracts on parasitism rates in relation to parasitoid age*

Twenty fresh irradiated *H. armigera* eggs were glued to a paper strip. In experimental sets, the strips received a spray of scale extracts (*H. armigera, C. cephalonica* separately), while control strips received only hexane. The egg strips were placed in a test tube, to which a mated *T. chilonis* female was introduced. The egg strips were replaced everyday with fresh egg strips until the fifth day of the experiment. The number of dark coloured eggs was counted in each strip during all the five days. The experiments were conducted simultaneously and include 5 observations. The method of Nordlund *et al* (1976) was followed with a slight modification for this test. The data obtained in this investigation were analysed by Student *t* test analysis.

## 3. Results

### 3.1 *Isolation of kairomonal compounds from scale extracts*

Figures 1 and 2 indicate the total ion chromatogram of scale extracts of *H. armigera* and *C. cephalonica* respectively. Comparison of the mass spectra obtained from various peaks matched with the standard kairomonal compounds in NBS 49K volatile chemical library in GC/MSD chemstation. The notable compounds identified were docosane, pentacosane, hexatriacontane, nonacosane, heptadecane 2, 6, 10, 15-tetramethyl etc. A list of compounds identified from the scales of *H. armigera* and *C. cephalonica* is provided in table 1 along with their matching

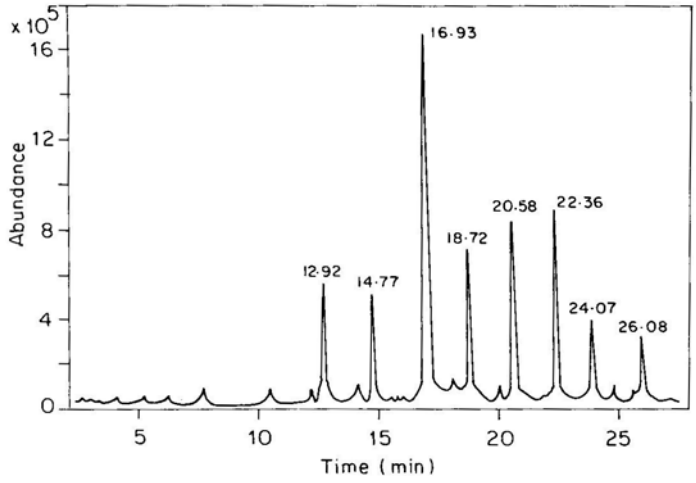


**Figure 1.** Gas chromatogram of *H. armigera* scale extracts.

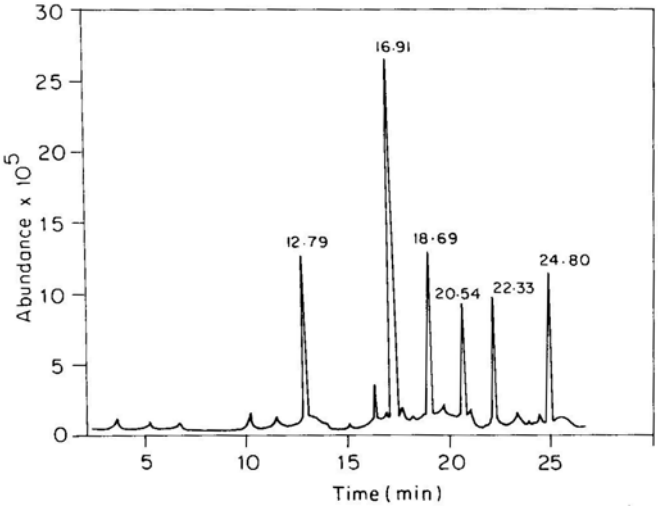


**Figure 2.** Gas chromatogram of *C. cephalonica* scale extracts.

**Table 1.** Compounds identified from *H. armigera* and *C. cephalonica* moth scales.

| Kairomone source | Retention time | Identified* compound | Quality (%) |
|---|---|---|---|
| *H. armigera* scales | 12·92 | 1, 2-Benzenedicarboxylic acid butyl octyl ester | 64 |
| | 14·77 | Docosane | 88 |
| | 16·93 | Hexatriacontane | 94 |
| | 18·72 | Tridecane, 1-iodo | 89 |
| | 20·58 | Hexatriacontane | 96 |
| | 22·36 | Heptadecane, 2, 6, 10, 15-tetramethyl | 92 |
| | 24·07 | Heptadecane, 2, 6, 10, 15-tetramethyl | 93 |
| | 26·08 | Heptadecane | 88 |
| | 12·79 | 2, 6, 10-Dodecatrienal 3, 7, 11-trimethyl | 88 |
| *C. cephalonica* scales | 16·91 | Pentacosane | 94 |
| | 18·69 | Pentacosane | 92 |
| | 20·54 | Hexatriacontane | 64 |
| | 22·33 | Nonacosane | 54 |
| | 24·80 | Hexadecane | 84 |

*Mass spectra of respective compounds are compared with NBS 49K library compounds for percentage matchability.

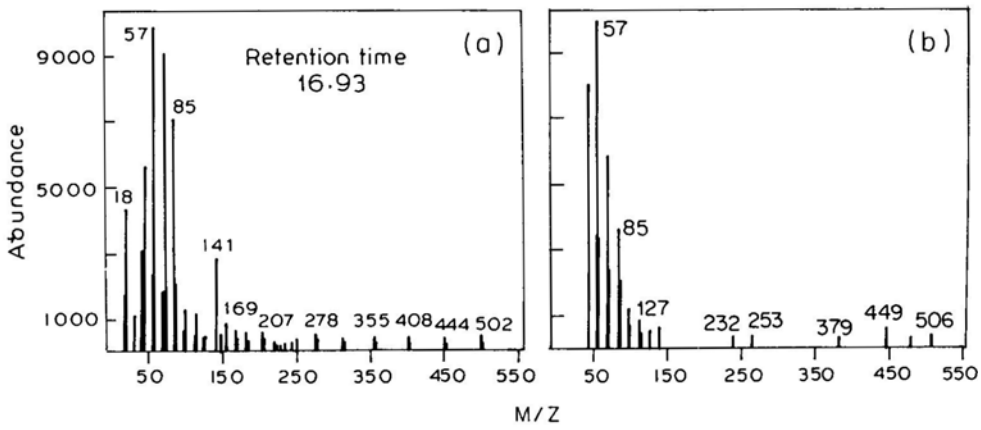


**Figure 3.** Mass spectrum of (**a**) isolated compound (**b**) standard hexatriacontane.

percentage with standard compounds. Figure 3 and 4 provide the mass spectra of authentic hexatriacontane and pentacosane compounds respectively along with compounds obtained in GC separation.

### 3.2 *Petri dish bioassay*

The results on Petri dish bioassays indicated highest parasitization ($\bar{X}$ = 83·4) when the whole filter paper had been treated with *H. armigera* scale extracts followed by SES ($\bar{X}$ = 74·6) as compared to random treatment. *C. cephalonica* moth scale extracts also increased parasitism rates when the entire surface ($\bar{X}$ = 68·5) was treated

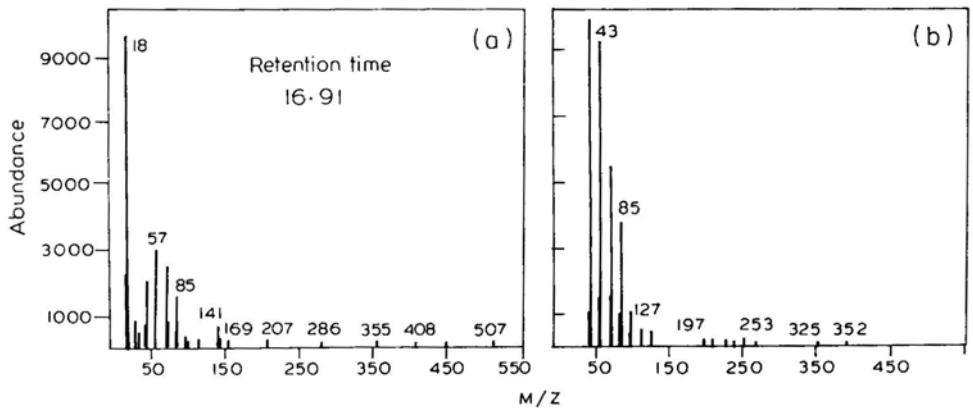


**Figure 4.** Mass spectrum of (**a**) isolated compound (**b**) standard pentacosane.

**Table 2.** Petri dish bioassay of moth scale extracts on parasitism rates on *T. chilonis* on *H. armigera* eggs.

| | Percentage parasitism | |
|---|---|---|
| | Scale extracts of | |
| Treatment | *H. armigera* | *C. cephalonica* |
| Control | 38·3 ± 1·77 | 38·3 ± 1·77 |
| SES | 74·6 ± 4·32 | 55·3 ± 2·99 |
| Random | 64·4 ± 3·32 | 54·3 ± 3·33 |
| Whole dish | 83·4 ± 5·17 | 68·5 ± 3·90 |
| Scales | 65·0 ± 3·34 | 53·5 ± 3·02 |
| CD at 5% level | 2·7 | 2·5 |

Data subjected to ANOVA ($P$ >0.05)

followed by SES (X = 55·3). Egg surfaces treated with fresh scales of *H. armigera* and *C. cephalonica* increased parasitism rates of *T. chilonis* and the values were 65 and 53·5% respectively. A comparison of data clearly demonstrate that parasitism by *T. chilonis* was significantly enhanced in moth scale extract treatments compared to control (X = 38·3) (table 2). The pattern of scale extract treatment exhibited a significant difference in the parasitism rates of *T. chilonis.*

### 3.3 *Potted plant bioassay*

Higher parasitism was recorded in plants treated completely with *H. armigera* and C. *cephalonica* moth scale extracts as compared to control ($\bar{X}$ = 58·4 and $\bar{X}$ = 47·7 respectively), SES form the next treatment to record higher parasitism and the values were 50·5 and 37·0% for *H. armigera* and *C. cephalonica* respectively compared to control ($\bar{X}$ = 12·4%). Random treatment of scale extracts as well as scales evoked lesser response among *T. chilonis* females compared to other treatment patterns (table 3).

**Table 3.** Potted plant bioassay of moth scale extracts on parasitism rates of *T. chilonis* on *H. armigera* eggs.

| Treatment | Percentage parasitism | |
|---|---|---|
| | Scale extracts of | |
| | *H. armigera* | *C. cephalonica* |
| Control | 13·97 ± 1·13 | 13·97 ± 1·13 |
| SES | 50·5 ± 2·68 | 37·0 ± 1·94 |
| Random | 41·3 ± 2·11 | 40·6 ± 2·28 |
| Whole dish | 58·4 ± 3·27 | 47·7 ± 2·46 |
| Scales | 45·4 ± 2·59 | 47·1 ± 2·97 |
| CD at 5% level | 1·15 | 1·12 |

Data subjected to ANOVA ($P > 0{\cdot}05$)

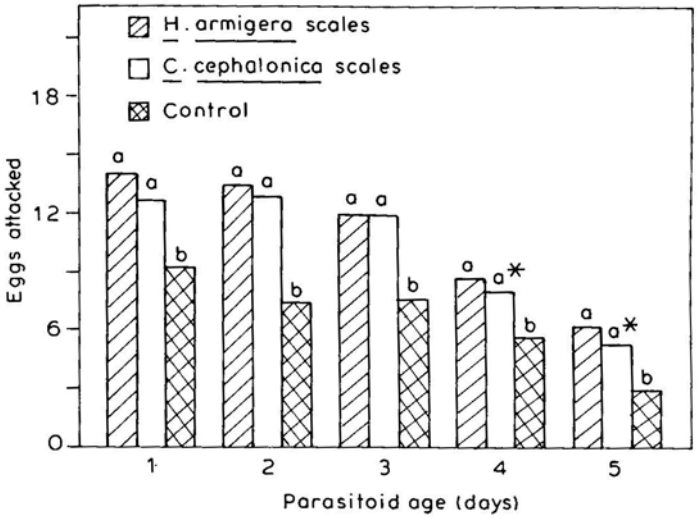


**Figure 5.** Parasitism by *T. chilonis* in moth scale treated and untreated egg cards at different parasitoid ages.
Data subjected to Students *'t'* test ($P$>0.05).
Columns followed by same alphabet letters and a* and b are not significantly different.

### 3.4 *Parasitism rates in relation to parasitoid age*

Figure 5 demonstrated that egg cards treated with *H. armigera* and *C. cephalonica* moth scale extracts increased parasitism rates of *T. chilonis* in relation to parasitoid age. The mean per cent parasitization was significantly different in *H. armigera* moth scale extract treated egg cards compared to control. The degree of parasitism in *C. cephalonica* moth scale treated egg cards was significantly different from control during the second and third day and no significant difference was noted during subsequent days.

## 4. Discussion

The present results indicate that kairomonal compounds from *H. armigera* and *C.*

*cephalonica* moth scales increased parasitization when applied over target sites. This investigation supports the earlier work in this direction in a number of host-parasitoid systems (Lewis *et al* 1975a, b; Elzen *et al* 1984; Nordlund *et al* 1984; Nordlund 1987; Shu *et al* 1990). An analysis of *H. armigera* and *C. cephalonica* moth scales for possible kairomonal substances using gas chromatography indicated the presence of hexatriacontane, nonacosane, docosane, pentacosane and heptadecane. The significance of these kairomonal substances in behavioural manipulation of entomophagous insects was earlier emphasized and reviewed by Lewis *et al* (1976).

The data presented here strongly indicate the role of moth scale extracts in enhancing the parasitization rate of *T. chilonis* on *H. armigera* eggs. Our results fully corroborate those of Jones *et al* (1973) who implicated the compounds such as tetracosane, docosane, tricosane and pentacosane from *H. zea* moth scales as a kairomone that influenced parasitization by *T. evanescens.* Gross *et al* (1975) and Lewis *et al* (1975a) demonstrated the role of tricosane as a kairomone to *T. evanescens* and *T. achaea* Nagarajan and Nagarkatti. The compounds from *H. virescens* scales, 11-methylhentriacontane, 16-methyldotriacontane and 11, 13-methyltriacontane were reported to act as kairomones and influence parasitism rates by *Cardiochiles nigriceps* Viereck. The treatment pattern of kairomones plays a major role in activating the natural enemies as is evident from this study. Lewis *et al* (1975b) attributed this differential parasitoid response to various kairomone treatment patterns to continuous behavioural stimulus available to parasitoids in target sites.

Laboratory observations on parasitism rates by *T. chilonis* in response to scale extract treatments reveal the importance of kairomones from moth scales. Similar observations by Nordlund *et al* (1976) in *T. pretiosum* Riley support our study on the role of kairomones in improving parasitism. Kairomone compounds from *H. armigera* and *C. cephalonica* moth scales in the manipulation of parasitoid activity could play a major role in future biological control programmes, since large cultures of moths are available in parasitoid breeding laboratories for efficient extraction and use of kairomones.

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