ENHANCING THE PERFORMANCE OF THE EGG PARASITOID, *T. chilonis* ISHII (Trichogrammatidae : Hymenoptera)

By

SHAJNA P. K.

THESIS

submitted in partial fulfilment of the requirement
for the degree of

Master of Science in Agriculture

(AGRICULTURAL ENTOMOLOGY)

Faculty of Agriculture
Kerala Agricultural University, Thrissur

Department of Agricultural Entomology

COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA

2006

DECLARATION

I, Shajna P.K. (2004-11-18) hereby declare that this thesis entitled 'Enhancing the performance of the egg parasitoid, *Trichogramma chilonis* Ishii (Trichogrammatidae: Hymenoptera)' is a bonafide record of research work done by me during the course of research and this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Vellanikkara
Date: 26/10/06

Shajna

SHAJNA P.K.

CERTIFICATE

Certified that this thesis, entitled **'Enhancing the performance of egg parasitoid, *Trichogramma chilonis* Ishii (Trichogrammatidae: Hymenoptera)'** is a record of research work done independently by **Ms. Shajna P.K.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Dr.A.M.Ranjith
Chairman, Advisory Committee
Associate Professor
Department of Agricultural Entomology
College of Horticulture
Kerala Agricultural University
Thrissur, Kerala

Vellanikkara
Date: 26/10/06

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Ms. Shajna P.K.**, a candidate for the degree of **Master of Science in Agriculture**, agree that this thesis entitled **'Enhancing the performance of the egg parasitoid, *T. chilonis* Ishii (Trichogrammatidae:Hymenoptera)'** may be submitted by **Ms. Shajna P.K.,** in partial fulfillment of the requirement for the degree.

Dr. A.M. Ranjith
Chairman, Advisory Committee
Associate Professor
Department of Agricultural Entomology
College of Horticulture
Vellanikkara

Dr. Jim Thomas
(Member, Advisory Committee)
Associate Professor and Head
Dept.of Entomology
College of Horticulture
Vellanikkara

Dr. Lyla K.R.
(Member, Advisory Committee)
Associate Professor
Dept. of Entomology
College of Horticulture
Vellanikkara

S. Krishnan
(Member, Advisory Committee)
Assistant Professor
Department of Agricultural Statistics
College of Horticulture
Vellanikkara

(EXTERNAL EXAMINER)
Dean & Professor &
HOD (Entomology)
Pandit Jawaharlal Nehru College
of Agriculture, Nedungadu P.O
Karaikal -609603

ACKNOWLEDGEMENT

And so comes the time to look back on the path traversed during the endeavour and to remember the faces and spirits behind the action with a sense of gratitude. Nothing of significance can be accomplished without the acts of assistance, words of encouragement and gestures of helpfulness from the other members of the society.

I would like to thank Almighty for being my guiding light who always led me through the right path. First and foremost I bow my head before the Almighty God who enabled me to successfully complete the thesis work in time.

I avail this opportunity to express my deep sense of reverence, gratitude and indebtedness to **Dr.A.M. Ranjith**, Associate Professor, Department of Agricultural Entomology and Chairperson of my advisory Committee for his inspiring guidance, critical comments, constructive suggestions, constant supervision, support and encouragement throughout the course of my study period and in the preparation of this thesis. I am very fortunate to be his student and without his cooperation and advice, I would not have been able to complete this work.

I consider it as my privilege to express my deep-felt gratitude to **Dr.Jim Thomas**, Associate Professor and Head, Department of Agricultural Entomology and member of my Advisory Committee, for his sustained interest and constructive criticisms throughout the course of my work.

I respectfully thank **Dr. Lyla K.R.**, Associate Professor, Department of Agricultural Entomology and member of my Advisory Committee for her ardent interest, valuable suggestions, critical scrutiny of the manuscript and ever willing help, which has helped a lot in the improvement of the thesis.

I am genuinely indebted to **Sri. S. Krishnan**, Assistant Professor, Department of Agricultural Statistics, College of Horticulture for his valuable assistance and guidance during the statistical analysis of the data. His friendly attitude and cooperation helped me a lot for the smooth conduct of my analysis part.

I wish to extend my cordial thanks to **Dr. R. Ushakumari, Dr. Sosamma Jacob, Dr. Susannamma Kurien, Dr. Maicykutty P Mathew, Dr. Mani Chellappan, Dr. Haseena Bhasker, Dr. D. V. Sairam Kumar and Dr. Pathummal Beevi** of Department of Agricultural Entomology for their constant inspiration and friendly suggestions during the period of my study.

I am thankful to **Shaiby, Omana chechi, Archana, Sindhu, Ratheesh, George chettan** and all other non teaching staff of the Department of Agricultural Entomology for their sincere help and valuable co-operation which helped me a lot to carry out this investigation effectively.

Thanks are due to my seniors **Rameash Sir, Ramu, Renitha, Amritha, Prathiba, Thygarajan, Karthikeyan Sir, Deepthy, Smitha** and also my juniors **Jyothi, Hema and Jagdish.**

With all regards I sincerely acknowledge the wholehearted co-operation and generous help rendered by the members of YESCOM.

I am grateful to Mr. Santhosh of Computer club for his valuable suggestions and timely help rendered during the preparation of the manuscript.

I am happy to place on record my sincere thanks to my batchmates ***Sreerekha, Smisha, Shaneeja, Cinu, Sini, Lincy, Nisha, Chitra, Smitha, Shibi, Sara, Gayathri, Maggi, Kavya, Veera, Grace, Meghna, Sani, Dhanya chechi, Santhosh*** *and* ***Premjith*** *.*

My heartfelt thanks are due to my dearest roommates ***Neema, Ramya*** *and* ***Anjali*** *for their constant support, patient listening, ever-willing help and understanding all through the course of my study.*

Words cannot really express the true friendship that I relished from my classmate ***Seena*** *and I am thankful to her for the ever-willing help, timely suggestions and encouragement which gave me enough mental strength to get through all tedious circumstances.*

The award of KAU merit scholarship is deeply acknowledged.

Above all, I am in dearth of words to express my love towards ***my parents, my sisters,*** *and* ***my husband*** *whose continuous inspiration and prayers gave me the strength to successfully complete the thesis work.*

Shajna P.K.

Affectionately dedicated to

Vappa, Umma

&

Ikka

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

INTRODUCTION

1. INTRODUCTION

Increased attention is now being given for the implementation of Integrated Pest Management in rice with specific thrust on promoting biocontrol and reducing insecticide use to the bare minimum. There is abundant evidence of the vital role of natural enemies in suppressing the pest population in rice (Chelliah *et al.*, 1989). Integrated pest management techniques in rice to check leaf folder, involving inundative release of egg parasitoid *Trichogramma chilonis* has been reported to be successful in different rice growing regions across the country (Misra *et al.*, 1994; Shrike and Bade, 1997; Brar *et al.*, 2001, Beevi *et al.*, 2002).

In nature, there is always a resident population of the parasitoid that spurts in response to a population upsurge of the host. Kairomones trigger the influx of *T. chilonis* into a new habitat. Volatiles released by damaged plants after insect feeding tend to increase the efficiency of natural enemies and plants producing such alarm pheromones, which in turn will attract more parasitoids and predators. Kairomones like plant volatiles from weeds in the rice ecosystem, food lures and host insect volatiles can be supplemented in a cropping system to enhance the efficiency of the natural population.

Shelf life of a biocontrol agent is an important consideration for commercial success of a mass production programme. Mass production of natural enemies with a predictable period of adult emergence is essential for effectiveness of the released natural enemies (Paul and Sreekumar, 1998). Short term storage of trichogrammatids for two to three weeks is common in many of the biocontrol laboratories, but it is generally found that after storage, mortality is high, fertility is reduced and sex ratio is adversely affected.

Different temperatures have different effects on the adult emergence, longevity and fecundity. Refrigerated storage can be tried to enhance the optimum storage conditions for maximum parasitization by *Trichogramma.*

In the backdrop of the above situation, the present study was taken up to fulfill the following objectives.

- Identify insect host substances or plants that could be used to enhance the retention time of trichogrammatids in the field.
- To validate the positive response to semiochemicals by using olfactometric responses.
- Identify the degree of efficiency of the parasitoid emergence and percentage parasitism in refrigerated storage for utilizing as nucleus culture in *Trichogramma* production centres and by the farmers.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Rice is an important crop, which is attacked by 33 species of insects. Among these, the leaf folder has gained the status of major pest in south and south east Asia in recent years (Ramasubaiah *et al.*,1980; Bentur *et al.*, 1989). Among four leaf folder species recorded in India, *Cnaphalocrocis medinalis* (Guenee) cause heavy yield losses (Pandya *et al.*, 1987; Srivastava, 1988).

Many scientists have stressed the importance of the leaf folder as a major pest. Field studies conducted in Bihar during the kharif season of 1993 revealed that the infestations of *C. medinalis* varied from 1.4 to 33.2 per cent from July to October (Kumar *et al.*, 1996). The paddy crop (varieties CR1009 and ADT36) was heavily damaged by leaf folder during 1995-96 (Manisegaran *et al.*, 1997). Field experiment conducted by Dodia *et al.* (1997) attributed the yield decrease with increase in leaf damage and the mean grain yield was highest (4807 kg ha^{-1}) when there was no leaf damage. The level of damage to the rice hybrids (ADRH1 and CORH1) exceeded the economic threshold level during August and September 1998 in Tamil Nadu. Leaf folder incidence was significantly higher at 77 per cent in shaded areas than 42 per cent in unshaded areas (Balasubramani *et al.*, 2000).

The extent of damage caused by the major pests of rice is governed largely by the activity of natural enemies. *Trichogramma chilonis, Cardiochiles philippinensis, Macrocentrus philippinensis, Apanteles cypris, Goniozus triangulifer* and *Temelucha philippinensis* were recorded as the major parasitoids of the leaf folder larva (Mishra and Mandal, 2003). Among them, *T. chilonis* parasitized significantly more number of *C. medinalis* eggs and emerged as the best candidate.

2.1 Effectiveness of *Trichogramma* spp. in the field and laboratory

2.1.1 Field studies

Field trials using *T. chilonis* against *C. medinalis* revealed that four to nine releases of the parasitoid at 1,00,000 adults ha^{-1} starting from 20 to 38 days after transplanting, with a mean of 6.6 to 9.8 days duration between the releases, resulted in a 3.7-59 per cent decrease in leaf damage and 1.3 to 10.2 fold increase in egg parasitism (Bentur *et al.*, 1994). Field experiments were conducted by Balasubramanian *et al.* (1994) in rice fields in Coimbatore to evaluate the efficiency of *T. chilonis* against *C. medinalis* and *Marasmia patnalis*. It was found that during the kharif season, inundative release of *T. chilonis* at 5 cc ha^{-1} on 65 and 75 DAT followed by an application of monocrotophos 360 ml a.i ha^{-1} against the lepidopteran pests significantly reduced the insect damage. This was on par with two applications each of phosphamidon and monocrotophos.

Field release of egg parasitoid, *T. chilonis* and applications of *Bacillus thuringiensis* (B.t.), buprofezin 25 WP, neem seed kernel extract (NSKE) and monocrotophos 36 WSC were evaluated for control of rice leaf folder, *C. medinalis* on two rice cultivars under irrigated ecosystem. Studies indicated that, either six releases of *T. chilonis* alone or four releases of the egg parasitoid followed by application of B.t., NSKE and buprofezin, alone or in combination, significantly reduced leaf folder damage and gave higher grain yield compared to control plots (Saikia and Parameswaran, 2002).

The efficacy of different species of *Trichogramma* (*T. chilonis, T. achaeae, T. brasiliense, T. pretiosum* and *T. japonicum*) on chilli fruit borer, *Helicoverpa armigera* (Hubner) was evaluated by Chandrasekhar *et al.* (2002). The results revealed that *T. chilonis* was most efficient, based on the highest percent parasitism, followed by *T. pretiosum* and *T. brasiliense*. It was found that *T. japonicum* and *T. achaeae* were inefficient in parasitism of *H. armigera* eggs on chilli.

Large scale field demonstrations using *T. chilonis* conducted against sugarcane stalk borer (*Chilo auricilius*) in Punjab during 2000-01 revealed that the mean stalk borer incidence was 4.6 per cent in the field compared to 11.7 percent in the control, resulting in a 60.9 per cent reduction in damage over the control (Shenhmar *et al.*, 2003).

An experiment was conducted during rabi 2001 in Kerala to evaluate the effectiveness of releasing *T. chilonis* and *T. japonicum* for pest management in rice

(Beevi *et al.*, 2003). It was observed that the yield obtained upon treatment with both *Trichogramma* spp. were higher than both chemical control and untreated control. At 6-7 releases, each of 1,00,000 parasitoids per hactare, the yield was highest (4512 kg ha^{-1}), recording a 20.3 per cent increase over untreated control and an 11.8 per cent increase over chemical control.

A study conducted to evaluate the bioefficacy of *T. chilonis* against rice leaf folder showed that reduction in leaf damage varied from 63.8 to 75.5 per cent when the egg parasitoids were released at 50,000 ha^{-1} and 72.6 to 81.8 per cent when released at 1,00,000 ha^{-1} (Kumar and Khan, 2005).

2.1.2 Laboratory studies

The performance of three species of trichogrammatids on the gram pod borer, *H. armigera* was evaluated in the laboratory by Ballal and Singh (2003) in Karnataka. Based on the maximum parasitism of eggs of *H. armigera,* these studies indicated that *T. chilonis* and *T. pretiosum* were more effective parasitoids than *T. brasiliense.*

2.2 Biology and life cycle

The biology of trichogrammatidae were studied on bollworm eggs (*H.armigera*).The adult female wasp uses chemical and visual cues to locate a boll worm egg. The chemical cues called kairomones are on the moth scale left near the egg by the female moth during oviposition (Nordlund *et al.*,1981). Egg shape and colour also may be visual cues to the wasp (Ruberson and Kring, 1993)

The life cycle of *Trichogramma* from egg to adult requires about nine days, but varies from eight days when mid-summer temperatures are high (90°F) to as many as 17 days at 60°F. Adults are most active at 75-85 °F (Knutson, 2000).

Egg period of trichogrammatids lasts for 16-24 hours, larval period 2-3 days, prepupal period 2 days and pupal period 2-3 days. Total development is completed in 8-10 days during summer and 9-12 days during winter (Anonymous, 2003).

2.3 Kairomones

2.3.1 Kairomones in host parasitoid interactions

Kairomones constitute an important group of allelochemicals which are adaptively favourable to the receiver of the stimuli (Brown *et al.*, 1970). Their role in interspecific interactions involving insects has now been recognized, especially in host location by natural enemies. They may be released either by the plant or the host insect and act either as long range guiding systems for the natural enemies or short range contact stimuli eliciting strong host seeking response in the parasitoids upon direct contact (Hendry *et al.*, 1976). Kairomones attract natural enemies from a distance or can elicit intensive searches in the vicinity of the contaminated substrate (Waage, 1978). Parasitoids detect chemical cues that are emanating from the host insects which help in their host location. These chemical cues which are often found in the host insect or their byproducts act as the search in the near vicinity (Tumlinson *et al.*, 1992). To enhance the efficiency of potential parasitoids, kairomones are often recommended (Bakthavatsalam and Singh, 1996).

2.3.2 Kairomones for Trichogrammatidae

The egg parasitoids belonging to the family Trichogrammatidae are also known to make use of a wide range of chemical stimuli in host searching, the most important being kairomones mediated by host insects (Lewis *et al.*, 1972). The attraction of trichogrammatids to kairomones mediated by plants were reported by Nordlund *et al.* (1985).

2.3.3 Moth Scale Extract (MSE)

The involvement of host mediated chemical cues in their host location by *Trichogramma* spp. was first suggested by Laing in 1937 who reported that *T.evanescens* Westwood perceived moth odour left by female *Helicoverpa zea* (Boddie) at oviposition sites. Further studies by Lewis *et al.* (1971) demonstrated that moth odour did attract the parasitoid *T. evanescens* to its host *H.zea* and *Plodia interpunctella* Hubner resulting in a higher degree of parasitism as compared to parasitism of host eggs in a moth odour free environment.

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).

Responses of *Trichogramma* spp. to chemicals from a number of lepidopterous pests such as *Ephestia kuehniella* Zell., *Mythimna unipuncta* (Haw.) (Ferreira *et al.*, 1979), *Mamestra brassicae* (L.) (Smits, 1982), *H. armigera* Hubner and *Earias insulana* (Boisduval) (Garcia and Piqueras, 1985), *Pieris brassicae* (L.) and *P. rapae* (L.) (Noldus and van Lenteren, 1985), *Choristoneura fumiferana* (Clemens) (Zaborski *et al.*, 1987), *Ostrinia nubilalis* (Hubner) (Shu and Jones, 1988) and *C. cephalonica* (Stainton) (Ananthakrishnan *et al.*, 1991) have been recorded.

Investigations into the nature of the host seeking stimulant by Lewis *et al.* (1972) revealed that the stimulant was contained in the scales of the host moths and that it was extractable with hexane. The hexane extract of the scales retained the biological activity and significantly improved the parasitization of host eggs by *T.evanescens* in the laboratory and field studies even at a thousand fold dilution. This is believed to be the first instance where a mediator was used to manipulate the field behaviour of a parasitoid.

Jones *et al.* (1973) succeeded in isolating and characterizing four hydrocarbon compounds from hexane extracts of *H. zea* scales. The compounds were docosane, tricosane, tetracosane and pentacosane, the most active being tricosane which elicited significant orientation and stimulated parasitization in both laboratory and small field studies.

Lewis *et al.* (1975) reported that kairomones helped in a better distribution of the parasitoid, thereby minimizing chances of superparasitism and they concluded that the kairomones present in moth scales function to increase parasitization rates of *Trichogramma* spp. by releasing and maintaining an intensive searching response in the wasp as they come in contact with the kairomone, rather than serve as an attractant. The exposure of *T. pretiosum* and *T. achaeae* Nagaraja and Nagarkatti to their host kairomones prior to release elicited an orientated host seeking bahaviour in the parasitoids, as reported by Gross *et al.* (1975). This response was manifested both in petri plate bioassay as well as field plot studies and could be of great value in release programmes.

Nordlund *et al.* (1976) reported that continuous exposure to kairomones present in the scales of *H. zea* improved parasitization, the number of progeny produced and the adult life span in females of *T. pretiosum.* Increased retention in the area treated with simulated moth scales of *H. zea* was attributed as a major factor in improving parasitization of *T. pretiosum* by Beevers *et al.* (1981).

Studies by Lewis *et al.* (1982), Nordlund *et al.* (1984), 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.

Gueldner *et al.* (1984) reported the presence of hexanoic, heptanoic, octanoic and nonanoic acids in scales of laboratory reared *H. zea* moths. They reported that the parasitoid *T. pretiosum* responded positively to some acids, but the parasitization was not improved over that of crude extract. The removal of acids from the scale extract did not influence the effectiveness of the extract in eliciting response from the prasitoids.

Zaborski *et al.* (1987) found that the scale extracts of spruce budworm, *C.fumiferana* elicited host seeking behaviour from its egg parasitoid *T. minutum*, while the scale extract of laboratory host *Sitotroga cerealella* Oliv. failed to elicit any such response from the parasitoid. Studies by Renou *et al.* (1989) confirmed earlier report of the presence of kairomones in scales of *O. nubilalis* deposited on egg surfaces and indicated them to be comprising chiefly of hydrocarbons.

Kaiser *et al.* (1989) reported that inexperienced females of *T. maidis* Pinturean and Voegele did not respond to odours from host eggs, synthetic pheromones or maize extract when offered alone while they responded to a mixture of all the three. Thomson and Stinner (1990) reported slight variations in response of different *Trichogramma* spp. to kairomones. The adults of *T. exiguum* Pinto and Platner and *Trichogramma* spp. near *T. pretiosum* showed distinct preference to scales of *H. zea* and *Manduca sexta* Johannsen as compared to the scales of *O. nubilalis*. The adult parasitoids of *T. minutum* and *T. maltbyi* did not discriminate between scales of the three hosts.

Shu *et al.* (1990) identified isolated and synthesized 11, 13, 7, 15, 10-dimethyl nonatriacontanes as kairomones for *T. nubilale.* Ananthakrishnan *et al.* (1991) attributed the kairomonal activity of *H. armigera* and *C. cephalonica* scales to a number of compounds they had isolated, such as decosane, pentacosane, hexatriacontane, nonacosane and heptadecane.

A kairomone eliciting host searching behaviour by *T. confusum* was extracted from scales of the gelechiid *Pectinophora gossypiella.* Moth scale extract increased the time spent by parasitoids and decreased the speed of movement in treated areas (Wang and Zong, 1991).

Chemically mediated interactions between the egg parasitoid *T. chilonis* and its host insect *H. assulta* were studied in laboratory experiments conducted by Boo and Yang (2000). It was found that *H. assulta* eggs were more parasitized by *T.chilonis* when the eggs were treated with male moth scale extract (MSE) of *H.assulta.* Parasitism was also affected by the age of the parasitoid, time of day and MSE concentration.

2.3.4 Plant volatiles

The fact that plants, along with the natural enemies are the ones to be benefited by the presence of kairomones together with the known presence of other major categories of chemical mediators such as pheromones, allomones etc., encouraged scientists to search for kairomones in plants. Several plant characters are known to affect the searching behaviour and parasitization efficiency of *Trichogramma* spp.

Hendry *et al.* (1976) analysed different plants like corn, green pepper, string bean, lettuce and celery for the presence of kairomonal principles. Gas chromatography-mass spectrometry indicated the presence of a series of hydrocarbons C_{21} to C_{25} in all the plants tested. However, corn was found to contain the highest quantity of all hydrocarbons, especially tricosane (153 ng g^{-1}) which is suggestive of a very direct relationship between host parasitoid signals and food-plant chemistry.

Altieri *et al.* (1981) carried out a series of experiments to study the influence of plant extracts on parasitization by *Trichogramma* spp. In studies where acqueous extracts of *Amaranthus* and corn were sprayed in plots of soybean, both the extracts led to an increase in parasitization over control. Similarly, suspending wicks impregnated with extracts of corn or amaranth improved the parasitization in soybean plots. They reported increased parasitization of *H. zea* eggs in soybean fields having weeds viz., *Desmodium, Croton* and *Cassia* as compared to weed free soybean plots, as well as in soybean and corn polycultures as compared to soybean monocultures. This finding was corroborated by Altieri and Todd (1981) who reported higher parasitization by *Trichogramma* spp. in soybean-corn polycultures as compared to soybean monocultures. Bhatnagar (1981) observed that parasitization of *H. armigera* eggs by *Trichogramma* spp. on favoured crops like sorghum and cowpea was up to 85 per cent while on hosts like pigeon pea and bengal gram, it was less than one per cent.

Nordlund *et al.* (1985) reported the presence of synomones in tomato plants which stimulated parasitism of *H. zea* eggs by *T. pretiosum* whereas maize leaf extract did not elicit any such response.

2.3.5 Honey

The behavioural response of adults of *Chrysoperla carnea* to some of the kairomonal compounds, including honey and fractionales from the scales of *H.armigera* was studied through EAG and orientational response studies. The male *C. carnea* showed highest response to honey and tricosane followed by dodecane, pentacosane and octane. In the case of females, honey recorded highest response. Pentasane, docosane, dodecane recorded highest EAG response (Anonymous, 2000).

2.4 Effect of adult nutrition on longevity of parasitoids

Parasitic wasps generally require food both for the production and maturation of eggs and as an energy source for flight. Parasitoids need nutritive compounds similar to other insects, e.g., amino acids, vitamins, minerals, cholesterol and a hexose monosaccharide (House, 1977). Timerak (1983) investigated the life-span of adult *Bracon brevicornis* Wesm. on various types of foods or the absence of foods, and found that when provided with external food (sucrose, molasses, honey or tap water), the female lived the longest on honey solution.

In a laboratory experiment, *T. japonicum* was given water, sugar solution, glucose solution, honey solution, protein hydrolysate solution or no food at temperatures of 14, 26, 29 or 31 °C. Honey, sugar and glucose increased the longevity of the adult *T. japonicum*. The longevity was highest with honey solution at 14 °C (Ram *et al.*, 1997).

An experiment was conducted by Islam (2001) to study the role of adult nutrition on longevity and fecundity of a hymenopteran parasitoid, *Dinarmus basalis*. According to that, honey-fed females lived a maximum of 31.2 days and produced 368.3 eggs whereas glucose-fed females lived for 26.75 days and produced 312.6 eggs. The minimum longevity and fecundity were observed for lactose-fed females. He concluded that honey solution proved to be the best for the survivability in *D. basalis*.

An experiment was conducted by Sheeba *et al.* (2002) to study the effect of honey on various biological parameters of *T. japonicum* under laboratory conditions. Honey feeding had a positive impact on the longevity and parasitic efficiency of *T.japonicum*. Honey feeding enhanced longevity irrespective of sex or host availability for parasitization.

The value of maize pollen as a food source for *T.brassicae* was assessed in the laboratory by testing the effect of different pollen diets on the longevity, capacity of parasitism and other life table parameters. The different pollen diets tested were maize pollen and water (wet filter paper dusted with pollen), honey, water, maize pollen and honey in the presence and absence of european corn borer eggs. As per the study, *T. brassicae* females that fed on honey alone (8.37 days) or maize pollen and honey (8.23 days) lived significantly longer than those feeding on maize pollen and water (4.97) or on water alone (2.67 days). Hence *T. brassicae* used maize pollen as food that significantly increased longevity and fecundity of the tested females. Pollen and honey supplied a complete diet for *T. brassicae,* compared to pollen and water or water alone (Guren *et al.*, 2004).

2.5 Olfactometer

Biocontrol organisms respond to olfactory cues with behaviour which can affect their efficacy as pest control agents. Olfactometry is commonly used in investigations of organism behaviour and responses to olfactory stimuli (Nettles, 1980; Janssen *et al.*, 1990; Martin *et al.*, 1990 and Delegue *et al.*, 1991). Four armed olfactory chambers (Vet *et al.*, 1983 and Bakchine *et al.*, 1990) provide a relatively unconfined central arena in which organisms can freely move and into which four olfactory treatments or controls can be introduced.

Renou *et al.* (1992) reported that in the presence of an extract of eggs of the pyralid *Ostrinia nubilalis* or the noctuid *Mamestra brassicae,* females of *T. brassicae* exhibited increased rates of upwind locomotion in the tubes of a linear olfactometer at 2400-2800 lux and 24 °C. They concluded that various compounds present on the surface of *O. nubilalis* egg masses may play a role in the orientation of *T. brassicae* to its host.

Studies in a four-armed airflow olfactometer by Romeis *et al.* (1998) indicated that *T. chilonis* was repelled by volatiles from pigeon pea pods but showed no response to volatiles derived from hexane extract of pod surfaces.

'Y' tube ofactometer studies conducted by Bakthavatsalam *et al.* (1999) revealed that hexane wash of gallery and body of *Opisinia arenosella* Walker elicited positive response from the parasitoids namely *Goniozus nephantidis*

(Muesebeck), *Brachymeria nephantidis* Gahan and *Elasmus nephantidis* Rohwer, in terms of more number of parasitoids entering the kairomone arm than hexane arm.

The olfactory responses of mealy bug predator, *Cryptolaemus montrouzieri* to the kairomones of prey arthropods and their host plants were studied by Kotikal and Sengonca (1999) using olfactometer and found that the larvae as well as adults of *C. montrouzieri* could sense the chemicals liberated by their prey and use them in reaching the source.

A study was conducted by Reddy *et al.* (2002) to find out the interactions between the bioagents (*T. chilonis, Cotesia plutellae, C. carnea*) and various host-associated volatiles of diamond back moth, *Plutella xylostella* using a Y olfactometer. The results indicated that the sex pheromone and larval frass volatiles from the diamond back moth, as well as relative compounds from cabbage may be used by these natural enemies to locate their diamond back moth host.

Host location and recognition by the egg parasitoid *Trissolcus brochymenae* were analysed in terms of response to kairomones from several stages of its host, *Murgantia histrionica* in a 'Y' tube olfactometer. Studies revealed that parasitoid females responded by increasing residence time and / or reducing linear speed to chemical cues from gravid females, virgin females and males, third and fifth instars and eggs (Conti *et al.*, 2003).

The olfactory response of *T. ostriniae* to kairomones emitted by Asian corn borer female adults at various stages, their accessory glands and eggs was measured using a four-arm olfactometer by Shuxiong *et al.* (2004). Their study revealed that the airborne chemicals from egg masses, mated-females before their first oviposition and their accessory glands stimulated an intensive search behaviour in *T. ostriniae* females.

A Y-tube olfactometer assay was done to test attractivity of *T. chilonis* to leaf and floral volatiles of *Tagetes erecta* and *Solanum viarum*. Parasitoids reaching the cue source within 10 minutes and remaining arrested for 15 seconds were recorded as having made choice. The olfactomeric responses revealed that *T.chilonis* had maximum net response to hexane extract of *T. erecta* flower bud (47.5 per cent) followed by floral and leaf volatiles (Tandon and Bakthavatsalam, 2005).

2.6 Wind tunnel

Tingle *et al.* (1989) showed that mated *H. subflexa* females flew upwind in the wind tunnel bioassays to a methanol wash of fresh whole leaves from its host plant, ground cherry *(P. angulata)* culminating in 50 per cent of the responding moths depositing one or more eggs. Wang *et al.* (1990) observed that *T. ostrinae* dispersed as far as 6 m off the releasing point in the field of corn plants, but was found more within the distance of 2 m.

Wind tunnel studies conducted by Noldus *et al.* (1991) demonstrated the behavioural response of male moths of *M. brassicae* and females of its egg parasitoid *T. evanescens*, when exposed to leaves of Brussels sprout. Male moths responded to odour treated leaves over a short distance (5 cm) in a wind tunnel, but were not attracted at a distance of 1 m. In contrast, sex pheromone adsorbed to and re-released from the glass wall of a wind tunnel compartment attracted male moths from 1 m.

Mitchell *et al.* (1991) used a plexiglass flight tunnel (195 cm length x 60 cm width x 60 cm height) to observe flight and ovipositional responses of *H. virescens* females to volatiles from crude extracts of three different plant hosts (cotton, tobacco and a weed *Desmodium tortuosum*). It was found that the females responded positively via upwind flight to volatiles emitted from methylene chloride washes of fresh whole leaves of host plants.

Singh *et al.* (1994) reported that adults of *T. chilonis* were able to easily locate host eggs within a distance of 5 m radius. Higher per cent parasitism was observed at 1 m radius towards the direction of the wind. Sohi *et al.* (1996) reported that the maximum distance of dispersal of *T. chilonis* in cotton was 10 m. In sugarcane field, *T. chilonis* dispersed upto 10 m and the parasitization in corcyra trap cards was 9.0 to 56.5 per cent at distance of 2-10 m (Anonymous, 1999).

The olfactory responses of male moths of mango fruit borer, *Deanolis albizonalis* to female sex pheromone was studied in the laboratory using the wind tunnel method by Sujatha *et al.* (2002). The males showed response towards the females by orientation with characteristic behaviour such as swift antennal movement and extension of proboscis.

2.7 Storage and Viability Studies

Storage of parasitized host eggs at low temperature is an essential pre-requisite in biological control. This retards the development of parasitoids so as to synchronize their emergence in large numbers with availability of vulnerable stage of the host in the fields. Efficient storage of the biocontrol agent could improve current parasitoid production methods by making the system more flexible and efficient.

Venkataraman and Govil (1952) had reported the storage of *T. chilonis* in prepupal stage at 12°C for 15 days to be safe and could be protracted to 25 days but at the cost of reduced survival and fecundity of emerged adults.

Gautam (1986) reported that seven days old parasitized host eggs of *S. litura* (Fabricius) by *T. remus* Nixon could be stored for 10 days at 10 °C. Killineer *et al.* (1990) however, reported that storage of the *Trichogramma* spp. as the parasitized *Sitotroga cerealella* Olivier eggs up to 30 days does not adversely affect their emergence.

A differential response was noticed when four species of *Trichogramma* were stored at 2, 5 and 10°C for 7-49 days. A temperature of 10°C gave a storage period of 49 days (Jalali, 1992). *Trichogrammatoidea eldanae* can be stored at 2 and 5°C for less than 14 days and at 10°C for less than 21 days without affecting longevity and fecundity of parasitoid. At 8°C storage, the parasitoid reached zero emergence after 60 days of storage and this period was found to be 49 days when stored at 10°C (Jalali and Singh, 1992). *T. eldanae* could be stored up to 80 days at 5°C and *T. brasiliense* up to 50 days at 10°C (Singh and Jalali, 1994).

Singh *et al.* (1997) reported that *T. chilonis* could be stored for 20 days without affecting the adult emergence. Khosa and Brar (2000) reported that different population (Muktsar, Sangrur, Ludhiana and laboratory reared) of *T. chilonis* can be stored for 22 days.

Farid *et al.* (2001) reported that percentage emergence of *T. chilonis* was detrimentally affected after cold storage of pupae at 5-8°C even for one week. Three week storage decreased emergence by more than 30 per cent. Four weeks of storage at this temperature led to almost total mortality of the parasitoid pupae. However, pupae stored at 5-8°C took shorter time for adult emergence than control.

Studies on *T. bactrae* Nagaraja were carried out with the objective of determination of storage condition conducive for holding the parasitoid. From parasitized eggs of *C. cephalonica* (Stainton), stored after 3-7 days of parasitization for five days at 10°C, above 50 per cent adult emergence occurred and eggs could not be stored for more than seven days. In another experiment, exposure of eggs to 10 and 15°C for 5, 10 and 15 days after 3, 5 and 7 days of parasitization, provided satisfactory adult emergence from eggs stored at 15°C for 5 days after 5 and 7 days of parasitization (72.3 and 72.1 %) and for 10 days after five days of parasitization (69.8 %) (Gupta and Bhardwaj, 2002).

Pitcher *et al.* (2002) compared emergence rates of *T. ostriniae* reared on *S. cerealella* eggs held at 6, 9, 12, 15 and 24°C for up to eight weeks after parasitism. At 15°C, emergence occurred in less than two weeks. Emergence was more than 80 per cent for parasitized egg stored at 9 and 12°C for four and six weeks respectively. Storage at 6°C caused a significant decline in emergence after two weeks.

Cold storage studies of various day age parasitoid cards of *T. ostriniae* at 6, 9 or 12°C were conducted by Yu and Chen (2003). Result showed that 12°C was not a suitable temperature for cold storage because the parasitoid adults could not emerge successively within stored period, and their potential parasitism were mostly significantly fewer than that stored at 6 and 9°C. Cold storage at 6 and 9°C achieved almost similar result, but both temperatures had various negative effects.

Shivaleela and Patil (2003) determined the effect of refrigeration on *T. achaeae* by exposing 5500 *C. cephalonica* eggs. Upon refrigerating the eggs for two days, a maximum of 97 per cent *T. achaeae* emergence was observed. In contrast, eggs stored for 80 days resulted in only 4.0 per cent emergence. Parasitoid emergence was quite high (more than 80 %) up to 30 days of storage and thereafter, it declined.

Rundle *et al.* (2004) reported that *T. carverae* can be successfully stored up to two weeks without detrimental effects and 10°C is the preferred storage temperature. A study conducted by Kumar *et al.* (2005) revealed that *T. chilonis* and *T. pretiosum* could be stored for 20 days at a low temperature of 4±0.5°C without adversely affecting their adult emergence and parasitization efficiency.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Trichogramma chilonis is one of the most effective parasitoids infesting *Cnaphalocrosis medinalis*. We have to design methods to increase the effectiveness of *T.chilonis* in the field. It is also necessary to improve the availability of trichocards at critical periods by storing them in refrigerator for better use by farmers. It is worthwhile to evaluate the response of trichogrammatids to host kairomones or plant volatiles. This will tell us whether their adult longevity was improved by providing adequate food sources. These olfaction studies involving semiochemicals require the standardization of olfactometers.The succeeding paragraphs describe the methodology adopted in the present study to validate the above objectives.

3.1 Designing of olfactometer

A working model of olfactometer (Plate 1) was at first made using mylar sheet (polyester film, Garware 200 microns) for standardizing the design. A rectangular mylar sheet of 50 cm x 20 cm was cut and four equidistant holes of 3.5 cm diameter was made at 7 cm height. This sheet was rolled into a cylinder of 20 cm length and 16 cm diameter. Four rectangular sheets of 45 cm x 11 cm was cut, rolled, pasted to form a tube and fitted into each of the four holes on the cylinder. The angle between each arm is 90°. A petriplate (16 cm diameter) was placed as the bottom of cylinder. The cylinder was closed with a removable lid made of mylar sheet facilitating easy placement of trichocards. A hole was made at the center of the lid to insert a tube (5 mm diameter) for sucking out air using an air flow regulator.

3.1.1 Validation of prototype

For testing the utility of the device, 10 percent honey solution, 10 percent sugar solution and *Corcyra* moth scales were kept in each of the three arms of the

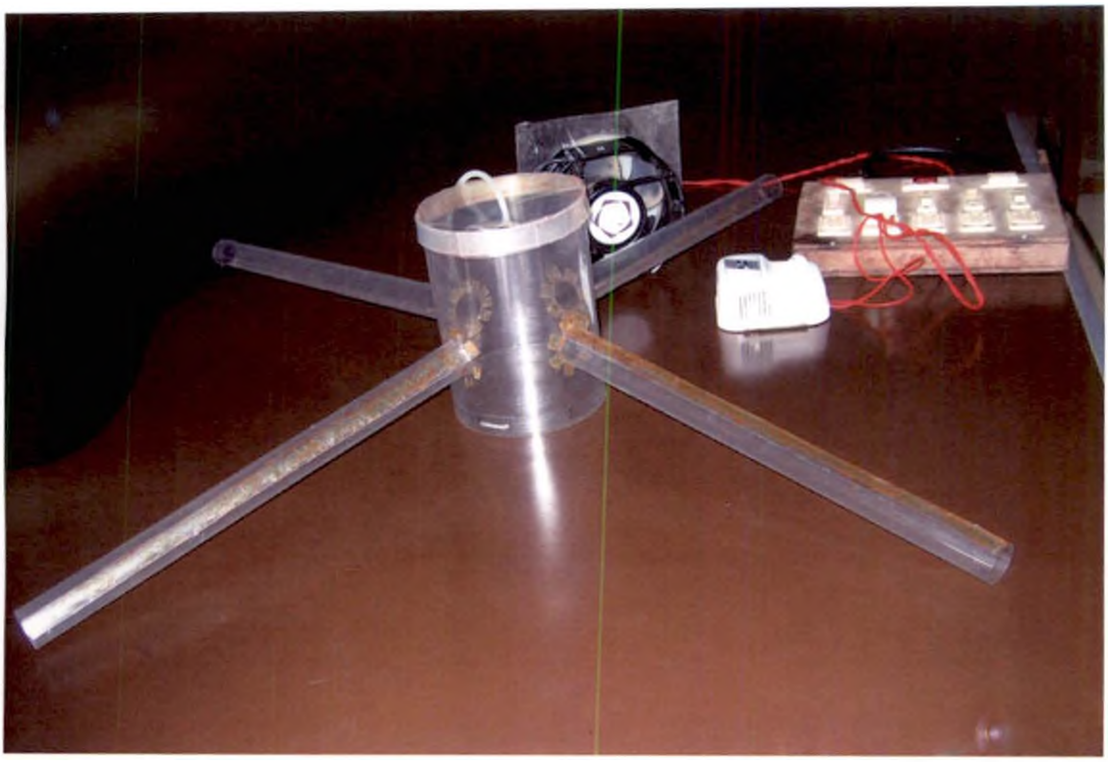

Plate 1. Polyester film prototype

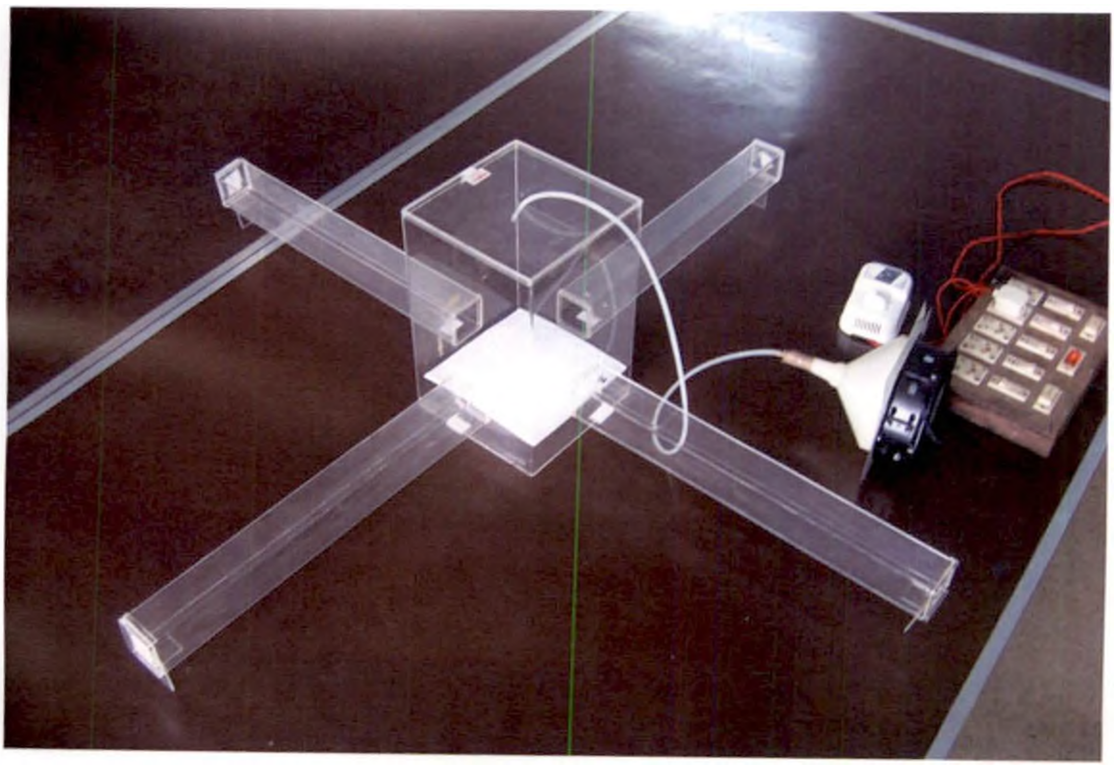

Plate 2. Acrylic olfactometer

prototype. The fourth arm was kept as control without any semiochemical cues. The distal end of
each arm was covered with a piece of muslin cloth. Trichocard with one cc egg load was kept on the petriplate in the bottom central region. The temperature was maintained at 28°C. The solutions of honey and sugar were dotted on a small piece of coconut leaf and kept at the distal end of the arms. *Corcyra* scales were placed inside a small plastic petridish and covered with muslin cloth. Constant and equal airflow was maintained in each arm by connecting a 5 mm flexible plastic hose to the upper central lid and sucking out air through this tube. The tube was connected to the stem of a 150 mm plastic funnel. Air was drawn out using an industrial fan (0.25/ 0.22 Amps, 230V, 50Hz, SUNON A2175-HBL) with a speed regulator. It was observed that there was a higher response towards honey solution and some were also attracted towards *Corcyra* moth scales. There was no response towards sugar solution and control. As this prototype was found to be a success, it was further fabricated in transparent acrylic sheet incorporating slight modifications.

3.1.2 Fabrication of acrylic olfactometer

A four armed olfactometer (Plate 2) was designed to find out the orientation responses of the adult parasitoids toward the kairomones. It was made of non-absorbent, transparent acrylic sheet of 2 mm thickness. The olfactometer consisted of a central rectangular chamber (20 cmx 20 cm x 30 cm height). The base was also with acrylic sheet (20 cm x 20 cm). From the centre of each face of the central chamber, at 50mm height from bottom, a 50mm square hole was cut. Into this, a 50 mm x 50 mm x 500 mm rectangular acrylic tube was connected. A small acrylic rim was provided at the connecting end for support and to keep the tube in position. This also prevented the tube from being pushed inside. The distal end was closed with an acrylic sliding door provided with a 5 mm hole at the centre. Moth scales, moth scale extracts, plant materials or other semiochemical emanating substances could be placed at the distal end through this window. The

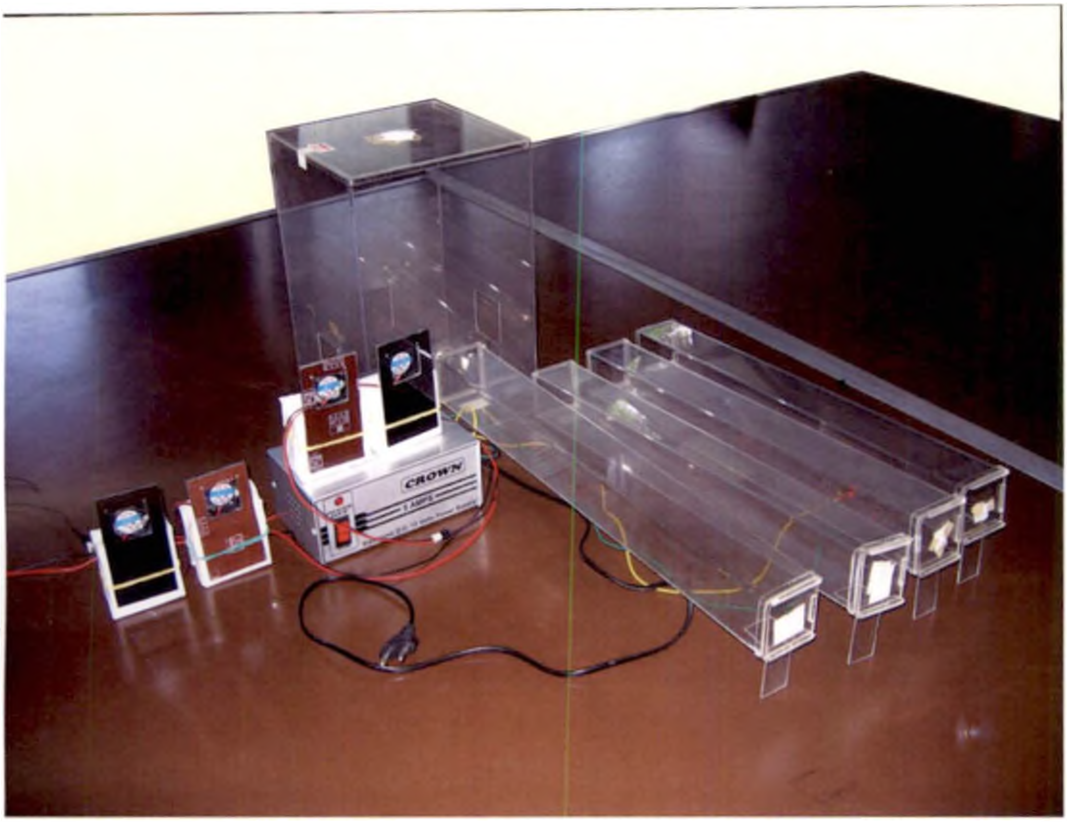

Plate 3a. Uniform air inflow olfactometer dismantled view

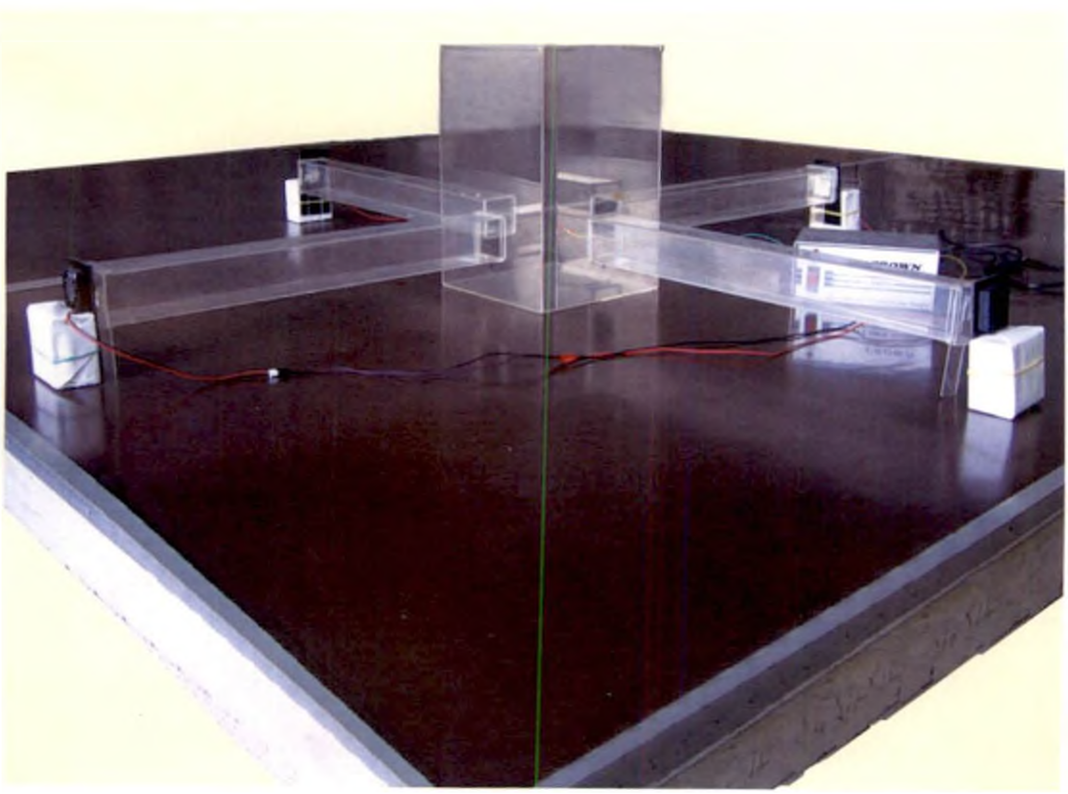

Plate 3b. Uniform air inflow olfactometer

opening can then be closed with the sliding door. An acrylic support at the distal end helped to keep the rectangular tube in position. To prevent the escape of attracted parasitoids to the outside, the central 5 mm hole was closed with a piece of muslin cloth. The top of the central chamber was closed with an acrylic lid (20 mm x 20 mm) and with a retaining rim on the inside. The lid was removable and was provided with a central 5mm hole. The inlet hose of the airflow regulator (industrial fan described as in 3.1.2) was connected to this 5 mm hole as in the case of the prototype. Trichocards were kept in the centre bottom of the chamber in a petriplate. The height of the petriplate with the trichocard was regulated to synchronise with the inner height of the four arms of the olfactometer. A 20 cm x 20 cm piece of sunpac was used as a central platform to keep the petriplate. During set up, vaseline was smeared at the connecting inlets of each arm to prevent entry of air. To facilitate the easy movement of *Trichogramma*, strips of thick paper card were kept at the inside entrance of each of the arms.

3.1.3 Validation of acrylic olfactometer

The olfactometer set up was validated using the same procedure as of the prototype. The attractants used in this case were also the same. During continued studies, it was noticed that the orientation responses to the kairomones were erratic. Various error factors were identified. These were then eliminated and the improved design was standardized as in 3.1.4.

3.1.4 Uniform air inflow olfactometer

In this set up, the air flow from inside the top central region of the olfactometer was dispensed with. Uniform flow towards the inside from the four different arms was assured by fixing a small axial flow fan at the four distal openings of each arm. The axial fan (DC 12V, 3.5 cm diameter) was screwed on to a laminate and housed on a wooden base to facilitate correct positioning of airflow towards the inside (Plate 3a and 3b). Air from these fans was directed towards the inside of olfactometer. The kairomones were kept at the distal end of each of these arms. The new set up allowed the insects to receive olfactory cues from these host kairomones. They could then travel upwind in response to the volatiles.

3.2 Wind tunnel set up

A wind tunnel set up was fabricated to evaluate the olfactory responses of *T.chilonis* to the best semiochemicals. The set up (Plate 4) had the following components.

1. Host odour chamber
2. Inflow fan
3. Tunnel
4. Inlet funnel
5. Sieve screen

Host odour chamber was made to a size of 35 cm x 35 cm x 35 cm. A circular hole (10 cm diameter) was given on one side of the chamber. The opposite side was provided with aluminium screen. The other four sides were covered with 'hylam' sheet.

Inflow fan (0.25/ 0.22 Amps, 230V, 50Hz, SUNON A2175-HBL) was kept at the back of the host odour chamber to provide an air flow into it through the circular hole on the other side of the chamber

A transparent plastic hose of 2 cm diameter and 2 m length was used as tunnel for the studies. The size of the tunnel was so fixed to study the easy movement of *Trichogramma*. One end of the tunnel was connected to an inlet funnel (15 cm diameter) and it was attached to the circular hole on one side of the host odour chamber.

A sieve screen with muslin cloth was placed at the stem of the funnel to allow the inlet of filtered air and to prevent the escape of *Trichogramma* adults. The other free end of the tunnel was closed with muslin cloth.

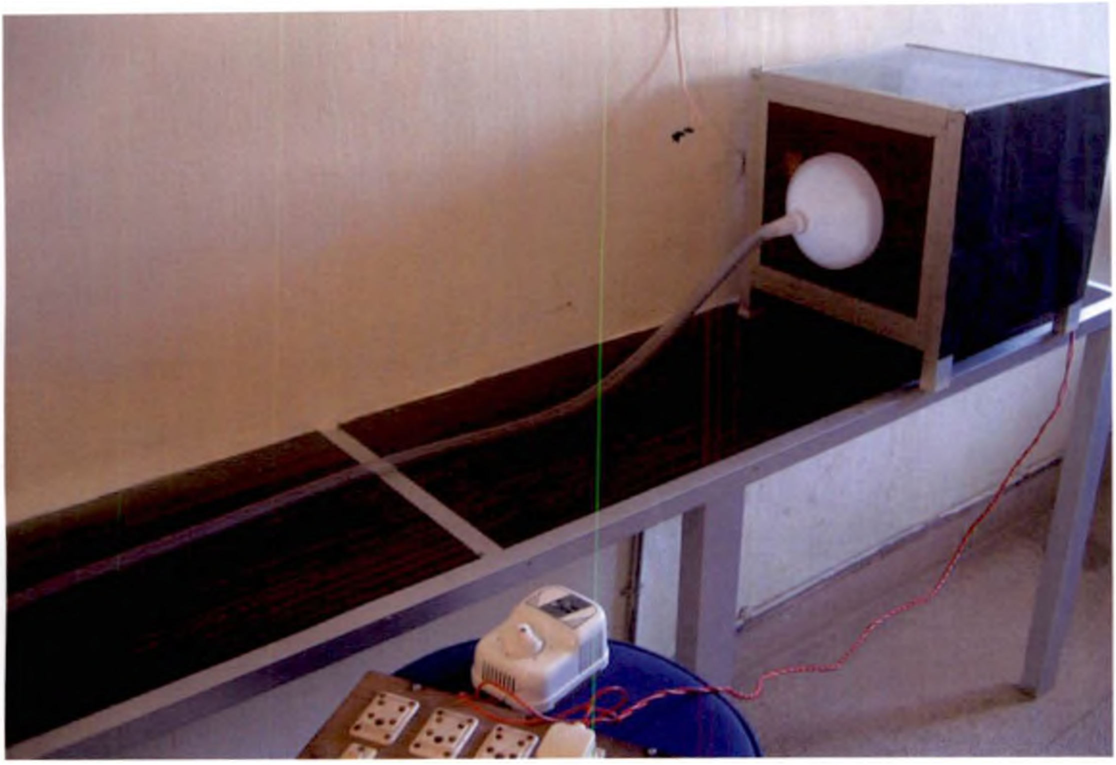

Plate 4a. Wind tunnel setup-funnel side view

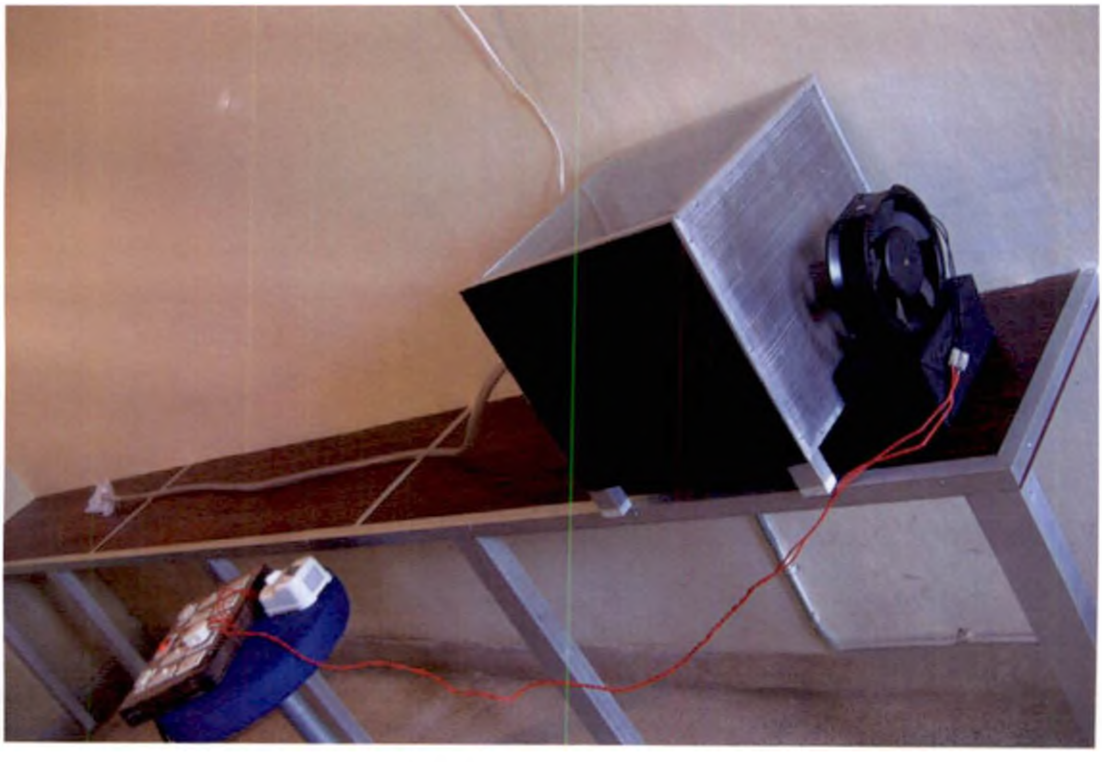

Plate 4b. Wind tunnel setup-fan side view

Host plants in bouquet or cotton wicks impregnated with kairomones were kept inside the host odour chamber. These wind tunnel setups allowed air to be draughted out through the funnel end to the free end.Trichogrammatids were kept at the free end. Five adult parasitoids were released at a time. They were thus free to respond to the volatiles in the wind tunnel. The setup allowed directed movement upwind. The inside of the wind tunnel was cleaned with methanol impregnated cotton after every exposure.

The cues from the best results of the olfactometer studies were used in the wind tunnel experimentation set up. Unlike the olfactometer, only one cue was kept at a time in the wind tunnel. The distance travelled by *Trichogramma* adults was observed at every thirty minutes, for a total duration of three hours.

3.3 Preparation of host extracts

3.3.1 Moth extracts

Moths of *C.cephalonica* and *C.medinalis* were collected and weighed. One gram of moths was homogenized with different solvents viz, acetone, ethanol, ether and hexane using a pestle and mortar. Solvents were added at the rate of 10ml per gram body weight of moths. They were then transferred to 125 ml stoppered bottles. The bottles were shaken vigorously and were kept overnight. The extract was filtered using Whatman no. 1 filter paper and was soaked in absorbent cotton wick. The wick was made with 0.5 g cotton and rolled using 1.5 g muslin cloth. This moth extract impregnated wick was used for the kairomone studies. The solvent was allowed to evaporate off completely by air drying.

3.3.2. Moth scale extracts

Scales of *C.cephalonica* were collected from State Biocontrol Laboratory, Mannuthy. One gram of scales were weighed and transferred into 125 ml stoppered bottles and different solvents (acetone, ethanol, ether and hexane) were added at the rate of 20 ml per gram of scales. The extraction was carried out as described in 3.2.1 above.

3.3.3. Response studies

The response of *Trichogramma* to moth and moth scales of *C.cephalonica* and *C.medinalis* extracted in different solvents were studied. For this the above described air dried cotton wicks were placed in each of the four arms of the olfactometer along with water soaked and dried wick as control. The different treatments were rotated in the different arms randomly and replicated four times.

Visual observations were made on the response of the adult parasitoids to the kairomones. The numbers of adults entering into the respective arms were counted at 30 minute intervals. The kairomonal wick plus control were interchanged among the different arms for each replication to eliminate any photo tactic or directional influence. Each treatment was replicated 4 times. At each exposure the treatment combinations were followed with different extracts out of

A-Acetone Extract
B- Ethanol extract
C - Ether extract
D- Hexane extract
E- Control

ABCD- acetone extract, ethanol extract, ether extract and hexane extract
ABCE- acetone extract, ethanol extract, ether extract and control
ACDE- acetone extract, ether extract, hexane extract and control
BCDE- ethanol extract, ether extract, hexane extract and control
ABDE- acetone extract, ethanol extract, hexane extract and control

3.3.4 Response of *T. chilonis* to plant volatiles

Ten different weed flora in the paddy ecosystem were selected for the study (Plate 5). The details of the plants used in the study are given in Table 1. The leaves of rice variety Jyothi served as the control 1 along with the leaf folder damaged leaf as control 2.

Plate 5. Different weeds in the rice ecosystem

O. rufipogon

S. interrupta

E. crusgalli

P. repens

C.dactylon

E. indica

D. ciliaris

C. distans

L. perennis

M. quadrinalis

Table 1. Weeds of rice ecosystem involved in the study.

Sl No.	Common name	Scientific name	Family
1	Wild rice (*Varinellu*)*	*Oryza rufipogon*	Poaceae
2	(*Polapullu*)*	*Sacciolepis interrupta*	Poaceae
3	Common barn - yard (*Kavada*)*	*Echinocloa crusgalli*	Poaceae
4	Torpedo grass (*Inchapullu*)*	*Panicum repens*	Poaceae
5	Doob grass (*Karukapullu*)*	*Cynodon dactylon*	Poaceae
6	Goose grass (*Mathangapullu*)*	*Eleusine indica*	Poaceae
7	Crab grass (*Njandu kalan pullu*)	*Digitaria ciliaris*	Poaceae
8	Nut sedge	*Cyperus distans*	Cyperaceae
9	Water primrose (*Neergramboo*)*	*Ludwigia perennis*	Onagraceae
10	Airy pepper wort (*Nalilakodiyan*)*	*Marsilea quadrifolia*	Marsileaceae
11	Rice	*Oryza sativa* (Control 1)	Poaceae
12	Rice	Oryza sativa damaged by leaf folder (Control 2)	Poaceae

* Names given in parenthesis indicate malayalam names.

10 g of fresh sample of the weed species was taken and kept in the arms of olfactometer. Four weeds were exposed at a time and replicated five times with rice leaves and leaf folder damaged rice leaves as control.

Out of the 12 plant volatile treatments which were first exposed, the best four treatments were again selected and re-exposed once again to confirm the responses.

3.3.5 Response of *T. chilonis* to semiochemicals

In order to ascertain the response of *T.chilonis* to food sources and host material, four different treatments were tried. The treatments were 10 per cent honey solution, 10 per cent sugar solution, *Corcyra* eggs and *Corcyra* scales.

10 ml of honey solution was impregnated in a cotton wick as described in 3.2.2. Similarly 10 ml sugar solution was also impregnated. Egg cards of *Corcyra* cut into small bits amounting to 0.5 cc (~10,000 eggs) were used in one treatment. In the case of *Corcyra* scales, one gram of scales were tied into a pouch in a piece of muslin cloth and used as the kairomone cue.

3.3.6 Statistical analysis

From the cumulative total of the succeeding observations, the incremental response during the particular half an hour time period was arrived at by calculating the difference between the initial and subsequent trichogrammatid numbers. This data was subjected to Kendall's test for obtaining the coefficient of concordance (w) to assess the most preferred semiochemical by *T. chilonis.*

3.4 Storage studies

An experiment was conducted to find out the suitable storage conditions of tricho card, containing eggs parasitized by *T.chilonis.* Cards (10 x 15 cm) glued with ~10,000 ultra violet irradiated eggs of *C.cephalonica* required for the studies were obtained from RARS, Pattambi. These cards were exposed to *T.chilonis* in the ratio of 8:1 for 48 hours. On the third day, a parasitized card (one day old parasitized card) was kept in the refrigerator at 7°C. On the succeeding days, parasitized cards of age 2-8 were kept under refrigeration. On every 7th day after storage in refrigerator, a small strip of 5.5 x 1.5 cm (here after denoted as tricho sub card) was extracted from each day old (1-8 day old) parasitized cards. These tricho sub cards are kept in open so that the *Trichogramma* adults emerged can be counted. From this observation, per cent emergence was recorded from one day old to eight day old cards. Two replications were maintained for the studies.

3.4.1 Parasitization efficiency

To study the parasitization efficiency of the parasitoid, a strip with about 100 good pupae of *T.chilonis* was cut and exposed to 500 eggs of *C.cephalonica* in a polythene cover of 12 x 7 cm.The number of *Trichogramma* adults emerged was counted and they were allowed inside the polythene bag to parasitize the host eggs until they died. After nine days, the number of adults emerged from the parasitized cards was counted. From these observations, the apparent per cent parasitism of *T. chilonis* were found out as

$$\text{Apparent per cent parasitism} = \frac{\text{Number of } \textit{Trichogramma} \text{ adults emerged from the strip with 100 eggs (A)}}{\text{Number of } \textit{Corcyra} \text{ eggs parasitized by A}} \times 100$$

Apparent per cent parasitism was necessary to understand the rate of emergence of the *Trichogramma* adults from different day old *Trichogramma* embryos kept under refrigeration. However in the field situation, in order to ascertain the exact number of adults that finally emerged, the absolute per cent parasitism was calculated as

$$\text{Absolute per cent parasitism} = \frac{\text{Number of parasitized eggs}}{500} \times 100$$

The quotient 500 was taken as a standard since each strip of *Corcyra* card is supposed to contain 500 eggs.

3.4.2 Statistical analysis

Statistical analysis on the per cent emergence and parasitism under different storage periods was done as completely randomized design (CRD).

RESULTS

4. RESULTS

The egg parasitoid, *T. chilonis* responds to the semiochemical cues from host insects and the host plants. The present study was designed to increase our knowledge about the responses towards semiochemicals and also to explore the possibilities of increasing the efficacy of the parasitoids. The results of the experiments are detailed in this chapter.

4.1 Olfactometer

The first prototype of the olfactometer was perfected and used for the preliminary trials.

4.1.1 Testing and validation of prototype

The prototype gave differential responses to the different olfactory cues eminating from the kairomones. This was only a preliminary study. The results with the four different kairomones are given as Table 2.

4.1.2 Testing of the acrylic olfactometer

Results using the host volatiles are given as Table 3. It was seen that the air draught from the four different arms were not uniform after the initial three four days. The small air outflow tube got partially blocked due to dust particles within this time. Also it was seen that positive phototrophic response made the southern and western arms were more attractive than others. The air flow and light arrangements were thus refined.

Table 2. Response of *T. chilonis* to kairomones in first prototype olfactometer

Cue	Number of adults in the kairomone arm at specific time periods							
	30 min	1 hr	1hr 30min	2 hr	2hr 30min	3 hr	3hr 30min	4 hr
10 % honey solution	10	24	32	41	49	50	53	48
10 % sugar solution	0	3	8	12	17	21	19	24
Corcyra moth scales	3	5	7	13	18	21	23	28
Control	0	0	0	0	0	0	0	0

Table 3. Response of *T. chilonis* to kairomones in acrylic olfactometer

Cue (MSE of *C. cephalonica*)	Number of adults in the kairomone arm at specific time periods							
	30 min	1 hr	1.30 hr	2 hr	2.30 hr	3 hr	3.30 hr	4 hr
Acetone extract	0	0	0	0	0	0	0	0
Ethanol extract	6	13	24	28	31	34	30	39
Ether extract	3	6	7	15	23	28	24	32
Hexane extract	0	0	0	0	0	0	0	0

4.2 Response of *T. chilonis* to semiochemicals in the standardized uniform air inflow olfactometer

4.2.1 Response of *T.chilonis* to moth extracts of *C. cephalonica* and *C. medinalis*

Extracts were prepared from whole moth of the leaf folder, *C. medinalis* and *C. cephalonica*. The parasitoids responded positively and differently to the moth extracts of both. Among the different solvent extracts, a significantly higher response was towards the hexane extract in both the cases (11.56, 10.04) respectively. Acetone extract (9.37, 8.0) was the next preferred kairomone followed by ether extract (7.62, 5.84) and there was only less attraction towards ethanol extract (3.98, 4.31) (Table 4a.).

4.2.2 Response of *T.chilonis* to moth scale extracts of *C. cephalonica*

Solvent extracts were prepared from the moth scales of *C. cephalonica*. The adult parasitoids got attracted to different extracts. Maximum attraction was found towards the hexane extract (12.36) followed by acetone extract (10.83), ether extract (6.94) and the least response was observed towards ethanol extract (6.04) (Table 4b.).

4.2.3 Response of *T.chilonis* to plant volatiles

Eight different weeds in the rice ecosystem and rice leaves as well as leaf folder damaged rice leaves were kept as different cues to the adult parasitoids. The results pertaining to the different sets of the four arms are summarized as Table 5a.

The statistical analysis of the data indicated that the most preferred weed is *E. crusgalli* (48.0) .The next order of preference was for *S. interrupta* (36.5) followed by wild rice, *O. rufipogon* (30.5). Leaf folder damaged leaf (24.25) and *C. distans* also attracted the parasitoids. An equal attraction was observed towards *M. quadrifolia* and *L. perennis*. Rice leaves had a very less attraction compared to

Table 4a. Olfactometric responses of *T. chilonis* to moth extracts of *C. cephalonica* and *C. medinalis*

Cue ***	Mean total of *Trichogramma* adults responded	Mean rank*	Cue ☆☆☆	Mean total of *Trichogramma* adults responded	Mean rank*
Acetone	22	8.0	Acetone	21.5	9.37
Ethanol	12.5	4.31	Ethanol	9.25	3.98
Ether	20.25	5.84	Ether	17	7.62
Hexane	29	10.04	Hexane	24	11.56
Control	0	0	Control	2	1.0

*** Moth extract of *C. cephalonica* in the specific solvents

☆☆☆ Moth extract of *C. medinalis* in the specific solvents

Table 4b. Olfactometric responses of *T. chilonis* to moth scale extracts of *C. cephalonica*

Cue	Mean total of *Trichogramma* adults responded	Mean rank*
Acetone	24.5	10.83
Ethanol	11	6.04
Ether	18	6.94
Hexane	32.25	12.36
Control	4.25	3.0

***Mean rank based on Kendall's test**

Table 5a. Olfactometric responses of *T. chilonis* to different weeds in the rice ecosystem

Sl no.	Weeds	Mean total of *Trichogramma* adults responded	Mean rank*
1	*Echinocloa crusgalli*	48	4.0
2	*Sacciolepis interrupta*	36.5	2.94
3	*Oryza rufipogon*	30.5	2.89
4	Leaf folder damaged leaf	24.25	2.50
5	*Cyperus distans*	10	2.44
6	*Digitaria ciliaris*	8.5	2.22
7	*Panicum repens*	6	2.17
8	*Ludwigia perennis*	3.5	1.78
9	*Marsilea quadrifolia*	2	1.78
10	*Oryza sativa*	4	1.67
11	*Eleusine indica*	1	1.61
12	*Cynodon dactylon*	0	0

***Mean ranks based on Kendall's test**

Table 5b. Olfactometric responses of *T. chilonis* to the preferred host plants

Sl no.	Weeds	Mean total of *Trichogramma* adults responded	Mean rank*
1	Leaf folder damaged leaf	44.25	3.56
2	*O. rufipogon*	32.25	3.06
3	*E. crusgalli*	25.5	2.54
4	*S. interrupta*	18	2.39

***Mean ranks based on Kendall's test**

leaf folder damaged leaves and the least preferred was *E.indica* (1.0). There was no attraction towards *C. dactylon.*

Data presented in Table 5b. indicated the order of preference to the weeds with highest mean ranking in the above set ups. Maximum attraction was towards the leaf folder damaged leaf (44.25) followed by *O. rufipogon* (32.25), *E. crusgali* (25.5) and least attraction was towards *S. interrupta* (18.0).

4.2.4 Response of *T.chilonis* to semiochemicals

Among the different choices for the adult parasitoids, significant attraction was found towards 10 per cent honey solution (2.44). The mean rank was same for both 10 per cent sugar solution and *Corcyra* scales (1.78) which indicated that there was an equal attraction towards both. No adults entered the control arm without any cues (Table 6).

4.2.5 Response of *T.chilonis* to different stages of rice leaf folder, *C. medinalis*

The attraction of *T. chilonis* to different stages of *C. medinalis* is summarized in Table 7. Highest response was towards the adult moths (31.0) and there was also attraction towards larva (26.0) and least preference was towards pupa (19.25)

4.3 Wind tunnel experimentation results

T. chilonis adults responded positively to the volatiles emitted from different semiochemicals (Table 8). The adults dispersed a distance of 135 cm towards 10 per cent honey solution. Maximum distance travelled towards the weed, *O. rufipogon* was 130 cm .The parasitoids travelled a distance of 99 cm towards *S. interrupta.* The adults responded to the hexane extract of *C. cephalonica* scales by reaching a distance of 98 cm.

Table 6. Olfactometric responses of *T.chilonis* to different kairomones

Cue	Mean total of *Trichogramma* adults responded	Mean rank*
10% honey solution	52.25	2.44
10% sugar solution	28	1.78
Corcyra scales	30.25	1.78
Control	0	0

***Mean ranks based on Kendall's test**

Table 7. Olfactometric responses of *T.chilonis* to different stages *C. medinalis*

Stages of *C.medinalis*	Mean total of *Trichogramma* adults responded	Mean rank*
Larva	26	2.17
Pupa	19.25	1.56
Adult	31	2.28
Control	0	0

***Mean ranks based on Kendall's test**

Table 8. Distance travelled by *T. chilonis* adults upwind in the wind tunnel set up.

Cue	Distance travelled by *T. chilonis* adults at specific time periods (cm)					
	30 min	1 hr	1 hr 30 min	2 hr	2 hr 30 min	3 hr
10 % honey solution	15.5	26	51	94	102	135
O. rufipogon	8	36	38	81	125	130
S. interrupta	9	16	22	40	76	99
MSE of *C. cephalonica* in hexane	12	21	59	71	83	98
Corcyra scales	8	15	29	30	69	85
E. crusgalli	5	8	17	34	44	78

4.4 Refrigerated storage studies

The effect of storage on the emergence of *T. chilonis* is summarized in Table 9.

Among the different day old parasitized cards, five day old card was found to have maximum emergence where as two day old card had minimum emergence. There was no blackening of eggs and emergence in the case of one day old parasitized cards.

4.4.1 Two day old card

The statistical analysis of the data in Table 9 indicated that the per cent emergence of parasitoids was 45.61 after one day and it was on par with 8th (49.61%), 15th (46.04%) and 22nd (40.18%) day respectively. Thereafter it declined considerably and it was only 10.82 per cent after 36 days of storage. There was no emergence from 43rd day onwards.

4.4.2 Three day old card

The adult emergence was 69.12 per cent on first day after storage and this was closely followed by emergence on 8th day (58.56%). The emergence on 15th day was 51.54 per cent which was statistically on par with 22nd day (50.45). The emergence varied significantly from 41.36 per cent to 8.59 per cent within 29 to 43 days and there was no emergence after 50th day.

4.4.3 Four day old card

The data revealed that 88.59 per cent emergence was noticed on first day after storage followed by 79.51, 66.04 and 60.98 per cent on 8th, 15th and 22nd day respectively. Per cent emergence on 29th day (53.98%) was found to be on par with that on 36th day (55.39%). More than 50 per cent emergence was noticed up to 36 days and subsequently it declined and was only 7.89 per cent after 50 days of storage.

Table 9. Effect of refrigerated storage on the number of *T.chilonis* adults emerged

Refrigerated storage period (days)	1 day	2 day	3 day	4 day	5 day	6 day	7 day	8 day
1	0	45.61[a] (6.78)	69.12[a] (8.34)	88.59[a] (9.44)	95.06[a] (9.77)	92.86[a] (9.66)	87.01[a] (9.35)	73.34[a] (8.59)
8	0	49.61[a] (7.08)	58.56[b] (7.69)	79.51[b] (8.95)	86.41[ab] (9.32)	81.65[a] (9.06)	87.93[a] (9.40)	60.62[b] (7.82)
15	0	46.04[a] (6.82)	51.54[c] (7.21)	66.04[c] (8.16)	80.93[b] (9.02)	82.49[a] (9.11)	80.64[b] (9.0)	50.12[c] (7.11)
22	0	40.18[a] (6.38)	50.45[c] (7.14)	60.98[d] (7.84)	82.44[b] (9.11)	81.57[a] (9.06)	76.41[c] (8.77)	41.43[d] (6.47)
29	0	21.52[b] (4.68)	41.36[d] (6.47)	53.98[e] (7.38)	65.999 c (8.15)	58.59[b] (7.67)	64.05[d] (8.03)	32.54[e] (5.75)
36	0	10.82[c] (3.36)	22.48[e] (4.79)	55.39[e] (7.48)	40.64[d] (6.41)	36.1[c] (6.05)	40.72[e] (6.42)	22.97[f] (4.84)
43	0	0[d] (0.71)	8.59[f] (3.0)	31.57[f] (5.66)	31.49[e] (5.65)	27.45[d] (5.28)	25.02[f] (5.05)	8.94[g] (3.07)
50	0	0[d] (0.71)	0[g] (0.71)	7.89[g] (2.89)	11.54[f] (3.45)	9.58[e] (3.17)	10.99[g] (3.39)	0[s] (0.71)

Values in parenthesis are the $\sqrt{x} + 0.5$ transformed values.

The values within the row and column followed by the same letters do not differ significantly in DMRT p=(0.05)

4.4.4 Five day old card

A highest per cent emergence of 95.06 was observed after one day of storage and it was on par with 8th day (86.41%). Per cent emergence on 22nd day (82.44%) was on par with 8th day (86.41%). Per cent emergence was 65.99 on 29th day and there after it decreased considerably and was only 11.54 per cent after 50 days of storage.

4.4.5 Six day old card

Statistical analysis of the data indicated that the per cent emergence up to 22nd day of storage was on par with each other and the values ranged from 92.86 per cent (1st day) to 81.57 per cent (22nd day).

4.4.6 Seven day old card

An 87.93 per cent of adult emergence was observed on 8th day which was statistically on par with the per cent emergence on first day of storage (87.01%). Per cent emergence on 15th day was 80.64 followed by 76.41 on 22nd day and thereafter a decreasing trend was observed.

4.4.7 Eight day old card

The data presented in Table 9 revealed that there was significant difference in the emergence during the storage periods. Fifty per cent emergence was noticed up to 15 days and later on the emergence reduced steeply and there was no emergence on 50th day.

4.5 The effect of refrigerated storage on the parasitization efficiency of *T. chilonis*

The effect of refrigerated storage on the parasitization efficiency of *T. chilonis* is summarized in Table 10. In the case of two day old card exposed for parasitization, 56.25 per cent of apparent ratio was <40 per cent but the absolute

Table 10. Effect of refrigerated storage on the parasitization efficiency of *T. chilonis*

Absolute% parasitism / Apparent % parasitism	Days	< 20 %	20-40 %	40-60 %	> 60 %	Total
< 40 %	2	3[a] 33.33[b]	6[a] 66.67[b]	0	0	9[a] 56.25[b]
	3	0	2[a] 66.67[b]	1[a] 33.13[b]	0	3[a] 18.75[b]
	4	0	3[a] 30.0[b]	3[a] 30[b]	4[a] 40.0[b]	10[a] 62.5[b]
	5	0	0	4[a] 44.64[b]	5[a] 55.56[b]	9[a] 56.25[b]
	6	0	0	4[a] 40.0[b]	6[a] 60.0[b]	10[a] 62.5[b]
	7	0	3[a] 27.27[b]	3[a] 27.27[b]	5[a] 45.45[b]	11[a] 68.75[b]
	8	0	6[a] 60.0[b]	4[a] 40.0[b]	0	10[a] 62.5[b]
40-60 %	2	5[a] 100[b]	0	0	0	5[a] 31.25
	3	6[a] 60.0[b]	4[a] 40.0[b]	0	0	10[a] 62.5[b]
	4	3[a] 60[b]	2[a] 40.0b	0	0	5[a] 31.25[b]
	5	2[a] 40.0[b]	3[a] 60.0[b]	0	0	5[a] 31.25[b]
	6	2[a] 66.67[b]	1[a] 33.33[b]	0	0	3[a] 18.75[b]
	7	3[a] 100[b]	0	0	0	3[a] 18.75[b]
	8	3[a] 100[b]	0	0	0	3[a] 18.75[b]
> 60 %	2	2[a] 100[b]	0	0	0	2[a] 12.5[b]
	3	3[a] 100[b]	0	0	0	3[a] 18.75[b]
	4	1[a] 100[b]	0	0	0	1[a] 6.25[b]
	5	2[a] 100[b]	0	0	0	2[a] 12.5[b]
	6	2[a] 66.67[b]	1[a] 33.33[b]	0	0	3[a] 18.75[b]
	7	2[a] 100[b]	0	0	0	2[a] 12.5[b]
	8	3[a] 100[b]	0	0	0	3[a] 18.75[b]

a : frequency b : relative percentage

The values within the row and column followed by the same letters do not differ significantly in DMRT p=(0.05) ratio in this group was all below 40 per cent. 31.25 per cent of the apparent ratio was between 40 and 60 per cent.and the remaining 12.5 per cent was >60 per cent but the absolute ratio was so poor that it was less than 20 per cent in all the cases.

In three day old card, 61.25 per cent of the apparent ratio was between 40 and 60 per cent but the absolute ratio was <40 per cent. But in this group, the absolute ratio between 20 and 40 per cent was only 40 per cent. Even when the apparent ratio of <40 per cent was noticed in three day old card, the absolute ratio was between 40 and 60 per cent in 33.13 per cent of the cases.

The same inference could be drawn from four day old card which means that there is no change in relation between apparent and absolute ratio with the expectation that in the apparent ratio group of <40 per cent, 40 per cent of the absolute ratio was >60 per cent. As we proceed to the case of five day old card, the same can be noticed with a slighter increment in >60 per cent absolute ratio under <40 per cent apparent ratio category.

The peak activity could be noticed in six day old parasitized cards with the tendency to taper down slowly in seven day old card and sharply in eight day old card.

DISCUSSION

5. DISCUSSION

The present problem was identified as a step towards optimizing the use of scarcely available trichocards and also to increase their field efficiency. Even though trichocards are available for the farmers, their use at the right time at the right situation is highly essential. Olfactometric responses to plant volatiles and kairomones were done towards this goal. Storage studies helped to extend the availability of *Trichogramma* adults under normally unavailable periods. The results of the studies are discussed in the following paragraphs.

5.1 Olfactometer

The prototype olfactometer using polyester film was utilized for initial response studies. This gave an idea on whether confined trichogrammatids would have a directed response to the semiochemical cues under laboratory situations.

5.1.1 Design testing and validation of prototype

The prototype olfactometer using polyester film was utilized for initial response studies. The trichogrammatid response over a period of four hours towards the four arms of the prototype gave a clear idea that the kairomones definitely attract the trichogrammatids. It was also seen that except stray movements backwards under some time periods, the attraction to 10 per cent honey solution, 10 per cent sugar solution and scales of the rearing host (*C. cephalonica*) was positive, consistent and cumulative.

5.1.2 Design and fabrication of the acrylic olfactometer

The acrylic olfactometer was designed, fabricated and was put to validation by using solvent extracts of moth scales of *C. cephalonica*. Initially the responses were definite, cumulative and consistent. However, due to the continued draught of air that is sucked out through the narrow tube, a lot of dust got accumulated inside. These were primarily the remnants of the *Corcyra* eggs and moth scales available on the trichocard. This lead to the partial blocking of the tube and consequent erratic responses. Hence the set up was changed to directed inflow of air through the individual arms of the olfactometer.

5.2 Response of *T. chilonis* to semiochemicals in the standardized uniform air inflow olfactometer

5.2.1 Response of *T. chilonis* to moth extracts of *C. medinalis* and *C. cephalonica*

Results revealed that hexane extract was highly responsive than the other three solvent extracts in both *C. medinalis* and *C. cephalonica* extracts(Table 4a, Fig.1). This may be because hexane could extract more concentration or wider range of the kairomones which attract the parasitoids. This is in agreement with the investigations by Lewis *et al.* (1972) who reported that the host seeking stimulant of *T. evanescens* was contained in the scales of the host moths and that it was extractable with hexane. They also found that the hexane extract of the scales retained the biological activity and significantly improved the parasitization of host eggs.

The results establish the affinity of the parasitoid to its most preferred field host, *C. medinalis*. Except in the case of ethanol extract, responses towards *C. medinalis* was distinct and superior to that of *C. cephalonica*. This finding confirms that even though *C. cephalonica* is the usual rearing host in the lab, *T. chilonis* will always prefer *C. medinalis* in the field. Hence it will be effective in the field.

5.2.2 Response of *T. chilonis* to moth scale extracts of *C. cephalonica*

The attraction of *T. chilonis* to extracts of moth scale in hexane and acetone was observed in the experiments (Fig.2). Jones *et al.* (1973) isolated tricosane from hexane extracts of *H. zea* scales which elicited significant orientation and stimulated parasitization in both laboratory and small field studies.

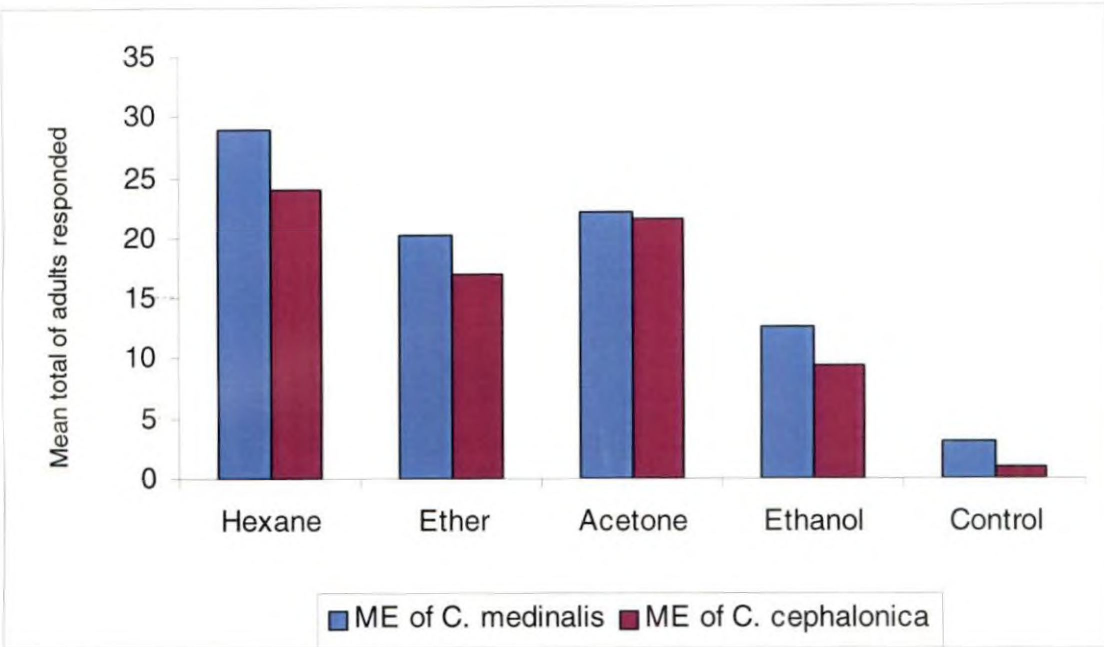

Fig. 1. Olfactometric responses of *T.chilonis* to moth extracts of *C. cephalonica* and *C. medinalis*

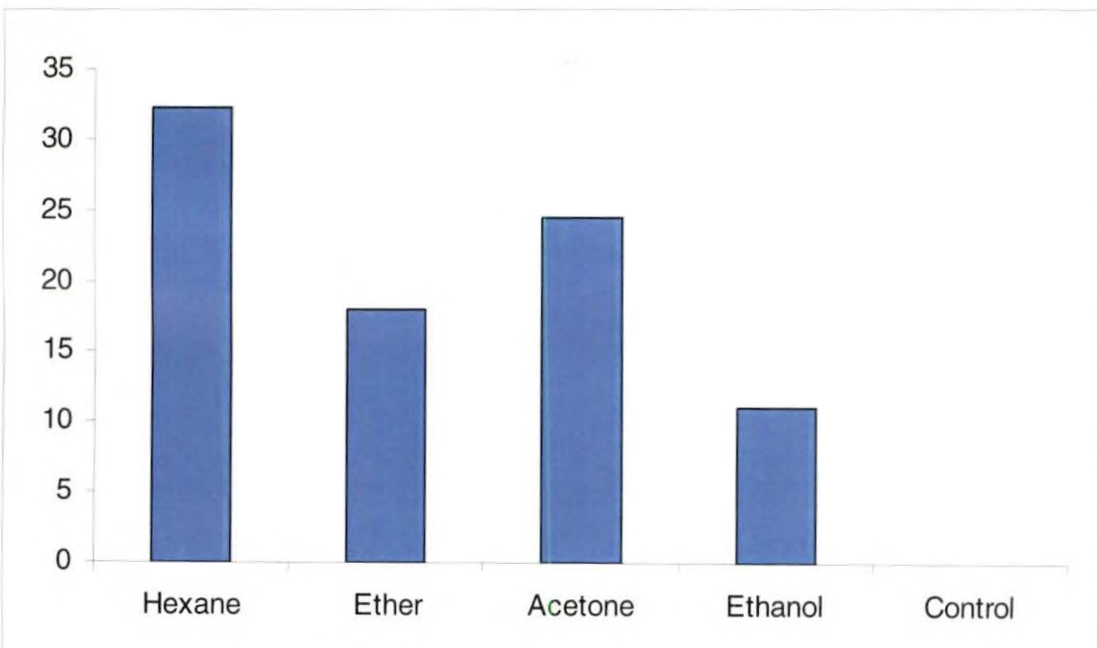

Fig. 2. Olfactometric responses of *T. chilonis* to MSE of *C. cephalonica*

Since moth scales of *C. cephalonica* are a bye-product of *Trichogramma* production, it would be worthwhile to utilize it for increasing field parasitization if possible. A practical utility of this finding is to use slow release absorptive wooden blocks impregnated with the solvent extract in the paddy field. It is also possible to add diluted honey solution in the impregnated blocks so as to increase the longevity of the adults. Farmers can make kairomone dispensers by solvent extracts of moth scales and impregnating on cotton wicks with honey solution. Wang and Zong (1991) also reported that moth scale extract of *P. gossypiella* increased the time spent by parasitoids and decreased the speed of movement in the treated areas.

5.2.3 Response of *T. chilonis* to plant volatiles

The attraction of *T. chilonis* to different weeds in the rice ecosystem as well as the leaf folder damaged leaves and rice leaves are presented in Table 5a and Fig 3a. It was seen that the highest response was towards *E. crusgalli* (4.0). Khan and Tiwari (2001) also reported that wild hosts have a positive effect on the emergence and parasitization efficiency of *T. chilonis*. According to their results, the egg cards treated with a fern, *Pteridium aquilinum*, resulted in maximum mean per cent parasitism and in the cards treated with *Eucalyptus rostrata* maximum mean per cent emergence was observed.

The results indicated that there was only very less attraction towards the rice leaves which is contradictory at the initial outset. However a scrutiny of the results revealed that this is an adaptation by the parasitoids. There is an increased preference towards the wild hosts where there will always be overlapping generations of leaf folder. In the main crop field, the availability of leaf folder is restricted to specific growth stages where there is a uniform system of crop growth. However, there is a clear preference of leaf folder damaged leaves. This means that there is migration towards the leaf folder damaged leaves under abundance of leaf folder infestation in the rice crop. Survival of the species in non cropped situations is assured by its positive response to the weeds.

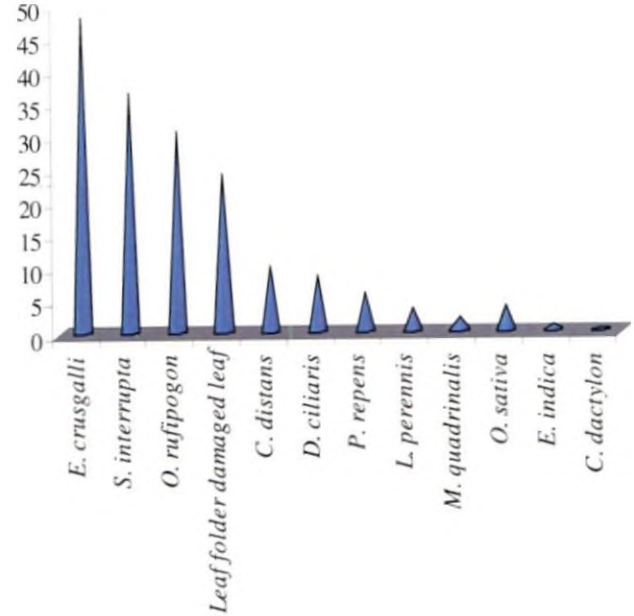

Fig. 3a. Olfactometric responses of *T. chilonis* to different weeds in the rice ecosystem

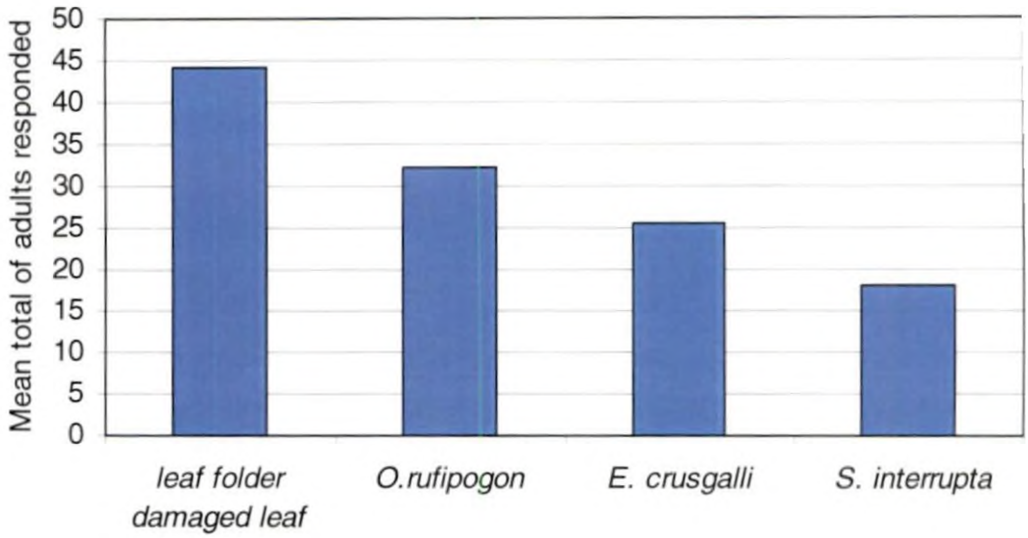

Fig. 3b. Olfactometric responses of *T. chilonis* to preferred host plants

From among the ten different weeds and two types of rice leaves set up as control, the maximum preferred weeds were again exposed in the olfactometer (Table 5b, Fig.3b). The results are a clear pointer to the ecological adaptation of *T. chilonis* for survival. The highest attraction was towards the leaf folder damaged leaf indicating an r-strategy where abundance of leaf folder in the paddy field is utilized for increasing the population to the maximum. The next preference to *O. rufipogon* is again very significant. This weed which is called as "*varinellu*" in malayalam is the most prominent companion weed in any rice ecosystem. The availability of this weed is hence almost assured. A distinct adaptability to varinellu makes host (*C. medinalis*) availability for *T. chilonis* a very good possibility. This would be one of the most adapted tritrophic interactions in the rice / weed – host insect-parasitoid regime.

5.2.4 Response of *T. chilonis* to semiochemicals

Results revealed that *T. chilonis* adults had a higher response towards 10 per cent honey solution (Table 6, Fig.4). This is in support to the results where *C.carnea* adults showed higher response to honey in EAG and orientational response studies (Anonymous, 2000).

In general, the flight range of trichogrammatids has not been reported beyond a normal range of 10 m. In those situations, the effectiveness of egg cards or natural parasitization in controlling widely dispersing populations of leaf roller may be limited. Then band or grid application of 10 per cent honey solution can substantially increase biocontrol by *Trichogramma*. Hence it is worthwhile to redesign the existing trichocard to contain more number of small strips with lesser parasitoids per grid. From these, more number of tricho sub cards can be cut and hung at more places within the same field. It is likely that it might increase the longevity of adults as well as the per cent parasitism. This result is in accordance with the findings of Sheeba *et al.* (2002) who concluded that honey feeding had a positive impact on the longevity and parasitic efficiency of *T. japonicum*. Ram *et*

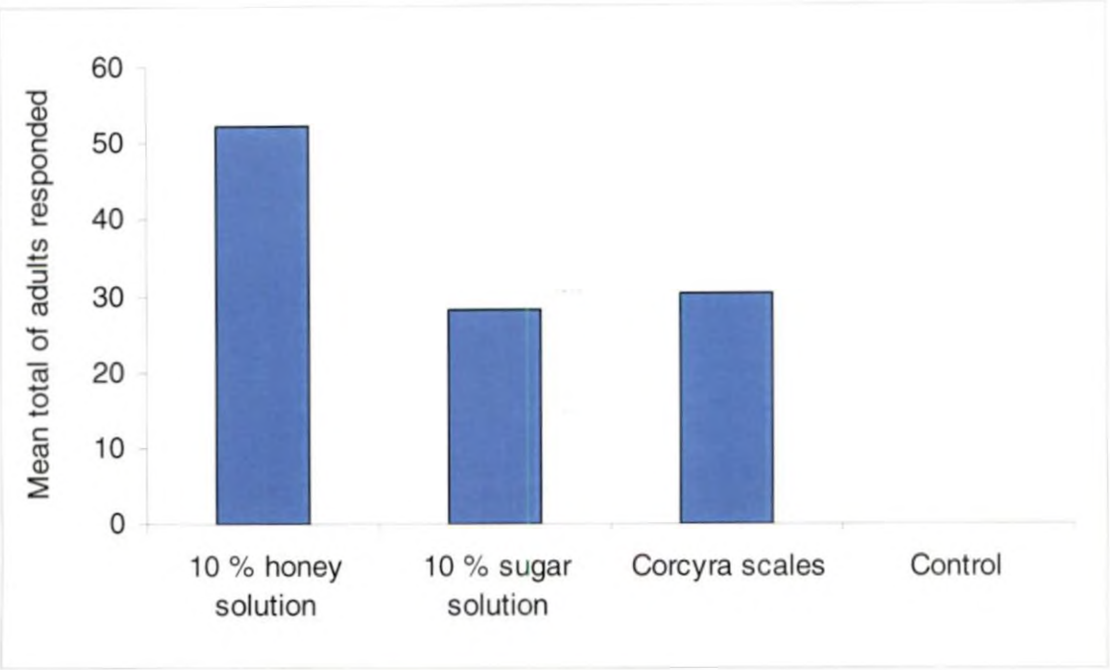

Fig. 4. Olfactometric responses of *T. chilonis* to different kairomones

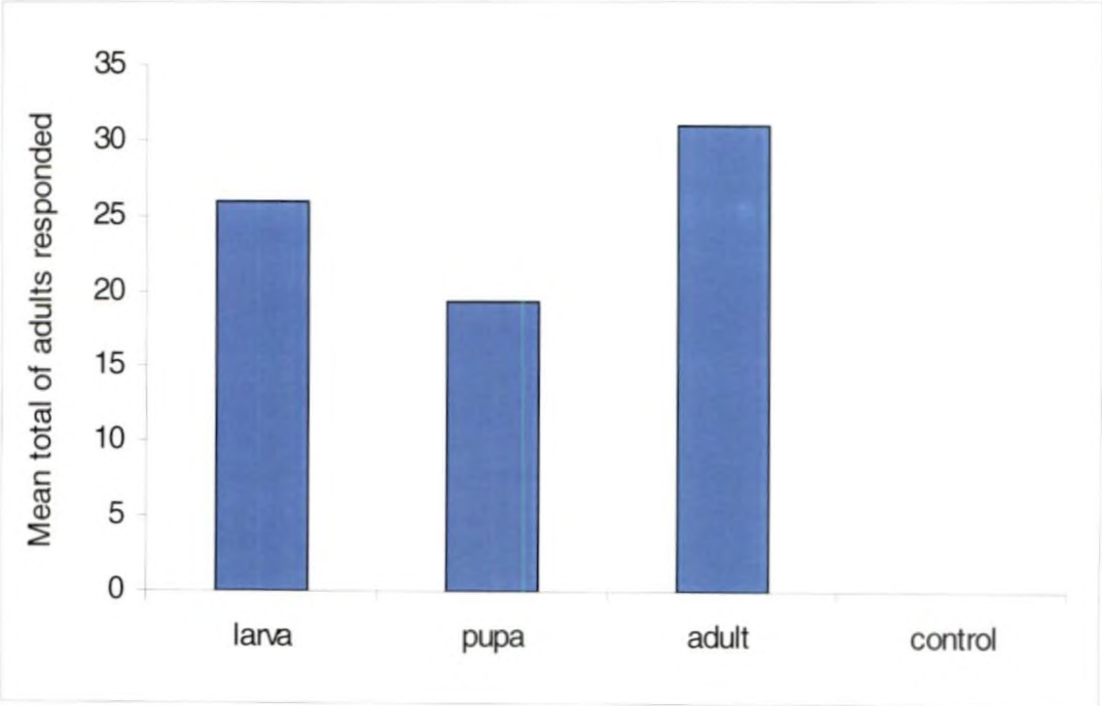

Fig. 5. Olfactometric responses of *T. chilonis* to different stages of *C. medinalis*

al. (1997) also reported that the longevity of *T. japonicum* adults was highest with honey solution as compared to solutions of sugar and glucose.

A second choice is to utilize 10 per cent sugar solution or the *Corcyra* scales which are wasted in the Biocontrol laboratories. Sugar solutions or moth scales can also be used for spot application in either the crop season amongst the rice area or in the weed areas during the fallow season. This strategy may be especially necessary during dry weather with no crop or very sparse weed population. At these times, the honey solution, sugar solution or moth scales make the parasitoid to remain in the vicinity for a longer time and also in better health.

5.2.5 Response of *T.chilonis* to different stages of rice leaf folder, *C. medinalis*

Table 7 depicts the results of olfactometric responses of *T. chilonis* to different stages of C. medinalis. Results of the experiment as picturised in Fig.5 validate the time tested theories regarding host parasitoid interaction. The adult moth which is the primary producer of eggs is the most preferred from among the different stages tried. The larval stage is the next preferred over the pupa. It may be probably due to a lower release of volatiles emanating from the inactive pupal stage.

5.3 Wind tunnel studies

The wind tunnel studies demonstrated the behavioural studies of *T. chilonis* adults towards different semiochemicals (Table 8). The adults responded positively by upwind flight to 10 per cent honey solution. The maximum distance covered within the stipulated time was to this food cue. The adults travelled a distance of 85 cm towards the *Corcyra* scales. So, it may be worthwhile to follow a strategy of grid or band application of 10 per cent honey solution and moth scales in the field for more retention and longevity and probably higher search efficiency of *T. chilonis* adults. This will ultimately increase the efficiency of leaf roller management.

The adults travelled a distance of 130 cm towards the weed, *O. rufipogon* and 99 cm towards *S. interrupta*. Mitchell *et al.* (1991) reported that *H. virescens* females responded positively to the volatiles emitted from crude extracts of three different plant hosts (cotton, tobacco and a weed *Desmodium tortuosum*). Tingle *et al.* (1989) also reported that mated *H. subflexa* females flew upwind in the wind tunnel bioassays to a methanol wash of fresh whole leaves from its host plant, ground cherry (*P. angulata)*.

5.4 Storage studies

Among the different day old parasitized cards, five day old card was found to have maximum emergence where as two day old card had minimum emergence (Fig.6). There was no emergence in the case of one day old parasitized card. The absence of emergence of the one day old card must be probably due to incomplete development of embryo to the organogenesis stage. At this time, it may only be in the blastoderm or germ band stage. Jalali and Singh also reported the failure of emergence in one day old cards. They concluded that it was due to the desiccation of host eggs when stored in the egg stage resulting in increased mortality.

The data (Table 9) revealed that the parasitized cards stored at prepupal and pupal stage (4-7 day old cards) had maximum emergence during the refrigerated storage periods. Similar observations were recorded in a study conducted by Curl and Burbutis (1977) which indicated that pupae of *T. nubilale* Ertle and Davis are more tolerant to relative long term exposure than other stages. Lopez and Morrison (1980) also reported that late larval and pupal stage of *T. pretiosum* were more tolerant to 0°C than other stages.

At room temperature, average emergence of *T. chilonis* was above 95 per cent. However, adults from different temperature and storage period emerged only when removed from storage to laboratory temperature. This is in accordance with Chen and Hung (1981) who reported that development ceased when *T.*

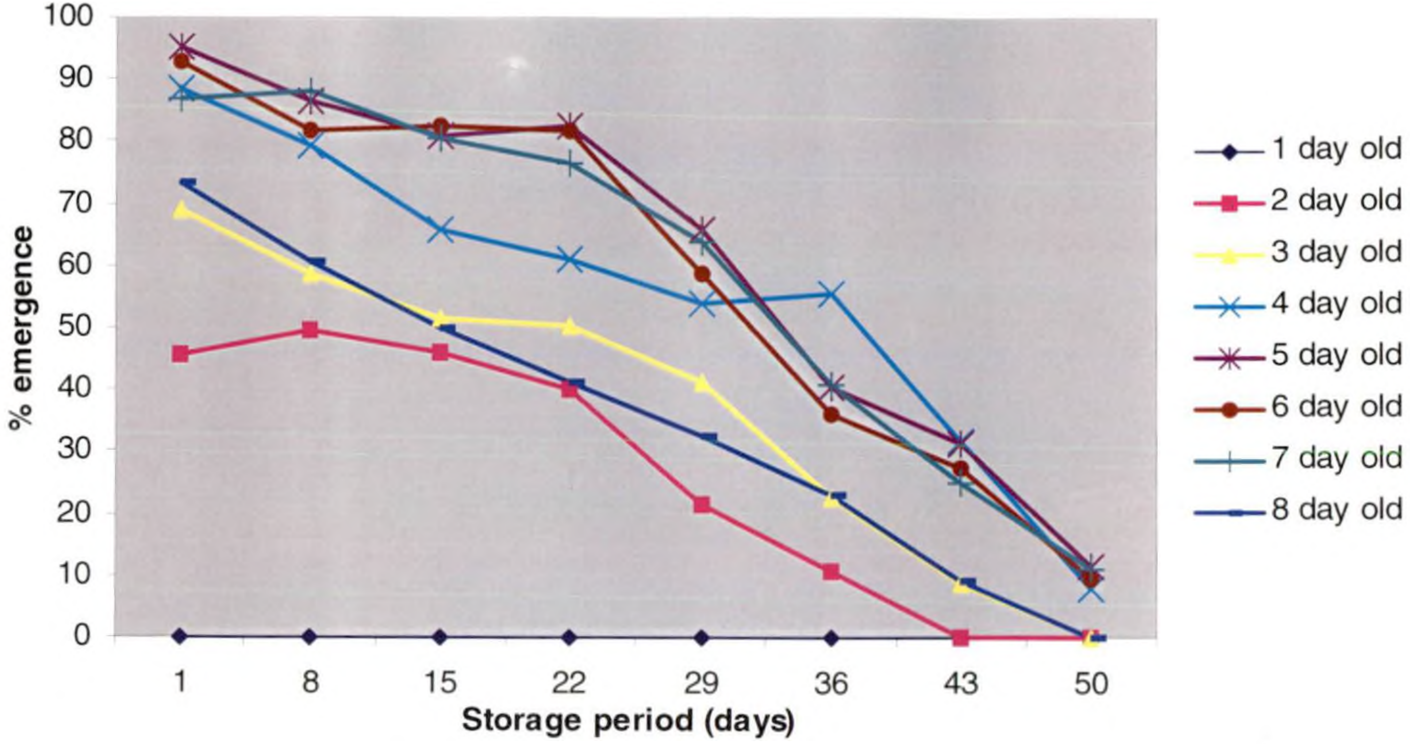

Fig. 6. Effect of refrigerated storage on the emergence of *T.chilonis*

australicum were kept at 6° and 10°C though it continued when removed to the room temperature.

Less than 50 per cent adult emergence was observed in the cards stored after two day of parasitization. Gupta and Bhardwaj (2002) also experienced drastic reduction (35 %) in emergence in the case of two day old parasitized card. It is clear from the graph (Fig. 6) that the adult emergence was >80 per cent when stored for 15 days in the case of 5-7 day old parasitized card. Venkataraman and Govil (1952) had reported that storage of *T. chilonis* in prepupal stage can be at 12°C for 15 days.

More than 50 per cent emergence was noticed on 4-7 day old parasitized cards upto 29 days after storage. This is in line with the findings of Killineer *et al.* (1990) who reported that storage of *Trichogramma* spp. as the parasitized *S. cerealella* eggs up to 30 days does not adversely affect their emergence. Khosa and Brar (2000) also reported that different populations of *T. chilonis* can be stored for 22 days under refrigeration.

There was no emergence after 50 days of storage in any of the parasitized cards. Jalali (1992) also observed that at 8°C storage, *T. chilonis* reached zero emergence after 60 days of storage and this period was found to be 49 days when stored at 10°C. On the other hand, Singh and Jalali (1994) reported that *T. eldanae* could be stored up to 80 days at 5°C.

The results of storage studies are also of practical significance in the actual laboratory situation. In many laboratory conditions, *Corcyra* culture may sometimes be completely rendered useless due to the attack by *Tribolium castaneum*, mites and *Bracon hebetor*. The results of our study have shown that the *Trichogramma* emergence can be delayed even up to 50 days under ordinary refrigerated storage. The per cent emergence however is only in the range of 9-11 per cent. This is in support to the findings of Singh and Jalali (1994) who reported that *T. brasiliense* can be stored up to 50 days at 10°C. But this is sufficient to start a new culture, may be with an increase in the *Trichogramma* – *Corcyra* ratio from 40:1 to about 4:1. At 10 per cent parasitoid emergence this is equivalent to the original ratio. Even for farmer situations, it may be more advisable to manage initial leaf folder populations at its first level of emergence itself. At this time the population is very low and even a 10 per cent parasitoid emergence can easily take care of first generation of leaf folder. Monitoring of initial broods of leaf folder can be easily done by installing light traps in water pan.

5.4.1 Time rider card

According to the time rider card (Fig.7), the maximum absolute emergence of the parasitoids (>75 %) are displayed as green areas. The emergence in between 50 – 75 per cent is represented as yellow and those below 50 per cent are displayed as red areas. It is seen that 5, 6 and 7 days after parasitization if they are subjected to refrigeration at 7°C, the absolute per cent emergence is not substantially changed as per statistical analysis. The perusal of the data (Table 8) shows that there is a progressive decline of parasite emergence with time. Maximum values have been obtained with 5 and 6 days after parasitization. The current recommendation is to use 7 day old card for storage. Since each egg card contains about 16-20,000 eggs, even a very small increase in percentage emergence would add substantially to the total *Trichogramma* output. For further refrigeration up to 36 days (yellow area), 4th day card perform well with 55.39 per cent. This is of interest to nucleus stock of *Trichogramma* being maintained at laboratories where they can go for a reasonably good per cent emergence, even after five weeks of refrigerated storage.

5.5 The effect of refrigerated storage on the parasitization efficiency of *T. chilonis*

The effect of refrigerated storage on the parasitization efficiency of *T. chilonis* is summarized in Table 10. The table explains the real biological situation. Apparent ratio is an indication of the actual number of *T. chilonis* adults

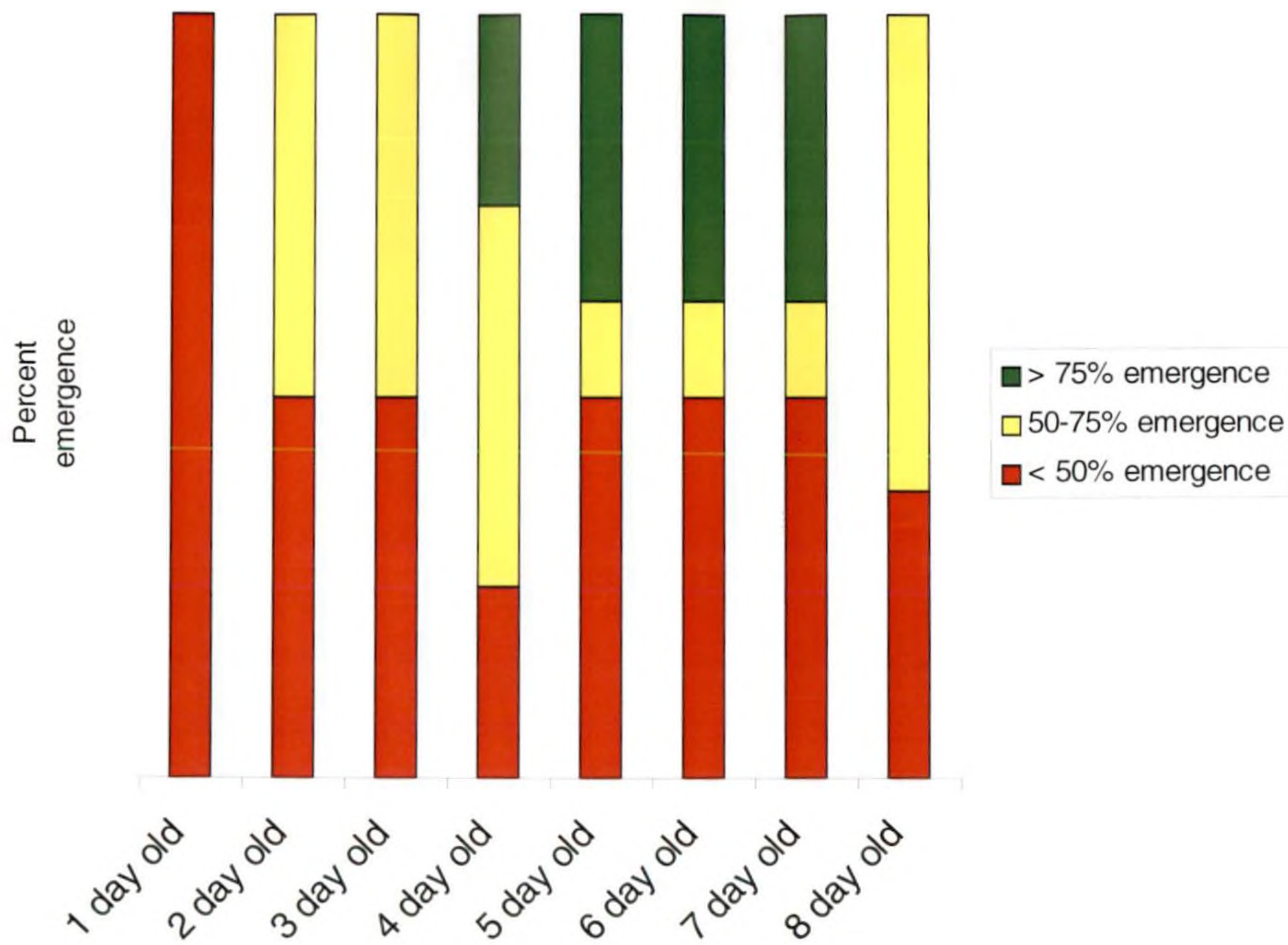

Fig. 7. Time rider card

that emerge. So the ratios show the relative utilization of *Corcyra* eggs for parasitization by the adults that have emerged during the particular time period. When a large number of adults have emerged, they would parasitize the available eggs and contribute to the absolute ratio. During the next time period, the ratios reflect the parasitization of the *Corcyra* eggs that are still available. This is the reason why there is a distribution of frequency and relative per cent of parasitization in the limited range of 20-40, 40-60 and >60 per cent with regard to absolute parasitism. The same reason holds good for limited parasitization with in the range of 40-60 per cent for apparent parasitization.

SUMMARY

6. SUMMARY

A study entitled 'Enhancing the effectiveness of the egg parasitoid *Trichogrmma chilonis* Ishii (Trichogrammatidae: Hymenoptera)' was carried out in the College of Horticulture, Vellanikkara with the objectives of increasing the effectiveness of *T. chilonis* and to standardize the optimum storage period of the trichocards.

A prototype of olfactometer suitable to monitor the movements of *T. chilonis* was at first made using polyester film for standardizing the design. The prototype was validated by keeping the kairomones in each of the four arms. As this was found to be a success, it was further fabricated with transparent acrylic sheet incorporating slight modifications. Various error factors were identified during the continued studies which were eliminated and an improved design was standardized as uniform air inflow olfactometer.

The responses of *T. chilonis* to different semiochemicals were studied in the uniform air inflow olfactometer. The semiochemicals used were moth extracts of *Cnaphalocrocis medinalis* and *Corcyra cephalonica* in different solvents (acetone, ethanol, ether and hexane), moth scale extracts of *C. cephalonica*, different weed plants in the paddy ecosystem, leaf folder damaged leaves, kairomones (honey solution, sugar solution, *Corcyra* scales) and different stages of *C. medinalis*. Observations were based on the number of adults entering into the respective arms at half an hour interval for a minimum period of 6 hours.

A higher response of adult parasitoids was noticed towards moth extracts of *C. medinalis* and *C. cephalonica* and also moth scale extracts of *C. cephalonica* in hexane solvent. This may be because hexane could extract the specific compound in more concentration or wider range of the kairomones which attract the parasitoids. A lower attraction of *T. chilonis* was observed in the ethanol extract. The results corresponding to the response to plant volatiles emanating from the weed plants are a clear pointer to the ecological adaptation of *T. chilonis* for survival. The highest attraction was towards the leaf folder damaged leaves followed by plant parts of *Oryza rufipogon*. No response was found towards the weed plant *Cynodon dactylon*. Among the different semiochemicals, significant attraction was towards 10 per cent honey solution. The results thus established that a band or grid application of honey solution will increase the longevity of adults as well as the per cent parasitism. Sugar solution (10 %) and *Corcyra* scales had an equal attraction for the adults when compared to control. The adult moth which is the primary producer of eggs is the most preferred from among the different life stages of *C.medinalis* tried. The larval stage is the next preferred over the pupa. It may probably be due to a lower release of volatiles emanating from the inactive pupal stage.

A wind tunnel set up was fabricated to evaluate the olfactory responses of *T. chilonis* to the best semiochemicals. The distance travelled by the parasitoids was observed for every 30 minutes, for a total duration of three hours. Maximum distance travelled by the adult parasitoids was towards honey solution followed by the weed *O. rufipogon*. It was seen that within a matter of few hours, the minute adult *Trichogramma* can travel a distance of 1.35 metres. The distance travelled was almost equal in MSE of *C. cephalonica* in hexane solvent and *Corcyra* scales. The results validate the findings observed using the four arm olfactometer (free choice test) with a single source wind tunnel (no choice test).

The effect of refrigerated storage on the emergence and parasitization efficiency of *T. chilonis* was studied. Among the different day old parasitized cards, five day old card was found to have maximum emergence where as two day old card had minimum emergence. There was no blackening of eggs and emergence in the case of one day old parasitized cards. More than 50 per cent emergence was noticed up to 36 days in four day old cards which is of interest to nucleus stock of *Trichogramma* being maintained at the laboratories. The results of the study have shown that the *Trichogramma* emergence can be delayed even up to 50 days under ordinary refrigerated storage. Even though the emergence is around 11 per cent, it is sufficient to start a new culture, may be with an increase in the *Trichogramma* – *Corcyra* ratio from 40:1 to about 4:1. Such cards even with around 10 per cent efficiency would be more economical than continuous rearing of *Corcyra* culture and production of trichogrammatids. Even for farmer situations, it may be more advisable to manage initial leaf folder populations at its first level of emergence itself. At this time the population is very low and even a 10 per cent parasitoid emergence can easily take care of first generation of leaf folder.

REFERENCES

REFERENCES

Altieri, M.A. and Todd, E.W. 1981. Some influences of vegetational diversity on insect communities of Geogia soybean fields. *Prot. Ecol.* 3: 333-338

Altieri, M.A., Lewis, W.J., Nordlund, D.A., Gueldner, R.C. and Todd, J.W. 1981. Chemical interactions between plants and *Trichogramma* wasps in Georgia soybean fields. *Prot. Ecol.* 3: 259-263

Ananthakrishnan, T.N., Senrayan, R., Murugesan, S. and Annadurai, R.S. 1991. Kairomones of *Heliothis armigera* and *Corcyra cephalonica* and their influence on the parasitic potential of *Trichogramma chilonis* (Trichogrammatidae: Hymenoptera). *J. Biosci.* 16: 111-119

Anonymous. 1999. Annual Report. Project Directorate of Biological Control. Bangalore. pp. 84

Anonymous. 2000. Annual Report. Project Directorate of Biological Control. Bangalore. pp. 32

Anonymous. 2003. Mass production of trichogrammatids and chrysopids. *tech. Bull.* Project Directorate of Biological Control, Bangalore. (eds: Jalali, S.K., Rabindra, R.J., Rao, N.S. and Dasan, C.B.). 33:7

Bakchine, E., Delegue, P.M.H., Kaiser, L. and Masson, C. 1990. Computer analysis of the exploratory behaviour of insects and mites in an olfactometer. *Physiol. Behaviour.* 48: 183-187

Bakthavatsalam, N. and Singh, S.P. 1996. L-Tryptophan as an ovipositional attractant for *Chrysoperla carnea* (Stephens)(Neuroptera:Chrysopidae). *J.Biol.Control* 10: 21-22

Bakthavatsalam, N., Singh, S.P., Tandon, P.L., Chaudhary, M. and Preethi, S. 1999. Behavioural responses of key parasitoids of *Opisina arenosella* Walker (Lepidoptera:Noctuidae) to the kairomones. *J. Biol. Control.* 13: 7-14

Balasubramani, V., Sridharan, S. and Sadakathulla, S. 2000. Effect of shade on leaf folder incidence in hybrid rice. *Insect Environ.* 6(1): 15-16

Balasubramanian, G., Babu, S.P.C., Venkatesan, S. and Gopalan, M. 1994. Evaluation of *Trichogramma japonicum* Ashmead against stem borer and *T. chilonis* Ishii against leaf folders in rice. *Pest Mgmt. Econ. Zool.* 2(2): 97-100

Ballal, C.R. and Singh, S.P. 2003. The effectiveness of *Trichogramma chilonis, Trichogramma pretiosum* and *Trichogramma brasiliense* (Hymenoptera: Trichogrammatidae) as parasitoids of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on sunflower (*Helianthus annus*) and redgram (*Cajanus cajan*). *Biocontrol Sci. Technol.* 13(2): 231-240

Beevers, M., Lewis, W.S., Gross, H.R. and Nordlund, D.A. 1981. Kairomones and their use for entomophagous insects: X. Laboratory studies on manipulation of host-finding behaviour of *Trichogramma pretiosum* Riley with kairomone extracted from *Heliothis zea* (Boddie) moth scales. *J. Chem.Ecol.* 7: 635-648

Beevi, S.P., Lyla, K.R. and Karthikeyan, K. 2002. Impact of the inundative release of egg parasitoids, *Trichogramma* spp. in rice pest management. *Proceedings of the Symposium of Biological Control of Lepidopteran Pests,* July 17-18, 2002. Bangalore, India. pp. 329-332

Bentur, J.S., Chelliah, S. and Rao, P. 1989. Approaches in rice pest management – Achievements and opportunities. *Oryza.* 26: 12-26

Bentur, J.S., Kalode, M.B., Rajendran, B. and Patel, V.S. 1994. Field evaluation of the egg-parasitoid, *Trichogramma chilonis* Ishii (Hym.:Trichogrammatidae) against the rice leaf folder, *Cnaphalocrocis medinalis* (Guen.) (Lep., Pyralide) in India. *J. appl. Ent.* 117(3): 257-261

Bhatnagar, V.S. 1981. Are effective parasites of *Heliothis* eggs found on pigeonpea and chickpea? *int. Pigeonpea Newsl.* 1:32

Boo, K.S. and Yang, J.P. 2000. Kairomones used by *Trichogramma chilonis* to find *Helicoverpa assulta* eggs. *J.chem.Ecol.* 26(2): 359-375

Brar, K.S., Singh, J. and Shenhmar, M. 2001. Field evaluation and demonstration of egg parasitoids for the control of leaf folder and stem borer of rice. *Proceedings of the symposium on Biocontrol based pest management for quality crop protection in the current millennium*, July 18-19, 2001. PAU, Ludhiana, India. pp. 33-34

Brown, W.L., Eisher, T. and Whittaker, R.H. 1970. Allomones and kairomones: Transpecific chemical messengers. *Biosci.* 20: 21-22

Chandrasekhar, K., Kulkarni, K.A. and Giraldi, R.S. 2002. Evaluation of parasitization efficiency of different species of *Trichogramma* on eggs of chilli fruit borer, *Helicoverpa armigera* (Hubner). *Proceedings of the symposium of Biological Control of Lepidopteran Pests,* July 17-18, 2002. Bangalore, India. pp.99

Chelliah, S., Bentur, J.S. and Rao, P. 1989. Approaches in rice pest management-achievements and opportunities. *Oryza* 26:121-126

*Chen, W.Y. and Hung, T.H. 1981. Effects of temperature on the development and the parasitism of *Trichogramma australicum* Girault. Rep. *Taiwan Sugar Res. Inst.* 93:29-37

Conti, E., Salerno, G., Bin, F., Williams, H.J. and Vinson, S.B. 2003. Chemical cues from *Murgantia histrionica* eliciting host location and recognition in the egg parasitoid *Trissolcus brochymenae*. *J. chem. Ecol.* 29(1): 115-130

Curl, G.D. and Burbutis, P.O. 1977. The mode of overwintering of *Trichogramma nubilale* Ertle and Davis. *Environ. Ent.* 6:629-632

Delegue, P.M.H., Trouiller, J., Bakchine, E., Roger, B. and Masson, C. 1991. Age dependency of worker bee response to queen pheromone in a four-armed olfactometer. *Insectes Soc.* 38: 283-292

Dodia, J.F., Patel, M.C., Patel, H.M., Korat, D.M. and Mehta, K.G. 1997. Determination of economic threshold level for rice leaf folder, *Cnaphalocrocis medinalis* Guen. in Gujarat. *Gujarat agric. Univ. Res. J.* 22(2): 51-56

Elzen, G.W., Williams, H.J. and Vinson, S.B. 1984. Isolation and identification of cotton synomones mediating searching behaviour by parasitoid *Campoletis sonorensis*. *J. chem. Ecol.* 19: 1251-1264

Farid, A., Tasbeehullah, A., Khan, A., Khattak, S.U. and Alamzeb, P.U. 2001. Effect of storage at low temperature on adult eclosion and longevity of adults of *Trichogramma chilonis*. *Pakist. J. Zool.* 33(3): 205-207

*Ferreira, L., Pintureau, B. and Voegele, J. 1979. A new type of olfactometer. Application to the measurement of the ability to search for and locate attractant substance in the host in the *Trichogramma*. *Annales de Zoologie Ecologie Animale.* 11: 271-279

*Garcia, C.T. and Piqueras, P.V. 1985. Olfactometer studies of the influence of the plant and of the host in the searching activity of *Trichogramma cordubensis* Vargas and Cabello and *Trichogramma* sp. near *buesi* (Hym:

Trichogrammatidae). *Boletin del Servicio de Defensa contra Plagas Inspeccion Fitopatologica.* 11: 237-241

Gautam, R.D. 1986. Effect of cold storage on adult parasitoid, *Telenomus remus* Nixon (Scelionidae, Hymenoptera) and the parasitized eggs of *Spodoptera litura* (Fabr.) (Noctuidae, Lepidoptera). *J. Ent. Res.* 10:125-131

Gross, H.R., Lewis, W.J., Jones, R.L. and Nordlund, D.A. 1975. Kairomones and their use for management of entamophagous insects: III. Stimulation of *Trichogramma achaeae, T. pretiosum* and *Microplitis croceips* with host seeking stimuli at time of release to improve their efficiency. *J. chem. Ecol.* 1: 431-438

Gueldner, R.C., Nordlund, D.A., Lewis, W.J., Thean, J.E. and Wilson, D.M. 1984. Kairomones and their use for management of entomophagous insects. XV. Identification of several acids in scales of *Heliothis zea* moths and comments on their possible rolë as kairomones for *Trichogramma pretiosum. J. chem. Ecol.* 10: 245-251

Gupta, P.R. and Bhardwaj, P.C. 2002. Storage conditions conducive for holding the egg parasitoid, *Trichogramma matoidea bactrae* Nagaraja. *J. Biol. Control* 16(1): 19-24

Guren, Z., Zimmermann, O. and Hassan, S.A. 2004. Pollen as a source of food for egg parasitoids of the genus *Trichogramma* (Hymenoptera:Trichogrammatidae). *J. chem.. Control* 14(2): 201-209

Hendry, L.B., Wichman, J.K., Hindenlang, D.M., Weaver, K.M. and Korzeniowski, S.H. 1976. Plants - the origin of kairomones utilized by parasitoids of phytophagous insects? *J. chem. Ecol.* 2: 271-283

House, H.L. 1977. Nutrition of natural enemies. *Biological control by augmentation of natural enemies* (eds. Ridgway, R.C. and Vinson, S.B.). Plenum Press, New York, pp.151-182

Islam, W. 2001. Effect of adult nutrition on longevity and fecundity of *Dinarmus basalis* (Rond.) (Hymenoptera:Pteromalidae). *J. Biol. Control.* 15(1): 5-10

Jalali, S.K. 1992. Differential response of four *Trichogramma* species to low temperatures for short-term storage. *Entomophaga.* 37(1): 159-165

Jalali, S.K. and Singh, S.P. 1992. Differential response of four *Trichogramma* species to low temperature for short-term storage. *Entomophaga.* 37: 159-165

Janssen, A., Hofker, C.D., Braun, A.R., Mesa, N., Sabelis, M.W. and Bellotti, A.C. 1990. Preselecting predatory mites for biological control: the use of an olfactometer. *Bull.Ent. Res.* 80: 177-181

Jones, R.L., Lewis, W.J., Beroza, M., Bierl, B.A. and Sparks, A.N. 1973. Host seeking stimulants (kairomones) for the egg parasite, *Trichogramma evanescens. Environ. Ent.* 2: 593-596

Kaiser, L., Delegue, P.M.H., Bakchine, M.E. and Masson, C. 1989. Olfactory responses of *Trichogramma maidis* Pint. Et. Voeg.: Effects of chemical cues and behavioural plasticity. *J. Insect. Behav.* 2: 701-712

Khan, M.A. and Tiwari, S. 2001. Effect of plant extracts on the parasitization efficiency of *Trichogramma chilonis* Ishii. *J.Biol. Control* 15(2): 133-137

Khosa, S.S. and Brar, K.S. 2000. Effect of storage on the emergence and parasitization efficiency of laboratory reared and field collected population of *Trichogramma chilonis* Ishii. *J. Biol. Control* 14: 71-74

Killineer, M., Gurkan, M.O. and Bulut, H. 1990. Studies on mass rearing and release techniques for *Trichogramma turkeiensis* Kostadinov and *T. embroyophagum* parasitoids of *Ephestia kuehniella* Zeller. *Proceedings of the Second Turkish National Congress on Biological Control.* pp.15-23

Knutson, A. 2000. A guide to the use of *Trichogramma* for biological control with special reference to augmentative releases for control of bollworm and budworm in cotton. *The Trichogramma Manual.* pp. 4

Kotikal, Y.K. and Sengonca, C. 1999. Olfactory responses of mealybug predator, *Cryptolaemus montrouzieri* Mulsant (Coleoptera:Coccinellidae) to the kairomones of prey arthropods, their host plants and predator itself. *J. Biol. Control* 13: 1-6

Kumar, S. and Khan, M.A. 2005. Bio-efficacy of *Trichogramma* spp. against yellow stem borer and leaf folder in rice ecosystem. *Ann. Plant Prot. Sci.* 13(1): 97-99

Kumar, P., Shenhmar, M. and Brar, K.S. 2005. Effect of low temperature storage on the efficiency of three species of trichogrammatids. *J. Biol. Control* 19(1): 17-22

Kumar, P., Singh, R., Pandey, S.K. and Kumar, P. 1996. Population dynamics of rice leaf folder, *Cnaphalocrocis medinalis* Guen, in relation to stage of the crop, weather factors and predatory spiders. *J. Ent. Res.* 20(3): 205-210

Laing, J. 1937. Host finding by insect parasites.I. Observations on finding of hosts by *Alysia manductor*, *Mormoniella vitripenosis* and *Trichogramma evanescens*. *J. Anim. Ecol.* 6:298-317

Lewis, W.J., Spark, A.N. and Redlinger, L.M. 1971. Moth Odor: A method of host finding by *Trichogramma evanescens*. *J. Econ. Ent.* 64: 557-558

Lewis, W.J., Jones, R.L. and Sparks, A.N, 1972. A host seeking stimulant for the egg parasite *Trichogramma evanescens*: Its source and a demonstration of its laboratory and field activity. *Ann. Ent. Soc. Am.* 65: 1087-1089

Lewis, W.J., Jones, R.L., Nordlund, D.A. and Gross, H.R. 1975. Kairomones and their use for management of entomophagous insects: II. Mechanisms causing increase in rate of parasitization by *Trichogramma* spp. *J. chem. Ecol.* 1: 349-360

Lewis, W.J., Jones, R.L., Nordlund, D.A. and Sparks, A.N. 1975a. Kairomones and their use for management of entomophagous insects I. Evaluation for increasing rates of parasitization by *Trichogramma* spp. in the field. *J. chem. Ecol.* 1: 343-347

Lewis, W.J., Nordlund, D.A., Guelder, R.C., Teal, P.E.A. and Tumlinson, J.H. 1982. Kairomones and their use for management of entomophagous insects. XIII. kairomonal activity for *Trichogramma* spp. of abdominal tips, excretion and a synthetic sex pheromone blend of *Heliothis zea* (Beddie) moths. *J. chem. Ecol.* 8: 1323-1331

Lopez, J.D. and Morrison, R.K. 1980. Suspectibility of immature *Trichogramma pretiosum* at freezing and sub- freezing temperatures. *Environ. Ent.* 9: 697-700

Manisegaran, S., Letchoumanane, S. and Hanifa, A.M. 1997. Natural parasitism of rice leaf folder *Cnaphalocrocis medinalis* (Guenee) in Karaikal region. *J.Biol.Control* 11: 73-75

Martin, W.R., Nordlund, D.A. and Nettles, W.C. 1990. Response of parasitoid *Eucelatoria bryani* to select plant material in an olfactometer. *J. chem. Ecol.* 16: 499-508

Mishra, B.K. and Mandal, S.M.A. 2003. Density dependant parasitization of rice leaf folder, *Cnaphalocrocis medinalis* (Guenee) in the coastal belt of Orissa. *J. appl. Zool. Res.* 14(1): 63-64

Misra, R.P., Arusummallaiah, L., Ravi, K. and Diwakar, M.C. 1994. Integrated pest management approach for rice- a case study in Karnataka. *Plant Prot. Bull.* 46:6-10

Mitchell, E.R. Tingle, F.C. and Heath, R.R. 1991. Flight activity of *Heliothis virescens* females (Lepidoptera:Noctuidae) with reference to host-plant volatiles. *J. chem. Ecol.* 17(2): 259-266

Nettles, W.C. 1980. Adult *Eucelatoria*: Response to volatiles from cotton and okra plants and from larvae of *Heliothis virescens, Spodoptera eridania* and *Estigmene acrea. Environ. Ent.* 9: 759-763

Noldus, L.P.J.J. and van Lenteren, J.C. 1985. Kairomones for the egg parasite *Trichogramma evanescens* Westwood. I. Effect of volatile substances released by two of its hosts, *Pieris brassicae* L. and *Mamestra brassicae* L. *J. chem. Ecol.* 11: 781-791

Noldus, L.P.J.J., Potting, R.P.J. and Barendregt, H.E. 199I. Moth sex pheromone adsorption to leaf surface: bridge in time for chemical spies. *Physiol. Ent.* 16(3): 329-344

Nordlund, D.A. 1987. Plant produced allelochemicals and their involvement in the host selection behaviour of parasitoids. *Insect Plants.*(eds: Labeyrie, V., Fabres, G. and Lachaise, D). Dr. W. Junk Publishers, London. pp. 302

Nordlund, D.A., Chalfant, R.B. and Lewis, W.J. 1984. Response of *Trichogramma pretiosum* females to extracts of two plants attacked by *Heliothis zea. Agric Ecosys. Environ.* 12: 127-133

Nordlund, D.A., Chalfant, R.B. and Lewis, W.J. 1985. Response of *Trichogramma pretiosum* female to volatile synomones from tomato plants. *J. Ent. Sci.* 20: 372-376

Nordlund, D.A., Lewis, W.J., Gross, H.R. and Beevers, M. 1981. Kairomones and their use for management of entomophagous insects. XII. The stimulatory effects of host eggs and the importance of host egg density to the effective use of kairomones for *Trichogramma pretiosum* Riley. *J. chem Ecol.* 7: 909-917

Nordlund, D.A., Lewis, W.J., Jones, R.L. and Gross, H.R. 1976. Kairomones and their use for management of entomophagous insects. IV. Effect of kairomones on productivity and longevity of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae). *J. chem. Ecol.* 2: 67-72

Pandya, H.V., Shah, A.H. and Purohit, M.S. 1987. Yield loss caused by leaf folder damage alone and combined with yellow stem borer damage. *Int. Rice Res. Newsl.* 12(5): 28

Paul, A.V.N. and Sreekumar, K.M. 1998. Improved technology for mass rearing of Trichogrammatids and their factitious host *Corcyra cephalonica*. *Technol. Biol. Control* 11:99-111

Pitcher, S.A., Hoffmann, M.P., Gardner, J., Wright, M.G. and Kuhar, T.P. 2002. Cold storage of *Trichogramma ostriniae* reared on *Sitotroga cerealella* eggs. *Biocontrol.* 47(5): 525-535

Ram, S., Patnaik, N.C., Sahoo, S., Mohapatra, A.K.B., Samal, K.C. and Mehta, S. 1997. Effects of food and temperature on the longevity of *Trichogramma japonicum* Ashmead, egg parasite of the yellow rice borer *Scirpophaga incertulas* Walker. *Environ. Ecol.* 15(3): 714-716

Ramasubaiah, K., Rao, N.V. and Rao, A.G. 1980. Nature of damage and control of rice leaf folder, *Cnaphalocrocis medinalis* Guen. *int. J. Ent.* 42: 214-227

Reddy, G.V.P., Holopainen, J.K. and Guerrero, A. 2002. Olfactory responses of *Plutella xylostella* natural enemies to host pheromone, larval frass, and green leaf cabbage volatiles. *J. chem. Ecol.* 28(1): 131-143

Renou, M., Nagnan, P., Berthier, A. and Durier, C. 1992. Identification of compounds from the eggs of *Ostrinia nubilalis* and *Mamestra brassicae* having kairomone activity on *Trichogramma brassicae. Ent. Exp. appl.* 63(3): 291-303

Renou, M., Hawlitzky, N., Bertheir, A., Malosse, C. and Ramiandrosa, F. 1989. Evidence of a kairomonal activity from eggs of the European corn borer on females of *Trichogramma maidis. Entomophaga.* 34: 569-580

Romeis, J., Shanower, T.G. and Zebitz, C.P.W. 1998. Physical and chemical plant characters inhibiting the searching behaviour of *Trichogramma chilonis. Ent. Exp. appl.* 87(3): 275-284

Ruberson, J.R. and Kring, T.J. 1993. Parasitism of developing eggs by *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae): Host age preference and suitability. *Biol. Control* 3: 39-46

Rundle, B.J., Thomson, L.J. and Hoffmann, A.A. 2004. Effects of cold storage on field and laboratory performance of *Trichogramma carverae* (Hymenoptera: Trichogrammatidae) and the response of three *Trichogramma* spp. (*T. carverae, T. brassicae* and *T. funiculatum*) to cold. *J. Economic Ent.* 97(2): 213-221

Saikia, P. and Parameswaran, S. 2002. Eco-friendly strategies for the management of rice leaf folder, *Cnaphalocrocis medinalis* Guenee. *Ann. Plant Prot. Sci.* 10(1): 12-16

Sheeba, S., Mohanraj, P., Singh, P.K. and Prasad, G.S. 2002. Effect of host and food on biological parameters of *Trichogramma japonicum* Ashmead (Hymenoptera: Trichogrammatidae) native to the Andaman Islands. *J. Ent. Res.* 26(2): 147-151

Shenhmar, M., Singh, J., Singh, S.P., Brar, K.S. and Singh, D. 2003. Effectiveness of *Trichogramma chilonis* Ishii for the management of *Chilo auricilius* Dudgeon on sugarcane in different sugar mill areas of the Punjab. *Proceedings of the Symposium of Biological Control of Lepidopteran Pests*, 17-18 July 2002. Bangalore, India. pp. 333-335

Shivaleela, P.M. and Patil, B.V. 2003. Effect of refrigeration on the emergence of egg parasitoid, *Trichogramma achaeae* Nagaraja and Nagarkatti and on its lab host, *Corcyra cephalonica* (Stainton). *Proceedings of the Symposium of Biological Control of Lepidopteran Pests*, July 17-18, 2002. Bangalore, India. pp. 71-74

Shrike, M.S. and Bade, B.A. 1997. Efficacy of *Trichogramma japonicum* against paddy stem borer. *J.Maharashtra agric. Univ.* 22:338-339

*Shu, S. and Jones, R.L. 1988. Laboratory studies of the host seeking behaviour of a parasitoid, *Trichogramma nubilale* and a kairomone from its host *Ostrinia nubilalis. Colloques de 'l' INRA*. No.43: 249-265

Shu, S., Swedenborg, P.D. and Jones, R.L. 1990. A kairomone for *Trichogramma nubilale* (Hymenoptera: Trichogrammatidae): Isolation, identification and synthesis. *J. chem. Ecol.* 16: 521-529

*Shuxiong, B., Zhemjing, W., Kanglai, H., Liping, W. and Darong, Z. 2004. Olfactory responses of *Trichogramma ostriniae* to kairomones from eggs and different stages of female adults of *Ostrinia furnacalis. Acta-Entomologica-Sinica.* 47(1): 48-54

Singh, J., Brar, K.S., Bakhetia, D.R.C. and Shenhmar, M. 1997. Effect of storage on the emergence, sex ratio and parasitisation efficiency of *Trichogramma chilonis* Ishii. *Proceedings of Third Agricultural Science Congress*, March 12-15, 1997, PAU, Ludhiana. pp. 249

Singh, S.P. and Jalali, S.K. 1994. Trichogrammatids. Project Directorate of Biological Control (ICAR), Bangalore. *tech. Bull.* 7: 93

Singh, S.P., Bhumannavar, B.S., Jalali, S.K.,Bhakthavatsalam, N. and Pushpalatha, A. 1994. Chrysopids and Trichogrammatids-strain selection and utilization Project Directorate of Biological Control (ICAR), Bangalore. *tech. Bull.*. 9: 45

*Smits, P.H. 1982. The influence of kairomones of *Mamestra brassicae* L. on the searching behaviour of *Trichogramma evanescens* Westwood. In *Les Trichogrammes.* Ier Symposium International, Antibes, 20-23 April, 1982. Paris, France; Institut Nationale de la Recharge Agronomique. pp.135-150

Sohi, A.S., Brar, K.S., Singh, J., Shenhmar, M. and Brar, D.S. 1996. Searching range of *Trichogramma chilonis* Ishii on cotton. *Insect Environ.* 1(4): 27

Srivastava, S.K. 1988. Leaf folder damage and yield loss on some selected rice varieties. *Int. Rice Res.Newsl.* 14(6): 10

Sujatha, A., Zaheruddeen, S.M., Rao, A.A. and Nagalingam, B. 2002. Electroantennogram and wind tunnel studies on olfactory responses of mango fruit borer, *Deanolis albizonalis* (Hampson) to female sex pheromone. *Pest Mgmt. Hort. Ecosyst.* 8(2): 83-89

Tandon, P.L. and Bakhtavatsalam, N. 2005. Electro-physiological and olfactometric responses of *Helicoverpa armigera* (Hubner) (Lepidoptera:Noctuidae) and *Trichogramma chilonis* Ishii (Hymenoptera:Trichogrammatidae) to volatiles of trap crops – *Tagetes erecta* and *Solanum viarum*. *J. chem. Ecol.* 19(1): 9-16

Thomson, M.S. and Stinner, R.E. 1990. The scale response of Trichogramma (Hym: Trichogrammatidae): Variation among species in host specificity and the effect of conditioning. *Entomophaga.* 35: 7-21

Timerak, S.A. 1983. Longevity of *Bracon brevicornis* (Hymneoptera:Braconidae) adults as influenced by nourishment on artificial and natural foods. *Entomophaga.* 28: 145-150

Tingle, F.C., Heath, R.R. and Mitchell, E.R. 1989. Flight response of *Heliothis subflexa* females to an attractant from groundcherry, *Physalis angulata. J. chem. Ecol.* 15: 221-231

Tumlinson, J.H., Turlings, T.C.J. and Lewis, W.J. 1992. The semiochemical complexes that mediate insect parasitoid foraging. *Agric Zool. Rev.* 5: 221-252

Venkataraman, T.V., and Govil, M.L. 1952. Some effect of low temperature on the biology of *Trichogramma evanescens minutum* Riley, in mass breeding. *Indian J. Ent.* 14: 215-227

Vet, L.E.M., Lenteren, V.J.C., Heymans, M. and Meelis, E. 1983. An airflow olfactometer for measuring olfactory responses of hymenopterous parasitoids and other small insects. *Physiol. Ent.* 8: 97-106

Waage, J.K. 1978. Arrestment responses of the parasitoid *Nemeritis canescens* to a contact chemical produced by its host *Plodia interpunctella. Physiol. Ent.* 3: 35-46

*Wang, J.J. and Zong, L.B. 1991. A study on host-seeking kairomone for *Trichogramma confusum* Viggiani. *Colloques-de-l'INRA.* 56: 93-96

Wang, J.L., Yang, Z.C. and Zhang, J. 1990. Study on the biological characters of *T.ostrinae* (Hymenoptera:Trichogrammatidae). *Natural Enemies of Insects.* 12(2): 56-61

Whittaker, R.H. and Feeny, P.O. 1971. Allelochemicals: chemical interaction between species. *Sci.* 17: 757-770

Yu, J.Z. and Chen, B.H. 2003. Emergence, fecundity and longevity of *Trichogramma ostriniae* (Hymenoptera: Trichogrammatida) after cold storage. *Plant Prot. Bull. Taipei.* 45(1): 35-44

Zaborski, E., Teal, P.E.A. and Laing, J.E. 1987. Kairomone mediated host finding by spruce budworm egg parasite, *Trichogramma minutum*. *J. chem. Ecol.* 13: 113-121

* Originals not seen

ENHANCING THE PERFORMANCE OF THE EGG PARASITOID, *T. chilonis* ISHII (Trichogrammatidae : Hymenoptera)

By

SHAJNA P. K.

ABSTRACT OF THE THESIS

submitted in partial fulfilment of the requirement
for the degree of

Master of Science in Agriculture

(AGRICULTURAL ENTOMOLOGY)

Faculty of Agriculture
Kerala Agricultural University, Thrissur

Department of Agricultural Entomology

COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA

2006

ABSTRACT

A study entitled 'Enhancing the effectiveness of the egg parasitoid *Trichogrmma chilonis* Ishii (Trichogrammatidae: Hymenoptera)' was carried out at the College of Horticulture, Vellanikkara with the objectives of increasing the effectiveness of *T. chilonis* and to standardize the optimum storage period of the trichocards.

A prototype of an olfactometer suitable to monitor the movements of *T. chilonis* was at first made using polyester film for standardizing the design. The prototype was validated by keeping the kairomones in each of the four arms. As this was found to be a success, it was further fabricated in transparent acrylic sheet incorporating slight modifications. Various error factors were identified during the continued studies and they were eliminated and an improved design was standardized as uniform air inflow olfactometer.

The responses of *T. chilonis* to different semiochemicals were studied in the uniform air inflow olfactometer. A higher response of adult parasitoids was noticed towards moth extracts of *C. medinalis* and *Corcyra cephalonica* and also moth scale extract of *C. cephalonica* in hexane solvent. This may be because hexane could extract more concentration or wider range of the kairomones which attract the parasitoids. The results corresponding to the response to plant volatiles emanating from the weeds in the paddy ecosystem are a clear pointer to the ecological adaptation of *T. chilonis* for survival. The highest attraction was towards the leaf folder damaged leaves followed by plant parts of *Oryza rufipogon*. Among the different semiochemicals, significant attraction was towards 10 per cent honey solution. The results thus established that a band or grid application of honey solution will increase the longevity of adults as well as the per cent parasitism. The adult moth which is the primary producer of eggs is the most preferred from among the different life stages of *C. medinalis* tried.

A wind tunnel set up was fabricated to evaluate the olfactory responses of *T. chilonis* to the best semiochemicals. The distance travelled by the parasitoids was observed for every 30 minutes, for a total duration of three hours. Maximum distance travelled by the adult parasitoids was towards honey solution. The results validate the findings observed using the four arm olfactometer (free choice test) with a single source wind tunnel (no choice test).

The effect of refrigerated storage on the emergence and parasitization efficiency of *T. chilonis* was studied. More than 50 per cent emergence was noticed up to 36 days in four day old cards which is of interest to nucleus stock of *Trichogramma* being maintained at the laboratories. The results of the study have shown that the *Trichogramma* emergence can be delayed even up to 50 days under ordinary refrigerated storage. Such cards even with around 10 per cent efficiency would be more economical than continuous rearing of *Corcyra* culture and production of trichogrammatids. Even for farmer situations, it may be more advisable to manage initial leaf folder populations at its first level of emergence itself. At this time the population is very low and even a 10 per cent parasitoid emergence can easily take care of first generation of leaf folder.