IMPACT OF NOVEL INSECTICIDES ON *Trichogramma chilonis* Ishii (Hymenoptera : Trichogrammatidae)

By

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(2011-11-113)

THESIS

Submitted in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University, Thrissur

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

COLLEGE OF HORTICULTURE

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KERALA, INDIA

2013

DECLARATION

I, **Uma S. (2011-12-104)**, hereby declare that this thesis entitled **"Impact of novel insecticides on *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae)"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title of any other University or Society.

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ACKNOWLEDGEMENT

First and foremost I bow my head the Almighty God whose grace has endowed me the strength and confidence to complete the thesis work successfully on time.

With immense pleasure I avail this opportunity to express my deep sense of gratitude and indebtness to Dr. Sosamma Jacob, Professor and Head, Department of Agrl. Entomology, College of Horticulture, Vellanikkara, and Chairman of my advisory committee for her sustained and valuable guidance, constructive suggestions, unfailing patience, friendly approach, constant support and encouragement during the conduct of this research work and preparation of the thesis. I gratefully remember her knowledge and wisdom, which nurtured this research project in the right direction without which fulfillment of this endeavour would not have been possible.

Words fail to express my indebtedness to Dr. A. M. Ranjith, Professor and Head, Communication Centre, Mannuthy and member of my advisory committee for the meticulous help, unwavering encouragement and close scrutiny throughout my study.

I also owe my sincere thanks to Dr. K. R. Lyla, Professor, Department of Agrl. Entomology, College of Horticulture, Vellanikkara and member of my advisory committee for her timely help, untiring support and valuable guidance during the course of my study.

I take this opportunity to express my heartfelt gratitude to Mr. S. Krishnan, Associate Professor, Department of Agricultural Statistics and member of my advisory committee for his suggestions and valuable guidance during the stastistical analysis of data.

I am thankful to Dr. Maicykutty P Mathew, Professor and Dr. Susanamma Kurien , Professor for their kind cooperation and ever willing help.

Words fall short as I place on record my indebtedness to Dr. Madhu Subramanian, Assosiate Professor, Dr. Mani Chellappan, Assosiate Professor and Dr. Haseena Bhaskar for their untiring support and immense help rendered during my course of study.

I extend my heartiest gratitude to Mrs. Sreeja. P and Vidya. C. V for their constructive criticism, generous support, sustained interest and creative suggestions throughout the period of my study.

My profound sense of gratitude to Omana chechi, Nabeeza chechi and Bindu chechi for their help and service during my work at BCCP

I express my sincere thanks to my beloved seniors Renjith chetan, Binisha chechi, Jimcy chechi, Swati chechi , Neena chechi, Najitha chechi, Jyothi chechi and Pramod chetan for their everwilling help and valuable guidance.

The encouragement rendered by my friends at various stages of this investigation was invaluable and I thank Maneesha, Roshna, Airi, Lakshmi, Achu and Subha from the bottom of my heart.

My heart never forgets my loving juniors Manju, Surya, Sandhya, Jijisha, Deepak and Maadhu for their valuable help.

The award of KAU junior fellowship is greatly acknowledged.

Above all, I am forever beholden to my achan, amma and Shambhu for their constant prayers, affection, moral support, personal sacrifice and sincere encouragement throughout the period of my studies. Words cannot really express the sincere support and encouragement that I relished from my better half Sreeraj.

Uma S.

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INTRODUCTION

INTRODUCTION

Trichogrammatid egg parasitoids are distributed worldwide and they play an important role in the biological control of lepidopteran pests of many crops. *Trichogramma* spp. parasitizes the eggs of over 400 species belonging to at least seven insect orders (Bao and Chen, 1989). In India, many species of *Trichogramma* have been successfully used for augmentative biological control programmes in Integrated Pest Management. *Trichogramma chilonis* Ishii. (Hymenoptera: Trichogrammatidae) is widely distributed almost throughout the country and its inundative field releases have achieved an appreciable success in suppressing the population density of rice leaf folders.

Despite the importance of biological control, the use of organic synthetic insecticides continues to be an important tool in the Integrated Pest Management system. The effectiveness of biological control can be improved by using insecticides in a compatible manner without causing any disruption to biocontrol agents. Knowledge of compatibility and impact of insecticides on the beneficial parasitoid species is highly essential for the effective integration of chemical and biological control programmes in rice IPM system. Moreover, the parasitoids are also considered as suitable bio-indicators for testing the safety of insecticides in ecosystems. Information on the effect of different insecticides on egg parasitoids will help to determine the type of spray and timing of spraying, thus avoiding contacts with their susceptible stages.

Lethal and sub lethal effects of insecticides are usually considered as a high risk to beneficial species (Croft, 1990 and Ruberson *et al.* 1998). Lethal effects of insecticides on beneficial arthropods are often expressed as acute or chronic mortality resulting from contact or ingestion with insecticides (Haseeb *et al.*, 2004). The sub lethal effects expressed as change in life history parameters such as parasitism rate, longevity, egg viability etc; are also to be considered for a complete analysis of the impact caused by insecticides (Desneux *et al.*, 2007).

The current trend in pesticide utilization pattern is a shift towards low dose, soft chemistry pesticides with reduced risk to human beings and other non target organisms in the environment. Most of the conventional insecticides are being replaced by the low dose and reduced risk insecticides with low mammalian toxicity. The conventional insecticides are mainly nerve poisons focussing on limited target sites such as Acetyl choline esterase (organophosphate, carbamates) and voltage gated sodium channels (DDT and pyrethroids) . The limited number of target sites leads to the development of insecticide resistance in insects.

Recently, novel insecticides, such as buprofezin, chlorantraniliprole, flubendiamide, spinosad and thiamethoxam belonging to newer chemical classes of thiadiazine, anthranilic diamide, phthalic acid diamide, macrocyclic lactone and neonicotinod, acting on unique target site of insect body without any cross resistance to conventional insecticides and possessing higher affinity to insect nerve receptors than to mammalian nerve receptors are being recommended as potential alternatives for the highly toxic organophosphate and carbamates in rice integrated pest management (IPM). All insecticides in general are designed to kill insects and hence are likely to be harmful to the natural enemies also. Therefore, systematic efforts are required to investigate the inherent toxicity and side-effects of the new generation insecticides to the egg parasitoid, *T. chilonis*. An important understanding of the parasitoid-insecticide interaction will assist in formulating more effective IPM strategies. It is very important to study the side effects of the novel insecticides on the egg parasitoid in order to exclude the ones that have detrimental effects on this hymenopteran wasp. Only limited information is available on the safety of the new insecticide molecules to *T. chilonis*. In this context, the present study is proposed to investigate the potential toxicity and safety of five important new generation insecticides *viz*., buprofezin 25 SC, chlorantraniliprole 18.5 SC, flubendiamide 480 SC, spinosad 2.5 SC and thiamethoxam 25 WG that are being recommended and commonly used against the key pests in the rice ecosystem in India. The study would serve as a pre-requisite to avoid those insecticides causing high ecological damage and it would

new insecticide molecules to *T. chilonis*. The study would serve as a pre-requisite to avoid those insecticides causing high ecological damage and it would also generate knowledge on the impact and selective use of novel insecticides against *T.chilonis* in rice ecosystem. In this background the present study entitled "Impact of novel insecticides on *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae)" has been undertaken with the following objectives

- Lethal effects and safety of five selected novel insecticides on the egg parasitoid, *T. chilonis*
- Potential compatibility of the selected new generation insecticides to the egg parasitoid

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

A comprehensive review of literature pertaining to the investigation entitled 'Impact of novel insecticides on *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae)' is presented in this chapter. The important works on the toxicity, safety and persistence of insecticides in general and newer molecules in particular are briefly reviewed hereunder.

2.1. TOXICITY OF CONVENTIONAL INSECTICIDES TO NON-TARGET ARTHROPODS

2.1.1.1. Compatibility of organochlorines to beneficial insects

The toxicity of insecticides to green lace wing *Mallada boninensis* (Okamoto) was studied by More *et al.* (2011) and endosulfan was rated as safe to the adults emergence from treated pupae and had no ovicidal action.

Neetan and Naveen (2013) studied the toxicity of insecticides against immature life stages of *Chrysoperla zastrowi* Sillemi under laboratory conditions and reported that endosulfan showed 70% hatching from treated eggs. Endosulfan treated pupae showed substantial higher per cent of adult emergence and survival.

2.1.1.2. Compatibility of organochlorines to *Trichogramma* spp.

Paul *et al.* (1976) reported endosulfan (0.1 %) and gamma -BHC (0.1 %) as the least toxic insecticides to *T. chilonis* Ishii and *Trichogramma japonicum.* Ashmead The safety of endosulfan was further studied and observed at lower rates of parasitism and adult emergence after spraying over *Corcyra* eggs with insecticides.

Kim-Chi (1978) evaluated toxicity of endosulfan in field and laboratory conditions on all the stages of the insect parasite *Trichogramma evanescens* Westw and concluded that preparations tested for endosulfan produced no effects

on the larval stage of the insect, but was slightly toxic for the pupal stage and the adult specimens were highly sensitive to them.

The effect of four insecticides was studied on the emergence of the parasite *T. chilonis* from eggs of the cotton pest *Earias vitella* (Fabricius) under laboratory conditions by Santharam and Kumaraswami (1985). It was reported that application of sprays of 0.035% and 0.07% endosulfan had little effect on parasite emergence to parasitized eggs. Treating host eggs before parasitization resulted in low (<25%) parasite emergence.

Laboratory studies were carried out to determine the effect of six insecticides on the emergence of *Trichogramma pretiosum* from parasitized eggs of *Ephestia kuehniella* Zeller of four different age groups by Hohmann (1991). Results revealed that the highest emergence rate was observed in endosulfan at 525 g a.i ha $^{-1}$.

Rajendran and Gahukar (2000) rated endosulfan (0.05 %) as the safest insecticide to *T. chilonis* with respect to reduction in emergence and parasitism among the four insecticides (0.05% endosulfan, 0.05% quinalphos, 0.04% monocrotophos and 0.04% fenvalerate) tested.

Singh and Gupta (2001) evaluated the toxicity of endosulfan to *T.chilonis* and reported that endosulfan was moderately toxic with reduction in parasitization with increase in concentration.

Singh and Shenhmar (2008) evaluated the impact of endosulfan on biological parameters of the egg parasitoid *T. japonicum*. Endosulfan (at 394 g a.i. ha^{-1}) was found to be comparatively safe to the parasitoid with the least effect on the emergence and parasitization of parents as well as their progeny.

Kaur *et al.* (2009a) conducted a study to select pesticides that are less inimical to *T. brasiliensis* Ashmead and concluded that endosulfan was slightly toxic for adult and pupa.

The LC_{50} values of endosulfan was 1.8501 mg a.i. l^{-1}, exhibiting low toxicity when compared with other insecticides tested. Thiamethoxam was found to be 1,322 times more toxic than endosulfan, as revealed by the LC_{50} values to *T. chilonis* (Preetha *et al.*, 2009).

The toxicity of seven insecticides was tested in net house and reported that endosulfan was the most toxic insecticide with 54.36 % mortality to *T. brasiliensis* and persistence for nine days after treatment (Kaur *et al.*, 2010).

2.1.2.1. Compatibility of organophosphates to beneficial insects

Soil-applied acephate and foliar-applied pyriproxyfen were tested against *Unaspis euonymi* (Comstock) and their potential impacts on the aphelinid parasitoid, *Encarsia citrina* (Crawford) by Rebek and Sadof (2003). Results revealed that these insecticides exhibited superior control of euonymus scale, but reduced numbers of surviving *E. citrina*.

Prabhakar *et al.* (2007) conducted laboratory studies to compare the toxicity of seven foliar insecticides to four species of adult beneficial insects representing two families of Hymenoptera: Aphelinidae (*Aphytis melinus* Debach, *Eretmocerus eremicus* Rose and Zolnerowich, and *Encarsia formosa* Gahan) and Mymaridae (*Gonatocerus ashmeadi* Girault) that attack California red scale. Insecticides *viz.*, acetamiprid (neonicotinoid), chlorpyriphos (organophosphate), bifenthrin, cyfluthrin, and fenpropathrin (pyrethroids), and buprofezin and pyriproxyfen (insect growth regulators (IGRs) were evaluated. Chlorpyriphos was consistently the most toxic pesticide to all four species of beneficial insects tested based on LC_{50} values recorded 24 hours post treatment compared with 48 hours LC_{50} values with the neonicotinoid and pyrethroids or 96 hours with the IGRs.

More *et al.* (2011) conducted studies to evaluate the toxicity of dimethoate to green lace wing (*Mallada boninensis*) and concluded that dimethoate treatment significantly reduced the adult emergence. There was no difference in fecundity as there was no significant difference in the eggs laid by female emerged from

insecticidal treatment. Dimethoate treatment had ovicidal action as maximum number of eggs did not hatch due to dimethoate treatment.

Neetan and Naveen (2013) evaluated safety of insecticides to immature stages of *Chrysoperla zastrowi* Sillemi. Acephate exhibited extremely high ovicidal action with no egg hatching. Chlorpyriphos and acephate were most toxic to second instar larvae and adults of the predator.

2.1.2.2. Compatibility of organophosphates to *Trichogramma* spp.

Paul *et al.* (1976) evaluated the effect of sprays of 0.1% endrin, 0.05% parathion and 0.1% malathion, on the survival and emergence of *Trichogramma australicum* Gir. and *T. japonicum* in the laboratory and concluded that only parathion had adverse effect on the emergence of adults.

Field and laboratory investigations were carried out to evaluate the toxic effects of insecticides *viz.*, methyl-parathion, dimethoate, mevinphos, endosulfan and entobacterin on the preimaginal stages of *T. evanescens* by Kim-Chi (1978) and concluded that methyl-parathion, dimethoate and mevinphos were safe to the larval stage of *T. chilonis*, slightly toxic for the pupal stage and highly sensitive to adults.

Laboratory studies were carried out to assess the effects of insecticidal sprays of 0.1% malathion, 0.05% parathion-methyl (methylparathion), 0.1% carbaryl, 0.07% phosalone and 0.04% monocrotophos on parasitism of eggs to *Corcyra cephalonica* (Stainton) by *T. brasiliensis* and subsequent emergence of the parasite by Paul *et al.* (1979). Malathion and parathion-methyl were reported to have no parasitism on eggs whereas eggs treated with phosalone or monocrotophos indicated high rate of parasitism and emergence.

Santharam and Kumaraswami (1985) evaluated the effect of four insecticides each at two different doses on the emergence of the parasite *T. chilonis* from eggs of the cotton pest *Earias vittella* in laboratory conditions. Results revealed that application of sprays of 0.025% monocrotophos or 0.05%

phosalone had little effect on parasite emergence (which was at least 46%). Treating host eggs before parasitization resulted in low (<25%) parasite emergence.

The effect of eight insecticides *viz;* parathion, malathion, carbaryl, BHC, endrin, DDT, endosulfan and diazinon were evaluated by Dutt and Somchoudhary (1986) against 3rd-instar larvae, pupae and adults of *Trichogramma perkinsi* Girault and *Trichogramma australicum* Girault, each at two concentrations of 0.05 and 0.1% (larvae and pupae) and 0.01 and 0.02% (adults)) and reported that the order of susceptibility of the different stages of the two parasitoids were adult > pupa > larva. The immature and adult forms of *T. australicum* were more susceptible to insecticides than those of *T. perkinsi,* and parathion and malathion caused higher mortality of *T. perkinsi* adults.

The effect of insecticides on the emergence of *T. pretiosum* from parasitized eggs of *Ephestia kuehniella* of four different age groups was evaluated by Hohmann (1991). Lowest emergence rates were observed in methomyl at 215 g a.i. ha^{-1} followed by chlorpyriphos (400 g a.i. ha^{-1}), deltamethrin (75g a.i. ha^{-1}), parathion-methyl (300 g a.i. ha^{-1}) and monocrotophos (420 g a.i. ha^{-1}).

Studies were conducted on the contact toxicity of insecticides on six ecotypes of *T. chilonis* (from Gudimangalam, Palladan, Udumalpet, Pongalur, Chingleput and Mettupalayam) and a laboratory strain of *T. chilonis* from Coimbatore in Tamil Nadu by Kumar *et al.* (1994). It was found that phosalone was most toxic to the ecotypes while quinalphos and monocrotophos were safer.

Borah and Basit (1996) studied the effects of insecticides *viz.*, chlorpyriphos, quinalphos, monocrotophos, cypermethrin, dimethoate, phosphamidon, fenvalerate, Biolep and Bioasp (both *Bacillus thuringiensis* subsp. *kurstaki*) and Neemazal-F on the emergence of *T. japonicum* from eggs of *C. cephalonica* treated on the 3rd or 6th days after parasitisation. It was concluded

that quinalphos had the highest toxicity whereas monocrotophos was the safest insecticide.

The toxic effects on parasitisation of *C. cephalonica* eggs by *T. chilonis* due to insecticides belonging to organophosphorus groups were reported by Sarode and Sonalkar (1999).

The deleterious effects of 0.05 % quinalphos and 0.04 % monocrotophos on *T. chilonis* were evaluated by Rajendran and Gahukar (2000) and reported that quinalphos and monocrotophos can cause significant reduction in the emergence and parasitism with respect to control.

The toxicity of acephate to *T.chilonis* was evaluated by Singh and Gupta (2001). The results showed that acephate was least toxic to natural enemies but showed reduction in percent parasitisation with increased in concentration. Among all insecticides, maximum parasitization (84.66 %) was observed in 50ppm dose of acephate and maximum adult emergence was found in all treatment of acephate. Among tested insecticides, acephate was the least toxic to *T. chilonis* in respect of LC_{50} value, percent parasitization and adult emergence.

Singh and Shenhmar (2008) reported that malathion (at 1250 g a.i. ha^{-1}) and chlorpyriphos (at 175 g a.i.ha^{-1}) were highly deleterious to *T. japonicum*.

A study was conducted to identify pesticides that are less inimical to *T. brasiliensis* by Kaur *et al.* (2009b) and concluded that monocrotophos was slightly toxic for adult and pupa.

The LC_{50} value of acephate was 4.4703 mg a.i. l^{-1} to *T. chilonis*, exhibiting low toxicity when compared with other insecticides tested (Preetha *et al.*, 2009).

The compatibility and toxicity of *T. brasiliensis* with insecticides were tested in net house by Kaur *et al.* (2010). Monocrotophos was reported to cause 43.30 % mortality and insecticide persistence for nine days after treatment.

Abdel-Rahman and El-Aziz. (2012) evaluated the effect of chlorpyriphos (organophosphate) on the pre-imaginal stages of the egg parasitoid, *Trichogramma cacoeciae* under laboratory conditions. Chlorpyriphos was relatively more compatible with least disruptive effect on *T. cacoeciae* emergence rate and was better suited for conserving natural or released populations of the *Trichogramma* wasps.

A study to assess the toxicity of insecticides on a biocontrol agent, *T. japonicum* Ahmead was undertaken by Zhou *et al.* (2012) using a dry film residue method. Results showed that among the seven classes of chemicals tested, organophosphates (chlorpyriphos, fenitrothion, phoxim, profenofos, and triazophos) exhibited the highest intrinsic toxicity to *T. japonicum*, with LC_{50} of 0.035 (0.029–0.044) to 0.49 (0.34–0.87) mg active ingredient (a.i.) l^{-1}. Risk quotient analysis showed that organophosphates were classified as slightly, moderately, or highly toxic agents to the parasitoid.

2.1.3.1. Compatibility of carbamates to beneficial insects

The sublethal effects of carbamates which can be as deleterious as mortality to the parasitoid *Microplitis croceipes* Cresson (Hymenoptera : Braconidae) were evaluated by Stapel *et al.* (2000). Results indicated the reduced host foraging ability and longevityof the parasitoid after feeding on extrafloral nectar from cotton (*Gossypium hirsutum* L.).The longevity of *M. croceipes* females that fed on nectar from cotton was affected for atleast 10 days after plants were treated with insecticides.

2.1.3.2. Compatibility of carbamates to *Trichogramma* spp.

Effect of carbosulfan was tested on pre-imaginal stages of the egg parasitoid, *Trichogramma cacoeciae* under laboratory (Abdel-Rahman and El-Aziz, 2012) and concluded that carbosulfan was the most toxic compound to *T. cacoeciae*.

Zhou *et al.* (2012) studied the toxicity of insecticides on *T. japonicum* Ahmead using a dry film residue method. Results showed that among the seven classes of chemicals tested, highest intrinsic toxicity to *T. japonicum* was exhibited by carbamates (carbaryl, carbsulfan, isoprocarb, metolcarb, and promecarb) with LC_{50} of 0.035 (0.029–0.044) to 0.49 (0.34–0.87) mg (a.i.) l^{-1}. Risk quotient analysis showed that carbamates are classified as slightly, moderately, or highly toxic agents to the parasitoid.

2.1.4.1. Compatibility of synthetic pyrethroids to beneficial insects

Laboratory studies were carried out by Prabhakar *et al.* (2007) to compare the toxicity of acetamiprid (neonicotinoid), chlorpyriphos (organophosphate), bifenthrin, cyfluthrin, and fenpropathrin (pyrethroids), and buprofezin and pyriproxyfen (insect growth regulators (IGRs) to four species of adult beneficial insects representing two families of Hymenoptera: Aphelinidae (*Aphytis melinus* Debach, *Eretmocerus eremicus* Rose and Zolnerowich, and *Encarsia formosa* Gahan) and Mymaridae (*Gonatocerus ashmeadi* Girault) that attack California red scale. Among the three pyrethroids, fenpropathrin was usually less toxic (except similar toxicity to *A. melinus)* than was cyfluthrin, and it was normally less toxic (except similar toxicity with *E. formosa*) than was bifenthrin.

More *et al.* (2011) investigated the toxic impact of cypermethrin on green lace wing (*Mallada boninensis*) and reported that cypermethrin treatment has significantly reduced the adult emergence with no difference in fecundity. Cypermethrin was toxic as maximum number of eggs did not hatch.

2.1.4.2. Compatibility of synthetic pyrethroids to *Trichogramma* spp.

Borah and Basit (1996) studied the effects cypermethrin along with other conventional insecticides on the emergence of *T. japonicum* from the eggs of *C. cephalonica* treated on the 3rd or 6th days after parasitisation and it was reported that among the conventional insecticides, fenvalerate had the least effect.

Sarode and Sonalkar (1999) reported the toxic effects of synthetic pyrethroids on parasitization of *C. cephalonica* eggs by *T. chilonis*.

Fenvalerate was reported to cause reduction in the emergence and parasitism of *T. chilonis* (Rajendran and Gahukar, 2000).

Singh and Gupta (2001) assessed the toxicity of fenvalerate to *T. chilonis* and observed that fenvalerate was more toxic than all other insecticide tested with the LC_{50} value was minimum 0.00274% indicating highest toxicity to natural enemies. Minimum percent parasitization was found in all concentration of fenvalerate. The minimum adult emergence of *T. chilonis* (only 1.66 adult) was found in fenvalerate at higher concentration of 1000ppm. Among the tested insecticides, fenvalerate was the most toxic in respect of LC_{50} value, percent parasitization and adult emergence.

Effect of some newer insecticides and biopesticides on parasitisation and survival of *T. chilonis* was evaluated by Fand *et al.* (2009) and revealed that lamda-cyhalothrin 0.0015% reduced the parasitization. The lambda-cyhalothrin 0.0015% adversely affected the emergence of parasitoid and was relatively less persistent over the other conventional insecticides.

Kaur *et al.* (2009a) conducted a study to select pesticides that are less inimical to *T. brasiliensis*, and concluded that cypermethrin was slightly toxic for adult and pupa with maximum survival in cypermethrin.

Kaur *et al.* (2010) studied the compatiblility of *T. brasiliensis* with seven insecticides in net house and cypermethrin was reported to cause 32.03 % mortality causing persistence for five days after treatment.

Laboratory bioassay was carried out by Ravivarman and Veeravel (2010) to determine the toxicity level of insecticides *viz.*, bifenthrin 10 EC at 25, 50 and 75 g a.i. ha^{-1}, lambda cyhalothrin 5 EC at 12.5 g a.i. ha^{-1} and chlorpyriphos 20 EC at 37.5 g a.i. ha^{-1} on the egg parasitoid, *T. chilonis*. The results revealed that maximum emergence of *T. chilonis* was noticed in control with 96.40 percent with

significantly less in bifenthrin 10 EC at the rate of 25 g a.i. ha^{-1} (88.14%), bifenthrin 10 EC @ 50 g a.i. ha^{-1} (86.42 per cent) and Bifenthrin 10 EC and 12.5 g a.i.ha^{-1} and chlorpyriphos 20 EC at 37.5 g a.i. ha^{-1} recorded 78.49 and 80.32 per cent adult emergence, respectively.

Fenvalerate was evaluated for toxicity on pre-imaginal stages of the egg parasitoid, *T. cacoeciae* under laboratory conditions and concluded the disruptive effect of fenvalerate on *T. cacoeciae* emergence rate, which increased when the parasitoid advanced in development in the host eggs. Abdel-Rahman and El-Aziz. (2012).

2.1.5.1. Compatibility of biological pesticides to beneficial insects

More *et al.* (2011) studied the impact of various insecticides and nimbecidine on the predator green lace wing (*Mallada boniensis*) and concluded that neem products are safer to the predator as it caused less ovicidal action on the eggs and did not reduce the fecundity.

2.1.5.2. Compatibility of biological pesticides to *Trichogramma* spp.

The effects of abamectin, *Bacillus thuringiensis*, cartap, deltamethrin, lambda-cyhalothrin and methamidophos on adults of *T. pretiosum* were evaluated in the laboratory by Branco and França (1995) and concluded that all the insecticides, except *B. thuringiensis*, were highly toxic to the parasitoid. The LC_{50} of abamectin to the parasitoid was 200 times lower than the recommended field rate.

Laboratory experiments were conducted to study the effects of insecticides Biolep and Bioasp (both *Bacillus thuringiensis* subsp. *kurstaki*) on the emergence of *T. japonicum* from eggs of *C. cephalonica* treated on the 3rd or 6th days after parasitisation and concluded that *B. thuringiensis* and neem products had the least effect on emergence of parasitoids (Borah and Basit, 1996).

Lakshmi *et al.* (1998) carried out laboratory experiments to study the effect of neem formulations compared with insecticides on *T. japonicum*, based on rate of parasitisation and emergence of adults from parasitised eggs. The results revealed that Econeem and Neem Azal T/S (0.1-1.0%) were safer compared to insecticides, particularly quinalphos and chlorpyriphos which had adverse effects on parasitization.

Seven eco-friendly insecticides, Dipel, Delfin, Biobit, Biolep, Bioasp (*Bacillus thuringiensis* formulations), Neemgold (botanical insecticide), Greencommandos (nematode formulation) were evaluated for their effects on *T. chilonis* (Ishii), by Malathe *et al.* (1999). Biobit and Greencommandos did not affect the parasitisation and development of *T. chilonis* on *C. cephalonica* when exposed before and after parasitisation. The insecticides had little effect on oviposition behaviour of *T. chilonis*, but not on further development and, hence, resulted in successful adult emergence of *T. chilonis*.

A study was conducted by Sarode and Sonalkar (1999) to evaluate the effect of neem seed extract on parasitisation of *C. cephalonica* eggs by *T. chilonis* and it was found to be moderately safe to the parasitoid.

Laboratory investigations were carried out to assess the toxicity of neem products and insecticides *viz.*, carbaryl 0.1%, endosulfan 0.07%, quinalphos 0.05%, quinalphos 0.04%, monocrotophos 0.07% and abamectin 0.003% against the egg parasitoids, *T. chilonis*, *T. japonicum* and *T. brasiliensis* reared on eggs of *C. cephalonica* by Srinivasan *et al.* (2001). Adult emergence and egg parasitization were greatly influenced by the insecticide treatments compared with the neem products, which were safer to the egg parasitoids.

Boomathi *et al.* (2005) evaluated microbial and botanical pesticide mixture to adults of *T. chilonis* and concluded that neem, sweet flag, pongamia and their mixtures produced 33.89 % adult mortality and 79.58 to 81 % adult emergence whereas spinosad and its combination products had poor adult emergence.

Laboratory experiment was conducted to compare the relative toxicity of bio pesticides against *T. chilonis* by Gandhi *et al.* (2005). They concluded that bio pesticides were safer than chemical insecticides in minimizing harmful effects on development and behaviour of natural enemies. Neem oil recorded 58.9 % parasitoid emergence, 59.3 % parasitism and 63.1 % egg hatchability.

Ramesh and Manickavasagam (2006) studied the effects of different insecticides and botanicals on adult parasitic features and developmental stages of *T. brasiliense*, *T. pretiosum*, *T. chilonis* and *T. japonicum*, reared on *C.cephalonica*, in laboratory conditions. The biopesticides Achook, Nimbicidine and Dipel were found to be safer to the parasitoids.

Effect of some newer insecticides and biopesticides on parasitization and survival of *T. chilonis* was evaluated by Fand *et al.* (2009) and revealed that HaNPV at 1×10^9 POBs/ml and *Bacillus thuringiensis* at 2 ml l^{-1} were safer to the parasitoid whereas neem oil 1.0% reduced the parasitization. The neem oil did not adversely affect the development and emergence of parasitoid although it had reduced the parasitisation.

Kaur *et al.* (2009a) evaluated the persistent toxicity of insecticides *viz.*, endosulfan, monocrotophos, cypermethrin, dimethoate, NSKE and *Bt* on biotic potential of an egg parasitoid, *T. brasiliensis*. Insecticides hamper the effectiveness of *T. brasiliensis* not only through direct mortality but also as a result of sublethal effects affecting the parasitisation capability and per cent emergence. Among all the tested insecticides only *Bt* was found non toxic to *T. brasiliensis* followed by NSKE which could be applied in conjunction of *T. brasiliensis* for controlling the insect pest.

The compatibility of NSKE and *Bt* was evaluated with *T. brasiliensis* by Kaur *et al.* (2010) and *Bt* was rated as the safest causing 4.75 % mortality .

Ksentini *et al.* (2010) evaluated biological insecticide, *Bacillus thuringiensis* and two chemical insecticides, deltamethrin and spinosad, for their effects on immature stages and adults survival of *T. cacoeciae* Marchal,

Trichogramma bourarachae Pintureau and *Trichogramma evanescens* Westwood. It was concluded that *B. thuringiensis* at the rate of 70 g ha^{-1} formulation was harmless towards all the development stages of the tested *T.* species and no persistence was reported by *Bt.*

Kaur *et al.* (2012) evaluated the residual toxicity and LT_{50} value of selected insecticides, NSKE 5.0% and *Bt* 0.2% on twenty freshly emerged adults of *T. brasiliensis*. The LT_{50} of NSKE was found to be 1.85 hrs and *Bt* was non significant on the day of application.

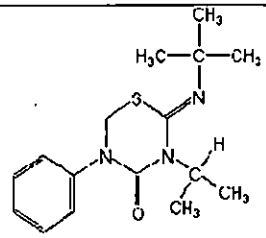
The toxicity of eight pest control products commonly used in vegetable crops, were evaluated by exposing the adults of the two species of *Trichogramma sp.* nr. mwanzai and *Trichogrammatoidea* sp. nr. *lutea* by Momanyi *et al.* (2012). They concluded that the biologically derived products *Bt* ssp. Kurstaki and neem-based products were found to be harmless and had no persistent toxicity on Trichogrammatid species.

2.2. SAFETY OF NEWER INSECTICIDES TO NON-TARGET ARTHROPODS

2.2.1. Buprofezin

Table 1. Basic details of buprofezin

ISO name	Buprofezin
IUPAC name	*(Z)*-2-*tert*-butylimino-3-isopropyl-5-phenyl-1,3,5-thiadiazinan-4-one
Proprietary name	Applaud 25 SC
Chemical class	Thiadiazine
Chemical Formula	$C_{16}H_{23}N_3OS$
Mode of Action	Inhibitor of Chitin biosynthesis
Oral LD_{50}	1635– 3847 mg/kg
Dermal LD_{50}	> 10 000 mg/kg

Structure	
Bioefficacy	Against the nymphal stages of whiteflies, scales, mealybugs, planthoppers and leafhoppers
Source	Rallis India Ltd.

2.2.1.1. Impact of buprofezin on beneficial insects

Prabhakar *et al.* (2007) conducted laboratory studies to compare the toxicity of seven foliar insecticides to four species of adult beneficial insects representing two families of Hymenoptera: Aphelinidae (*Aphytis melinus* Debach, *Eretmocerus eremicus* Rose and Zolnerowich, and *Encarsia formosa* Gahan) and Mymaridae (*Gonatocerus ashmeadi* Girault). Results highlighted that the IGRs buprofezin and pyriproxyfen were usually less toxic than the contact pesticides.

The toxicological effects of buprofezin, on the fecundity, development and pest control potential of the wolf spider *Pirata piratoides* (Schenkel) (Araneae: Lycosidae) were investigated in the laboratory by Lingling Deng *et al.* (2008). It was reported that buprofezin had low toxicity to *P. piratoides*. Buprofezin significantly reduced the percent hatching of spiders' eggs but slightly affected egg production. No negative effects on the development and growth were observed and were thus rated as safe.

Based on the toxicity studies conducted by Neetan and Naveen (2013) on the immature stages of *Chrysoperla zastrowi*, buprofezin treatment at the egg stage resulted in 73.33 % hatching. Adult emergence and survival from buprofezin treated pupae indicated its safety with 76.67 % adult emergence and 70 .00 % adult survival.

Pandi *et al.* (2013) evaluated the toxicity of buprofezin to grubs of *Cheilomenes sexmaculata* and concluded that buprofezin is safe at recommended dose of 0.013% because it produced 10, 16, and 22% mortality at 24, 48, and 72 HAT, respectively under laboratory conditions. Buprofezin was found to be 14.8 times more toxic in comparison to imidachloprid.

2.2.1.2. Impact of buprofezin on *Trichogramma* spp.

Guifen and Hirai (1997) assessed the toxicity buprofezin along with imidachloprid, fenitrothion and cartap on the preimaginal stages (eggs, larvae, pre-pupae and pupae) of *T. japonicum* in the laboratory and concluded that all insecticides penetrated into the egg shell and showed different degrees of toxicity. Buprofezin showed least toxicity among all the tested insecticides.

Nasreen *et al.* (2004) evaluated insecticides *viz.* diafenthiuron, buprofezin for their toxicity against egg parasitoid *T. chilonis* through leaf dip bioassay method at three levels of concentration *viz.*, low, recommended and high under laboratory conditions. It was reported that all concentrations of buprofezin remained harmless even at 48 hours after treatment. Parasitism rate of *T. chilonis* was highest (100%) in buprofezin with the lowest toxicity to the wasps with a LC_{50} of 3,383 (2406–5499) to 30206 (23107–41008) mg a.i. l^{-1}

The lethal and sublethal effects of six groups of insecticides were evaluated by Sun Chao *et al.* (2008) against *T. japonicum* under the laboratory conditions. Buprofezin, tebufenozide, furan tebufenozide and hexaflumuron had no effect on survival and parasitism capacity of adults of *T. japonicum* when they were exposed to the leaves with insecticide residues at various intervals after spraying. It was concluded that buprofezin had no significant direct or indirect harmful effects on parasitism capacity. By dip method also buprofezin were found to have little effect on adult emergence at different developmental stages (egg, larva, prepupa and pupa), with the emergence rates of 81.4%-91.8%.

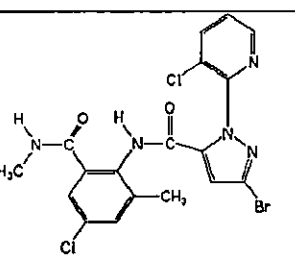
Sun Chao *et al.* (2008) reported that hexaflumuronron was harmless to pupae. Based on the results, the insect growth regulators buprofezin, tebufenozide

and Furan Tebufenozide were not directly or indirectly harmful to *T. japonicum*, suggesting that these insecticides are compatible to this parasitoid when being used for control of rice pests.

Insect growth regulator (chlorfluazuron, fufenozide, hexaflumuron and tebufenozide) were evaluated for their toxicity to *T. japonicum* Ahmead using a dry film residue method by Zhou *et al.* (2012). Among the seven classes of chemicals tested, insect growth regulator (chlorfluazuron, fufenozide, hexaflumuron and tebufenozide) exhibited the lowest toxicity to the wasps with an LC_{50} of 3,383 (2406–5499) to 30206 (23107–41008) mg a.i. L^{-1}. Risk quotient analysis revealed the safety of insect growth regulators to *T. japonicum.*

2.2.2. Chlorantraniliprole

Table 2. Basic details of chlorantraniliprole

ISO name	Chlorantraniliprole
IUPAC name	3-bromo-4′-chloro-1-(3-chloro-2-pyridyl)-2′-methyl-6′-(methylcarbamoyl)pyrazole-5-carboxanilide
Proprietary name	Coragen 18.5 SC
Chemical class	Anthranilic diamide
Chemical formula	$C_{18}H_{14}BrCl_2N_5O_2$
Mode of Action	Modulates ryanodine receptor
Oral LD_{50} and dermal LD_{50}	> 5000 mg/kg body weight
Structure	
Bioefficacy	Lepidopteran pests of rice, sugarcane, cabbage, blackgram,vegetables, cotton

Source	E I DuPont India Private Limited	

2.2.2.1. Impact of chlorantraniliprole to beneficial insects

Chlorantraniliprole has an excellent profile of safety to beneficial arthropods (Dinter *et al.*, 2008), pollinators and non-target organisms such as earthworms and soil microorganisms. The product effects on honeybees have been studied extensively, demonstrating low intrinsic toxicity of chlorantraniliprole and no negative effects were observed under worst-case semi-field conditions on foraging honey bees in numerous tunnel tests.

Lefebvre *et al.* (2012) evaluated the safety of newer molecules to predatory mite *Neoseiulus fallacis* (Garman) and concluded that no significant egg mortality was caused by chlorantraniliprole treatment. Chlorantraniliprole was found to be virtually nontoxic to larvae with no such adverse effect on the fecundity.

2.2.2.2. Impact of chlorantraniliprole to *T. chilonis*

Chlorantraniliprole is an anthranilic diamide insecticide with a novel mode of action. Chlorantraniliprole induces the activation of insect ryanodine receptors located in the sarcoendoplasmic reticulum to release stored intracellular calcium in the cell's cytoplasm (Lahm *et al.*, 2005). Sustained exposure to chlorantraniliprole leads to impaired regulation of the muscle excitation, contraction and relaxation cycle which leads to complete muscle contraction, paralysis and death of the insect (Cordova *et al.*, 2006). Comparative studies with mammalian cell lines that endogenously express ryanodine receptors demonstrate that chlorantraniliprole exhibits a greater than 350-fold differential selectivity for insect receptors relative to mammalian receptors (Cordova *et al.*, 2007). Differences in specificity and potency of effects distinguish the mammalian ryanodine receptor response from that of the insect and these differences appear to be the major contributing factor in the low

mammalian toxicity exhibited for chlorantraniliprole.

Diamides have proven to be highly efficacious against several lepidopteran species at low application rates (Lahm *et al.*, 2005, Cordova *et al.*, 2006, Seo *et al.*, 2007). Additionally, anthranilic diamides have demonstrated a favorable eco-toxicological profile with low impact on fish, birds, and common beneficial natural enemy arthropod species (Anonymous., 2007, Cordova *et al.*, 2006).

Chlorantraniliprole was reported to cause no toxic impact by acute contact or oral exposure to honeybees. A concentrations up to 0.0274 µg a.i./bee and 0.125 µg a.i./bee for acute oral and contact exposure, respectively did not exerted any toxic effect on bees at 48 hour after treatment whereas other non-target arthropods(ladybird beetle, hoverfly and *Orius laevigatus*), were found to be acutely affected by chlorantraniliprole (Seo *et al.*, 2007).

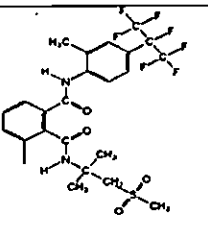
Chlorantraniliprole belongs to the "ryanodine receptor modulators," namely "Group 28 Insecticide" according to IRAC International Mode of Action classification.

Preetha *et al.* (2009) evaluated chlorantraniliprole along with other eight insecticides for their toxicity to the parasitoid *T. chilonis* using an insecticide-coated vial residue bioassay. The LC_{50} value of chlotrananiliprole to *T. chilonis* was found to be 1.9530 mg a.i. l^{-1} and based on the risk quotient chlorantraniliprole was found to be harmless to *T. chilonis.*

2.2.3. Flubendiamide

Table 3. Details of flubendiamide

ISO name	Flubendiamide
IUPAC name	3-iodo-*N'*-(2-mesyl-1,1-dimethylethyl)-*N*-{4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl) ethyl]-*o*-tolyl}phthalamide

Proprietary name	Fame 480 SC
Chemical class	Phthalic acid diamide
Chemical Formula	$C_{23}H_{22}F_7IN_2O_4S$
Mode of Action	Modulates ryanodine receptor
Oral LD_{50} and dermal LD_{50}	> 5000 mg/kg body weight
Structure	
Bioefficacy	Lepidopteran pests of rice, cotton, cabbage
Source	Bayer Crop Science Limited

2.2.3.1. Impact of flubendiamide to beneficial insects

Gradish *et al.* (2012) evaluated the susceptibility of *Megachile rotundata* (Fabricius), a pollinator of lowbush blue berry to insecticides and reported that flubendiamide was not toxic to adult *M. rotundata* by direct contact and had no effect on larval survivorship or time to complete cocoon spinning. Emergence after overwintering was relatively poor but there was no significant effect of treatment. Flubendiamide was rated as safe to *M. rotundata.*

Lefebvre *et al.* (2012) conducted laboratory studies to evaluate the impact of newer molecules *viz.*, flubendiamide, chlorantraniliprole, chlothianidin, novaluron, Spinetoram, and spirotetramat on *Neoseiulus fallacis* (Garman). It was reported that the overall egg mortality caused by flubendiamide was < 10% and flubendiamide were virtually nontoxic to larvae. Also, flubendiamide had no adverse effect on the fecundity.

2.2.3.3. Impact of flubendiamide to *T. chilonis*

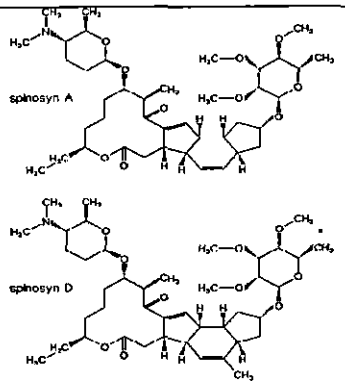
Sattar *et al.* (2011) studied the toxicity of flubendiamide and six other

insecticides against *T. chilonis* under laboratory and extended laboratory conditions following the guidelines recommended by International Organization for Biological Control (IOBC) against all the life stages of the parasitoid. Results regarding the harmful effects of the insecticides on the different life stages of *T. chilonis* revealed that flubendiamide was the most selective of all the tested insecticides for the development, survival and fecundity of the wasp. Persistency test showed that flubendiamide was "short lived". On the basis of this study, it was concluded that flubendiamide is considerably safe insecticide.

2.2.4. Spinosad

Table 4. Details of spinosad

ISO name	Spinosad
IUPAC name	mixture of 50–95% (2*R*,3a*S*,5a*R*,5b *S*,9*S*,13*S*, 14*R*, 16a*S*,1 erythro pyranosyloxy) - 9 -ethyl-2,3,3a,5a,5b, 6,7,9,10,11, (2*S*,3a*R*,5a*S*,5b*S*, 9*S*,13*S*,14*R*,16a*S*,16b*S*)-2 -(6-deoxy-2,3, 2,3, 3a, 5a, 5b, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16a, 16b–hexa
Proprietary name	Success 2.5 SC
Chemical class	Macrocyclic lactone
Chemical formula	$C_{41}H_{65}NO_{10}$ (spinosyn A) + $C_{42}H_{67}NO_{10}$ (spinosyn D)
Mode of Action	Acts on GABA receptor
Oral LD_{50} and dermal LD_{50}	>2000 mg/body weight

Structure		
Bioefficacy	Acts against sucking pests like aphids, whiteflies, scales	
Source	Dow Agro Science India Private Limited	

2.2.4.1. Impact of spinosad to beneficial insects

Sterk *et al.* (2001) studied the toxicity of insecticides to bumblebee larvae, influence on their behaviour and side-effects of pesticides on honeybees, *Aphis mellifera* L. It was concluded that spinosad was toxic if bees were treated directly or fed a mixture of product and sugar water.

Side-effect trials with new compounds were carried out under semi-field conditions, to evaluate its safety to first and second nymphal instars of *Orius laevigatus* (Fieber) (Anthocoridae) and *Macrolophus caliginosus* Wagner (Miridae), adult females of *Phytoseiulus persimilis* Athias-Henriot (Phytoseiidae), and pupae and adults of *Encarsia formosa* Gahan (Aphelinidae) (Sterk *et al.,* 2001). Spinosad was cateogarised as safe to all the organisms tested except to adult of *E. formosa* to which spinosad was moderately toxic. However, this effect was brief due to the short persistence of this compound.

The effects of spinosad to beneficial and non-target arthropods has been extensively studied under laboratory, semi-field and field conditions on a wide range of predatory and parasitic taxa in a variety of geographical regions and

crop types by Miles and Eelen (2009). Research has demonstrated that spinosad is of low risk to predatory mites and beneficial insect populations when used according to good agricultural or horticultural practice. Toxicity has been reported to certain parasitic hymenoptera but due to the very short persistence of the product any effects are short lived and followed by rapid recovery.

2.2.4.2. Impact of spinosad to *T. chilonis*

Spinosad, was reported to be a mixture of the spinosyns A and D having allosteric activator of the nicotinic acetylcholine receptor that was isolated as a mixture of secondary metabolites during fermentation of the soil actinomycete *Saccharopolyspora spinosa* (Mertz and Yao, 1990).

Three new insecticide chemistries were evaluated for their effects on survival on predaceous flower bug *Orius insidiosus* (Say) (Hemiptera : Anthocoridae) and parasitoid *Cotesia marginiventris* and *C. plutella* (Kurdjumov) (Hymenoptera: Braconidae) by Pietrantonio and Benedict (1999). They reported that the residues of spinosad caused significant mortality to the parasitoid by causing 50.00 % mortality in *C. plutella* by dried residue. Also *C. marginiventris* was found more susceptible to spinosad than *C. plutella.* Based on the percent mortality, spinosad was rated as moderately harmful (rank 2) for *C. plutella* and as harmful (rank 4) for *C. marginiventris.*

The effect of spinosad on *T. exiguum* Pinto and Platner emergence, adult survival, and fitness parameters were investigated by Suh *et al.* (2000). The mean life span of emerged *T. exiguum* females significantly varied among insecticide treatments, and was significantly affected by the developmental stage of parasitoid when treated. Based on LC_{50} values, spinosad was the categorized as the most toxic compound to female *T. exiguum* adults.

The effect of insecticides *viz.*, lambda-cyhalothrin, cypermethrin, thiodicarb, profenofos, spinosad, methoxyfenozide and tebufenozide on

emergence of *T. exiguum*, adult survival and fitness parameters were investigated by Suh *et al,* (2000) and it was concluded that spinosad adversely affected the emergence of *T. exiguum* from *Helicoverpa zea* host eggs when different pre-imaginal stages of development (larval, prepupal or pupal) were exposed. Based on LC_{50} values, spinosad was the most toxic compounds to female *T. exiguum* adults.

In a key review of spinosad compatibility with natural enemies, Williams *et al.* (2003) found that from 228 observations on 52 species of natural enemies, spinosad was ranked in the lowest IOBC risk category (Class 1, harmless) in 71% of laboratory studies and 79% of field-type studies. However, hymenopteran parasitoids are significantly more susceptible than predatory insects, with 78% of laboratory studies and 86% of field-type studies returning a moderately harmful (IOBC Class 3) or harmful (IOBC Class 4) result.

Williams *et al.* (2003) evaluated differential susceptibility of *T. pretiosum* to spinosad, thiamethoxam, and oxamyl and confirmed the toxicity order of thiamethoxam>spinosad>oxamyl. The results underscored the danger of generalizing pesticide effects.

Schneider *et al.* (2004) evaluated the topical toxicity of spinosad on *Hyposoter didymator* (Thunberg) (Hymenoptera: Ichneumonidae), a larval parasitoid of several lepidopteran pests under laboratory conditions. They reported that spinosad was harmful to the parasitoid (IOBC toxicity class 4). This rating was based on the reduction in rate of: (i) pupae formation, (ii) pupal mortality, (iii) adult longevity, (iv) parasitism, and (v) adult emergence.

Yong-yu xu *et al.* (2004) carried out laboratory experiments to study the effects of eight insecticides on *Diadegma insulare* (Cresson), a parasitoid of the diamondback moth, *Plutella xylostella* L. They concluded that spinosad caused 100 % adult mortality to *D. insulare* (24 h) in Petri dishes sprayed with

insecticides in the contact bioassays, and 95.8 and 100 % adult mortality in ingestion bioassays (24 h), respectively.

Shanmugam *et al.* (2006) studied the safety of some newer insecticides against *T. chilonis* under laboratory conditions. It was reported the microbial insecticide spinosad was safer than the newer insecticides.

Fernández *et al.* (2010) reported the toxicity of spinosad to the larval stage stage of the parasitic wasp *Eretmocerus mundus* by evaluating adult emergence as well as sub lethal effects on its reproductive performance in laboratory. Parasitized *Bemisia tabaci* pupae were placed on glass surfaces and directly sprayed with the maximum field recommended concentrations of the pesticides. Spinosad was given an IOBC toxicity rating of '4' indicating that it is harmful (high mortality).

Hussain *et al.* (2010) studied the effect of spinosad 240 SC at 200 ppm on the pre-imaginal development and adult survival of *T. chilonis*. Spinosad was reported to have a significant adverse effect on the emergence of *T. chilonis* when exposed to all immature stages of development (egg, larvae, pre-pupae, early pupae and pupae) in the host eggs of *Sitotroga cerealella*.

The effect of five insecticides on the immature stages of the first and the second generations of the egg parasitoid *T. evanescens* was studied in laboratory by Shoeb *et al.* (2010).The emergence rate for spinosad treatment was very low (3-5%) and the emerged adults died within 6-12 hrs after emergence. It was noticed that the two concentrations of spinosad gave the same effect on the immature stages of the egg parasitoid *T. evanescens* (same number of black eggs – same emergence rate – same percentage of females – same longevity for females and males).

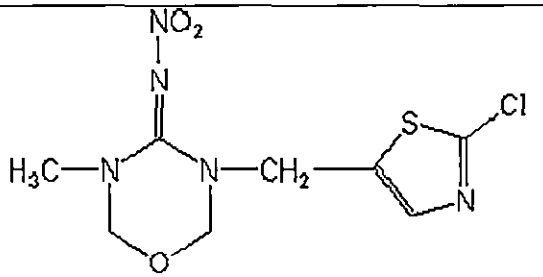
Wang DeSen *et al.* (2012) evaluated the acute toxicities and sublethal effects of ten conventional insecticides to adult of *T. chilonis*. Based on sublethal

concentration (LC_{30}) values at 8 hours after treatment it was reported that adult *T. chilonis* were susceptible to spinosad at 0.3269 mg/*l*. Spinosad reduced longevity (7.9 days) and fecundity (117.2) of treated adults.

Saljoqi *et al.* (2012) studied the compatibility of different concentrations of spinosad (0.2, 0.15, 0.1, 0.05 and 0.01 %). The results showed that 0.20 % gave the minimum parasitism in egg, larva and pupa.

2.2.5. Thiamethoxam

Table 5. Details of thiamethoxam

ISO name	Thiamethoxam
IUPAC name	(*EZ*)-3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene (nitro) amine
Proprietary name	Actara 25 WG
Chemical class	Neonicotinoid
Chemical formula	$C_8H_{10}ClN_5O_3S$
Mode of action	Irreversible blockage of post synaptic Acetyl choline receptor
Oral LD_{50} and dermal LD_{50}	> 2000 mg/kg body weight
Structure	
Bioefficacy	Effective against thrips, scales, aphids
Source	Syngenta India Limited

2.2.5.1. Impact of thiamethoxam to beneficial insects

Sterk *et al.* (2001) evaluated the side-effect of new compounds in IPM programs under semi-field conditions following protocols developed by the IOBC group (Sterk *et al.* 1999). The effect of new compounds were tested on first and second nymphal instars of *Orius laevigatus* (Fieber) (Anthocoridae) and *Macrolophus caliginosus* Wagner (Miridae), adult females of *Phytoseiulus persimilis* Athias-Henriot (Phytoseiidae), and pupae and adults of *Encarsia formosa* Gahan (Aphelinidae) and it was reported that thimethoxam were very toxic to all the arthropods tested.

The toxic impact of newer insecticides to bumble bee larvae, influence their behavior and side-effects of pesticides on honeybees, *Aphis mellifera* L were studied by Sterk *et al*, (2001) and it was concluded that thiamethoxam was toxic to bumblebees in all trials.

The safety of thiamethoxam to green lace wing (*Mallada boniensis*) was evaluated by More *et al.* (2011) and concluded that adult emergence due to thiamethoxam treatment was comparatively less. Also the egg hatchability was reduced due to thiamethoxam treatment.

The toxicity of thiamethoxam to the grubs of *Cheilomenes sexmaculata*, was evaluated by Pandi *et al.* (2013) and it was reported that thiamethoxam at field recommended dosage (0.005%) caused almost 50% mortality and was thus rated as toxic to the grubs. Neonicotinoid acetamiprid showed high toxic to grubs followed by thiamethoxam, imidacloprid, buprofezin and neembaan.

2.2.5.2. Impact of thiamethoxam to *T. chilonis*

Differential toxicity of insecticides *viz.*, spinosad, thiamethoxam, and oxamyl were evaluated by Williams and Price (2003) against the egg parasitoids *T. pretiosum* and reported the toxicity in the order of thiamethoxam>spinosad>oxamyl.

Nasreen *et al.* (2004) evaluated eight insecticides *viz.* diafenthiuron, buprofezin, thiodicarb, imidacloprid, carbosulfan, methamidophos, acetamiprid and thiamethoxam for their toxicity against egg parasitoid *T. chilonis* through leaf dip bioassay method at three levels of concentration *viz.*, low, recommended and high under laboratory conditions.. The results revealed that lower concentrations of acetamiprid and thiamethoxam were slightly harmful, while recommended and higher concentrations were found moderately harmful and harmful, respectively.

Shanmugam *et al.* (2006) studied the safety of some newer insecticides against *T. chilonis* under laboratory conditions. The microbial insecticide spinosad was safer than the newer insecticides, imidacloprid, indoxacarb and thiamethoxam. Imidacloprid and indoxacarb were moderately toxic with 47 and 37 % parasitism. The *T. chilonis* adult survival also showed a similar trend.

Nine insecticides, namely, imidacloprid, thiamethoxam, chlorantraniliprole clothianidin, pymetrozine, ethofenprox, BPMC, endosulfan, acephate and the product Virtako® (Syngenta; chlorantraniliprole 20%+thiamethoxam 20%) were tested for their toxicity to the parasitoid *T. chilonis* using an insecticide-coated vial residue bioassay by Preetha *et al.* (2009). Thiamethoxam was reported to have highest toxicity to *T. chilonis* with an LC_{50} of 0.0014 mg a.i. l^{-1} Thiamethoxam was found to be 3,195, 1,395 and 1,322 times more toxic than acephate, chlorantraniliprole and endosulfan, respectively, as revealed by the LC_{50} values to *T. chilonis*. Based on risk quotient, which is the ratio between the field-recommended doses and the LC_{50} of the beneficial, thiamethoxam was found to be dangerous to the parasitoid.

Zhou *et al.* (2012) studied the toxicity of seven classes of chemicals to, *T japonicum* Ahmead using a dry film residue method. Results showed that neonicotinoids (acetamiprid, imidacloprid, imidaclothiz, nitenpyram thiacloprid, and thiamethoxam) exhibited lowest intrinsic toxicity and risk

quotient analysis showed that thiamethoxam was comparatively harmful than other neonicotinoids to *T. japonicum*.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present investigation entitled 'Impact of novel insecticides on *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae)' was undertaken in the Department of Agricultural Entomology, College of Horticulture, Vellanikkara, Thrissur during 2012-13. The toxicity, safety and persistence of five novel insecticides *viz.* buprofezin, chlorantraniliprole, flubendiamide, spinosad and thiamethoxam from newer chemical groups with novel modes of action on *T. chilonis* were studied in the Insecticide Toxicology laboratory by adopting internationally approved and standardised protocols developed by the Pesticides and Beneficial Organisms Working Group of the International Organization for Biological Control of Noxious Plants and Animals / West Palaerctic Region Selection (IOBC/WPRS) (Sterk *et al.* 1999). The materials used and the methodologies adopted are elucidated hereunder.

3.1. MASS PRODUCTION OF *T. chilonis*

T. chilonis was mass produced on the factitious host, rice moth *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) reared in the laboratory at temperature 28 ± 2 ^{0} C and relative humidity 68 ± 2 %.

3.1.1. Rearing and maintenance of *C. cephalonica* culture in the laboratory

3.1.1.1. Preparation of rearing medium for *C. cephalonica*

The ingredients used for preparing rearing media for *C.cephalonica* are given below.

Bajra (*Pennisetum glaucum*)	-	2. 5 kg
Raw groundnut (*Arachis hypogaea*)	-	100.0 g
Yeast	-	5.0g

Corcyra eggs - 0.5 cc

C. cephalonica was reared on food quality bajra (*Pennisetum glaucum*) that was procured, after ensuring the absence of storage pests, from the local market. The stock culture of *Corcyra* eggs was procured from the State Biocontrol Laboratory, Mannuthy, Thrissur.

Half crushed bajra grains weighing 2.5 kg were taken in plastic basins of 5*l* capacity (30 cm diameter and 15 cm height). Crushed groundnut (100 g), yeast (5 g), sulphur (5 g) and streptomycin (40 ml) were added into the plastic basins containing bajra grains and mixed thoroughly. Groundnut was added to supplement the protein requirement of developing larvae and yeast to enhance the egg laying capacity of adult moths. Attack of mite was kept under check by the addition of sulphur. Streptomycin 0.1% was prepared by adding 1 g streptomycin in 1 *l* of water and 40 ml of this solution was poured into the media to prevent bacterial infection. Care was taken to protect the culture from the attack of ants and rodents also. After mixing all the ingredients thoroughly, 0.5 cc *Corcyra* eggs were sprinkled into the medium in the basin (Plate 1) and the mouth of the basin was covered with muslin cloth and tightly secured with a piece of muslin cloth. The basins containing the rearing media inoculated with *Corcyra* eggs were kept on a steel rack for the development of *Corcyra* culture in the laboratory (Plate 2).

3.1.1.2. *Corcyra* oviposition cage

Adult moths (Plate 3a) emerging) from the *Corcyra* culture (after about 40 days of inoculation) in the basins were collected daily using a test tube (Plate 3b) and transferred to an oviposition cage for egg laying. The oviposition cage (Plate 4) was fabricated with a plastic container (14 X 10 cm) having a lid. The bottom end of the plastic container was cut and a plastic net mesh was tied tightly to the cut end and a Petri plate (10 cm diameter) was fitted with a cello tape to the plastic net mesh for collecting the eggs laid by moths in the container. Adult moths were provided with 50 % honey solution and vitamin E drops on a cotton swab kept inside the oviposition cage to enhance the egg laying. The eggs laid by

Plate 1. Rearing media

Plate 2. Rearing room and arrangement

3a. *Corcyra* adults emerging from rearing media

3b. *Corcyra* adults collected in test tubes

Plate 3. Corcyra adult emergence

moths in the plastic container were dropped down through the mesh and collected in the Petri plate that served as an egg collecting tray. Eggs collected from the oviposition cage were then cleaned with a tea strainer for removing the body scales of the moths. The eggs thus cleaned were further used for culturing both *C. cephalonica* and *T. chilonis.*

3.1.2 Mass culturing of *T. chilonis*

The egg parasitoid *T. chilonis* was mass cultured on the eggs of rice moth, *C. cephalonica* in the laboratory. Freshly laid eggs of *Corcyra* were collected in the morning (Plate 3b) and cleaned from scales and other dust particles (Plate 3c). The cleaned *Corcya* eggs were sprinkled on sticky cards of 10 X 5 cm^2 size and were irradiated with UV rays of 30 Watts for 45 minutes at a distance of 2 ft, to prevent hatching of *Corcyra* larvae. These *Corcyra* egg cards were then kept in polythene bags along with a nucleus trichocard at 6:1 ratio for 3-4 days to get parasitized by *T. chilonis*. The *Corcyra* eggs, after parasitisation by *T. chilonis,* containing the pupae of *T. chilonis* appear black and plumpy. *T. chilonis* adults emerging from the trichocards were further used to maintain the culture in the laboratory at ambient temperature and humidity.

3.2. INSECTICIDES SELECTED FOR THE STUDY

Five novel insecticides *viz.*, buprofezin (Applaud 25 SC), chlorantraniliprole (Coragen 18.5 SC), flubendiamide (Fame 480 SC), spinosad (Success 2.5 SC) and thiamethoxam (Actara 25 WG) belonging to newer chemical classes *viz.*, thiadiazine, anthranilic diamide, phthalic acid diamide, macrocyclic lactone and neonicotinod respectively were evaluated for their impact on *T. chilonis*. The commercial formulations were used for conducting different experiments.

3.3. EXPERIMENTS UNDERTAKEN

The experiments carried out are listed below:

- Life cycle and developmental period of *T. chilonis*
- Acute contact toxicity of insecticides to adults of *T. chilonis*
- Dosage – mortality response of *T. chilonis* to insecticides
- Effect of insecticides on the pre- imaginal life stages of *T. chilonis*
- Parasitisation efficiency of adult *T. chilonis* emerging from insecticide treated immature life stages
- Parasitisation efficiency of *T. chilonis* as influenced by insecticide treatment on factitious host eggs
- Side- effects of insecticides to *T. chilonis*
- Persistent toxicity of insecticides to *T. chilonis*

3.3.1. Life cycle of *T. chilonis*

Freshly laid *Corcyra* eggs were collected from oviposition cages and were sieved to make them free from scales. The cleaned *Corcyra* eggs were glued on the cards of size 10 X 2 cm using gum. After drying, the *Corcyra* egg cards were irradiated with UV radiation to arrest the growth of *Corcyra* embryo. The cards were cut uniformly into smaller bits (5 X 1cm). Each card bit was placed in a polythene cover into which twenty newly emerged adults of *T. chilonis* were introduced for parasitisation. The polythene cover was air filled and then sealed using a sealer. The adults were allowed to parasitize *Corcyra* eggs for 24 hours and then were removed from the polythene cover. Ten replications were maintained. The parasitized *Corcyra* eggs were studied for the biology of *T. chilonis* and developmental period of the different stages was recorded.

3.3.2. Acute contact toxicity

The direct acute contact toxicity of five insecticides *viz.* buprofezin 25 SC, chlorantraniliprole 18.5 SC, flubendiamide 480 SC, spinosad 2.5 SC and thiamethoxam 25 WG at field recommended concentrations to the adults of *T. chilonis* was studied by bioassay (Plate 5). Commercial formulations of the insecticides were diluted with water at recommended concentration to obtain the different treatments (Table 6) for the experiment.

Table 6. Insecticide concentrations used for bioassay against *T. chilonis* adults

Insecticide formulation	Field recommeneded concentration		
	%	g a.i. ha^{-1}	ml/g l^{-1}
Buprofezin 25 SC (Applaud 25 SC)	0.04	200	1.60
Chlorantraniliprole 18.5 SC (Coragen 18.5 SC)	0.06	30	0.30
Flubendiamide 240 SC (Fame 240 SC)	0.005	25	0.13
Spinosad 2.5 SC (Success 2.5 SC)	0.015	75	0.50
Thiamethoxam 25 WG (Actara 25 WG)	0.005	25	0.20

Procedure of bioassay

Dry film residue method of bioassay (Plate 6) was followed to assess the contact toxicity of insecticides to adults of *T. chilonis*. Test tubes of 45 ml capacity with an internal surface area of 80 cm^2 were used for preparing dry film residue. The test tubes were cleaned by soaking overnight in soapy water, rinsed with acetone and air dried for four hours before use. The test tubes were coated

Plate 4a. Oviposition cage

Plate 4b. Corcyra eggs collected

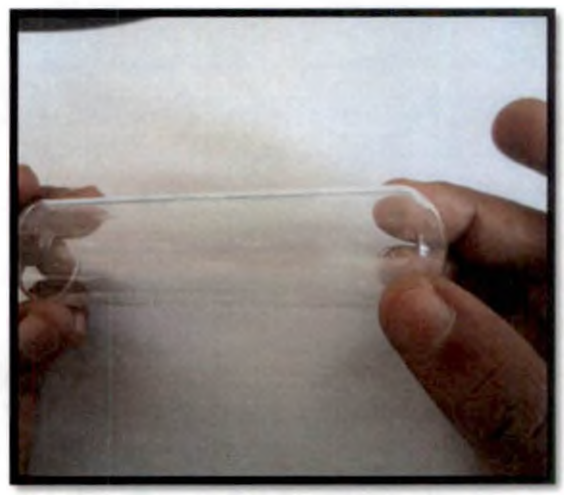
4c. Egg cleaning and sieving

Plate 4. *Corcyra* egg collection procedure

Plate 5. Preparation of field recommended concentration of insecticides using micropippette

Plate 6. Dry film residue method

6a. Rolling amidst fingers



6b. Swirling amidst fingers

evenly with 0.5 ml of different concentrations of insecticides and dried thoroughly by rotating the test tubes between the palms. For the untreated control, 0.5 ml of water alone was used. Twenty freshly emerged adult wasps were transferred into each test tube and covered with a muslin cloth secured with a rubber band. The adults were anaesthetised by keeping in refrigerator for 15 minutes to enable counting and transfer of wasps to test tubes. After four hours of exposure to the insecticide residue in the treated test tube, the wasps were transferred to other clean test tubes and observed for the lethal effects. Cotton swabbed with 50 % honey was kept inside the tube as adult food. Mortality of adult wasps was recorded at 24 and 48 hours after treatment (HAT). The experiment was carried out in Completely Randomized Design (CRD) with five insecticide treatments along with an untreated control and four replications for each treatment.

3.3.3. Dosage – mortality response of *T. chilonis*

3.3.3.1. Estimation of LC_{50} value of insecticides to *T. chilonis*

Dosage mortality response of *T. chilonis* to five insecticides was studied by dry film residue bioassay technique. Preliminary tests were initially carried out to fix the test concentrations that caused 20 to 90 % mortality of *T. chilonis*. Accordingly, different concentrations of commercial formulations were prepared with distilled water and used for bioassay (Table 7). Test tubes of 45 ml capacity with an internal surface area of 80 cm^2 were used as bioassay tubes. The test tubes were cleaned by soaking overnight in soapy water, rinsed with acetone and air dried for four hours before use. The cleaned test tubes were evenly coated with 0.5 ml of different concentrations of insecticides and dried thoroughly by rotating the test tubes with hand. Four replications were kept for each treatment along with an untreated control with water only. Twenty adults of *T. chilonis* emerging from the parasitized egg cards were released into each test tube and covered with a muslin cloth secured by a rubber band to allow air circulation. After 4 h of exposure, the wasps were transferred to a clean test tube. Observations on the mortality of *T. chilonis* were recorded at 24 hours after treatment (HAT).

Plate 7. Dip method

Plate 7a. Dip method

Plate 7b. Experimental arrangement

Table 7. Concentration of insecticides used in bioassays to determine median lethal concentration (LC_{50})

Insecticides formulation	Concentration (mg a.i l^{-1})															
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16
Buprofezin 25 SC	0.1000	0.2000	0.4000	0.6000	0.8000	1.000	1.200	1.4000	1.6000	1.800	2.000	2.2	2.4	2.6	2.8	3.00
Chlorantraniliprole 18.5 SC	0.1000	0.5000	1.0000	1.5000	2.0000	2.500	3.000	3.5000	4.0000	4.500	5.000	5.5	-	-	-	-
Flubendiamide 480 SC	0.1000	0.2000	0.3000	0.4000	0.5000	0.600	0.7000	0.8000	0.9000	1.000	-	-	-	-	-	-
Spinosad 2.5 SC	0.1500	0.3000	0.6000	0.9000	1.2000	1.5000	1.800	2.100	2.400	2.700	3.000	-	-	-	-	-
Thiamethoxam 25WG	0.0005	0.0006	0.0007	0.0008	0.0009	0.001	0.0011	0.0012	0.0013	0.0014	.0015	-	-	-	-	-

Statistical analysis

Necessary corrections were made for natural mortality in the untreated control using Abbott's formulae (Abbott, 1925). The data on percentage mortality were then subjected to probit analysis as per Finney (1971) and the log concentration probit mortality (LCP) lines were fitted.

3.3.3.2. Risk quotient of insecticides to *T. chilonis*

The risk quotient of different insecticides was determined to assess the risk to non target arthropods and thus the ecological risk of pesticides (Peterson, 2006). Risk quotient is the ratio between the field recommended dose and the LC_{50} to the beneficial insect. Risk quotients of the tested insecticides were worked out based on the LC_{50} values (24 HAT) obtained from the above experiment (3.3.3.1).

$$\text{Risk quotient} = \frac{\text{Recommended field rate (g a.i. ha}^{-1})}{LC_{50} \text{ of beneficial insect (mg a.i. } l^{-1})}$$

Based on the risk quotient values, the tested insecticides were categorised into different groups (Preetha *et al.*, 2010).

Risk quotient values	Category of insecticides
<50	Harmless
50 – 2500	Slightly to moderately toxic
>2500	Dangerous

3.3.4. INSECTICIDE EFFECT ON PRE-IMAGINAL DEVELOPMENT OF *T. chilonis*

The effect of insecticides on the development of immature stages of *T. chilonis* was studied by exposing the pre- imaginal stages (eggs, larvae and pupae) to insecticides. Fresh eggs of *C. cephalonica* were collected from oviposition cages and were glued on cards of size 10 X 2 cm^2 and irradiated with UV radiations. These cards were placed along with nucleus trichocards at 6: 1 ratio for parasitisation in polythene covers for 24 hours. Such parasitized egg cards were used for further experiments.

3.3.4.1. Exposure of egg stage of *T. chilonis* to insecticides

The *Corcyra* egg cards, one day after parasitisation (as described in 3.1.2.) were used for the experiment. Each egg card (10 X 2 cm^2), with *Corcyra* eggs containing *Trichogramma* eggs, was cut into four bits of 5 X 1 cm^2 size. The egg card pieces were treated by dipping in solution of five insecticides prepared at field recommended concentrations for three seconds (Plate 7). Four replications were maintained for each treatment and untreated control was maintained by dipping the egg card in water alone. The total number of eggs in each card bit was counted before insecticide treatment. After insecticide treatment, the egg card pieces were air dried at room temperature and kept in test tubes (45 ml capacity) closed with muslin cloth secured by a rubber band. The number of eggs parasitized, as indicated by black colouration of eggs, was observed at five days after treatment and the number of adults emerged from different treatments were also recorded at eight days after treatment. The longevity of adults and developmental period of parasitoids emerging from insecticide treated eggs of *T. chilonis* were also determined. The rate of parasitism and adult emergence were computed based on the formula

$$\text{Parasitisation per cent} = \frac{\text{Number of blackened eggs}}{\text{Total number of } \textit{Corcyra} \text{ eggs}} \times 100$$

$$\text{Per cent of adult emergence} = \frac{\text{Number of adults emerged}}{\text{Total number of blackened eggs}} \times 100$$

The effect of insecticides (E) on *T. chilonis* was also measured in terms of the reduction in parasitism (RP) and adult emergence i.e. parasitism viability (RPV) as compared to untreated control as per the formulae given below.

$$E\ (RP)\ \% = (1 - Pt / Pc) \times 100$$

$$E\ (RPV)\ \% = (1\text{-}Pt/ Pc) \times 100$$

where Pt is the rate of parasitism/ adult emergence in treatment

Pc is the rate of parasitism/ adult emergence in control

Based on the values of E, insecticides were categorised into following classes according to the IOBC standards (Hassan, 1992)

Class 1 – Harmless (E < 30 %)

Class 2 - Slightly harmful (50 – 79 % mortality)

Class 3 - Moderately harmful (80 –99 % mortality)

Class 4 - Harmful (> 99% mortality)

3.3.4.2. Exposure of larval stage of *T. chilonis* to insecticides

Trichocards with *Corcyra* eggs containing larval stages of *T. chilonis* (three days after parasitisation) were uniformly cut into four bits of size 5 x1 cm^2 and these card bits were treated by dipping them in the recommended concentration of selected insecticides for three seconds. Each treatment was replicated four times and an untreated check using water alone was maintained as control. The card bits were allowed to air dry at room temperature and each bit was kept in a test tube (45 ml), the mouth of which were secured tightly using

muslin cloth and rubber band. Total number of *Corcyra* eggs in each egg card bit was counted before giving the treatment. Observations on the number of eggs pupated (black plumpy eggs) and adults emerged were recorded. The adult longevity and developmental period from larvae to adults were also studied.

3.3.4.3. Exposure of pupal stage *T. chilonis* to insecticides

Corcyra cards (10 x 2 cm^2) with *Corcyra* eggs containing the *T. chilonis* pupae (five days after parasitisation) were cut into four bits (5x1cm^2). Total number of *Corcyra* eggs in each bit was counted and was treated by dipping in respective insecticide solution at recommended field concentration for three seconds. Insecticide residue was allowed to air dry at room temperature and the bits were then kept in test tubes (45 ml), the mouth of which were secured tightly using muslin cloth and rubber band. Four replications were kept for each treatment along with an untreated control. Adult emergence was observed three days after treatment and recorded. The longevity and developmental period of adults emerging from insecticide treated pupal stage of *T. chilonis* were also observed. The rate of parasitisation and adult emergence was worked out.

3.3.5. PARASITISATION EFFICIENCY OF ADULT PARASITOIDS EMERGING FROM INSECTICIDE TREATED LIFE STAGES

3.3.5.1. Parasitisation efficiency of second generation of *T. chilonis* as influenced by insecticide exposure of parental egg stage

The adults emerging from experiment 3.3.4.1. (exposure of egg stage of *T. chilonis* to insecticides) were collected and were further evaluated for their parasitisation efficiency and rate of adult emergence in second generation. UV irradiated *Corcyra* egg card bits (5 X 1 cm^2) were placed in test tubes of 45 ml capacity after counting total number of *Corcyra* eggs in each bit. Twenty adults emerging from experiment 3.3.4.1. were released in the test tube for parasitisation. Cotton swabbed with 50 % honey was kept inside the tube as adult food. As in 3.3.4.1, the same biological aspects of second generation of *T. chilonis* were studied.

3.3.5.2. Parasitisation efficiency of second generation as influenced by insecticide exposure of parental larval stage

The adults emerging from experiment 3.3.4.2, i.e. exposure of larval stage of *T. chilonis* to insecticides were further evaluated for their parasitisation efficiency and adult emergence. UV irradiated *Corcyra* egg card bits (5 X 1 cm^2) were placed in test tubes (45 ml capacity) after counting the *Corcyra* eggs in each bit. Twenty adults emerging from experiment 3.3.4.2. were released into the test tube containing *Corcyra* egg card bits for parasitisation. Cotton swabbed with 50 % honey was kept inside the tube as adult food. Number of eggs containing *T. chilonis* pupae i.e. blackened *Corcyra* eggs and number of adults emerged from these eggs were recorded. The longevity of the emerging adults was also observed. The rates of pupation and adult emergence were computed.

3.3.5.3. Impact of insecticide treatment of the pupal stage of *T. chilonis* on the parasitisation efficiency of second generation

The adults that had emerged from insecticide treated pupae from the above experiment (3.3.4.3.) were further studied for their parasitisation capacity. Twenty adults emerging from experiment 3.3.4.3. were introduced into a test tube (45 ml capacity) containing UV irradiated *Corcyra* egg card bits (5 X 1 cm^2) for parasitisation. Cotton swabbed with 50 % honey was kept inside the tube as adult food. Total number of *Corcyra* eggs in each bit was counted before adults were released. The parasitisation was assessed by counting black and plumpy eggs on the card bits. Number of adults emerged was also recorded.

3.3.6. PARASITISATION EFFICIENCY OF *T. chilonis* AS INFLUENCED BY INSECTICIDE TREATMENT ON FACTITIOUS HOST EGGS

Freshly laid *Corcyra* eggs collected from oviposition cage were cleaned and glued on paper cards (10 X 2 cm^2). The cards were irradiated with UV rays to prevent the hatching of *Corcyra* eggs and were cut into bits of size 5 X 1 cm^2. Number of *Corcyra* eggs in each bit was counted and the bits were treated with different insecticides by dipping them in respective insecticide solution prepared

at field recommended concentrations for three seconds. The insecticide residues on the card bits were air dried and the card bits were placed in test tube (45 ml capacity). Four replications were kept for each insecticide treatment along with an untreated control wherein the card was dipped in water only. Twenty adults of *T. chilonis* were released in each tube and the mouth of the test tube was secured tightly using muslin cloth and rubber band. Cotton swabbed with 50 % honey was kept inside the tube as adult food. The adults were allowed to oviposit on the insecticide treated eggs for three days. The number of eggs parasitized and adult emergence was observed. . The rate of pupation and adult emergence was worked out. The longevity of emerging adults was also recorded.

3.3.7. SIDE- EFFECTS OF INSECTICIDES TO *T. chilonis*

The side- effects of insecticides with respect to reduction in parasitisation, adult emergence and adult survival of *T. chilonis* as compared with the untreated control was studied for all the above experiments as given below.

3.3.7.1. Reduction in parasitism

The side- effects of insecticides in terms of reducing the parasitism of *T. chilonis* were worked out (Hassan *et al.*, 2000). It was determined by counting the number of parasitized eggs per card in insecticide treated and untreated control. The reduction in parasitism was calculated by the equation given below.

$$RP\ (\%) = (1 - Pt / Pc) \times 100$$

RP % - Percentage reduction in parasitism

Pt - Parasitism in insecticide treatment

Pc - Parasitism in untreated control

3.3.7.2. Reduction in parasitism viability

From the data on the number of adult parasitoids emerged from insecticide treated and untreated control in different experiments, the reduction in adult emergence or parasitism viability was computed (Manzoni *et al.*, 2007).

RPV (%) = (1 – AEt / AEc) X 100

RPV (%) – Percentage of reduction in parasitism viability (adult emergence)

AEt – Adult emergence in treatment

AEc – Adult emergence in control

3.3.7.3. Reduction in adult survival

The reduction in adult survival was computed from the data on the longevity of adults emerged in different treatments and untreated control of different experiments.

RAS % = (1 – ASt/ ASc) X 100

RAS % – Reduction in adult survival

ASt – Adult survival in treatment

ASc – Adult survival in control

The values of RP and RPV were calculated for each insecticide treatment in different experiments and the insecticides were classified for their side-effects as per the IOBC standards as follows.

Class 1 – Harmless (E < 30%)

Class 2 – Slightly harmful ($30 \leq E \leq 79\%$)

Class 3 – Moderately harmful ($80 \leq E \leq 99\%$)

Class 4 – Harmful (E>99%)

3.3.8. PERSISTENT TOXICITY OF INSECTICIDES TO *T. chilonis*

Insecticide solutions at their field recommended concentrations were prepared with water. Dry film residue technique of bioassay was adopted to study the persistent toxicity of insecticides to *T. chilonis*. Test tubes (45 ml size and internal surface area 80 cm^2) were coated evenly with 0.5 ml of different insecticides and allowed to air dry. Twenty freshly emerged *T. chilonis* adults were released into the insecticide treated test tubes at 1, 3, 5, 7 and 14 days after application of insecticides inside the test tube. Cotton swabbed with 50 % honey was kept inside the tube as adult food. Four replications were maintained for each treatment with a total of 80 adults per treatment. Observations on the mortality of wasps at 24 hours after release were recorded. Persistent toxicity of the insecticides was worked out as PT index (Sarup *et al.* 1970) where P is the period up to which the toxicity persisted and T is the average toxicity.

$$T = \frac{\text{Sum of percentage mortality}}{\text{Number of observations}} \times 100$$

The insecticide persistence was also computed by another method developed by members of the IOBC/WPRS Working Group for the evaluation of harmful activity duration (persistence) of insecticides against parasitoid under laboratory conditions (Sterk *et al.* 1999). Accordingly, insecticides were classified into categories based on the interval of time in which insecticide residues caused 30% mortality (the minimum level of toxicity as described for laboratory tests by IOBC) to *T. chilonis*. The insecticides were categorised based on persistence as:

A - Short lived (<5 days)

B - Slightly persistent (5–15 days)

C - Moderately persistent (16–30 days)

D - Persistent (>30 days)

RESULTS

4. RESULTS

The results obtained in the different experiments of the study entitled 'Impact of novel insecticides on *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae)' are presented in this chapter.

4.1. BIOLOGY STUDY OF *Trichogramma chilonis*

The life-cycle of *T.chilonis* was studied in the laboratory under the ambient conditions of 28 ± 2 ^{0}C and 68 ± 2 % relative humidity. The developmental stages *viz.* egg, larva and pupa of *T. chilonis* were observed and studied under the stereomicroscope. Egg stage was identified by the presence of amniotic fluid and liquid mass inside *Corcyra* eggs. The results of biology are summarised in Table 8. The egg stage lasted for a mean period of 2.7 days and after that *Corcyra* eggs were seen to form a condensed and hardened mass indicating the larval stage of *T. chilonis*. After a mean period of 2.2 days, the larvae of *T. chilonis* became pupae which were identified by the black colouration of *Corcyra* eggs. The mean period of pupal stage was 2.8 days. The total period required for the development from egg to adult *T. chilonis* was 7.3 days and the adults lived for about four days. Adults are identified by red coloured eyes and row of hairs present along the forewings (Plate 8).

4.2. ACUTE CONTACT TOXICITY OF INSECTICIDES TO *T. chilonis*

Direct contact toxicity of buprofezin 25 SC, chlorantraniliprole 18.5 SC, flubendiamide 480 SC, spinosad 2.5 SC and thiamethoxam 25 WG at their field recommended concentrations to adults of *T. chilonis* was studied by residue film bioassay. The lethal effects of different insecticides to *T. chilonis* was recorded in terms of adult mortality at 24 and 48 HAT after making necessary correction by Abbott's formula. Based on the adult mortality data, the insecticides were rated as harmless (< 30% mortality), slightly harmful (30 – 79 % mortality), moderately harmful (80- 90 % mortality) or harmful (> 90 % moratality).

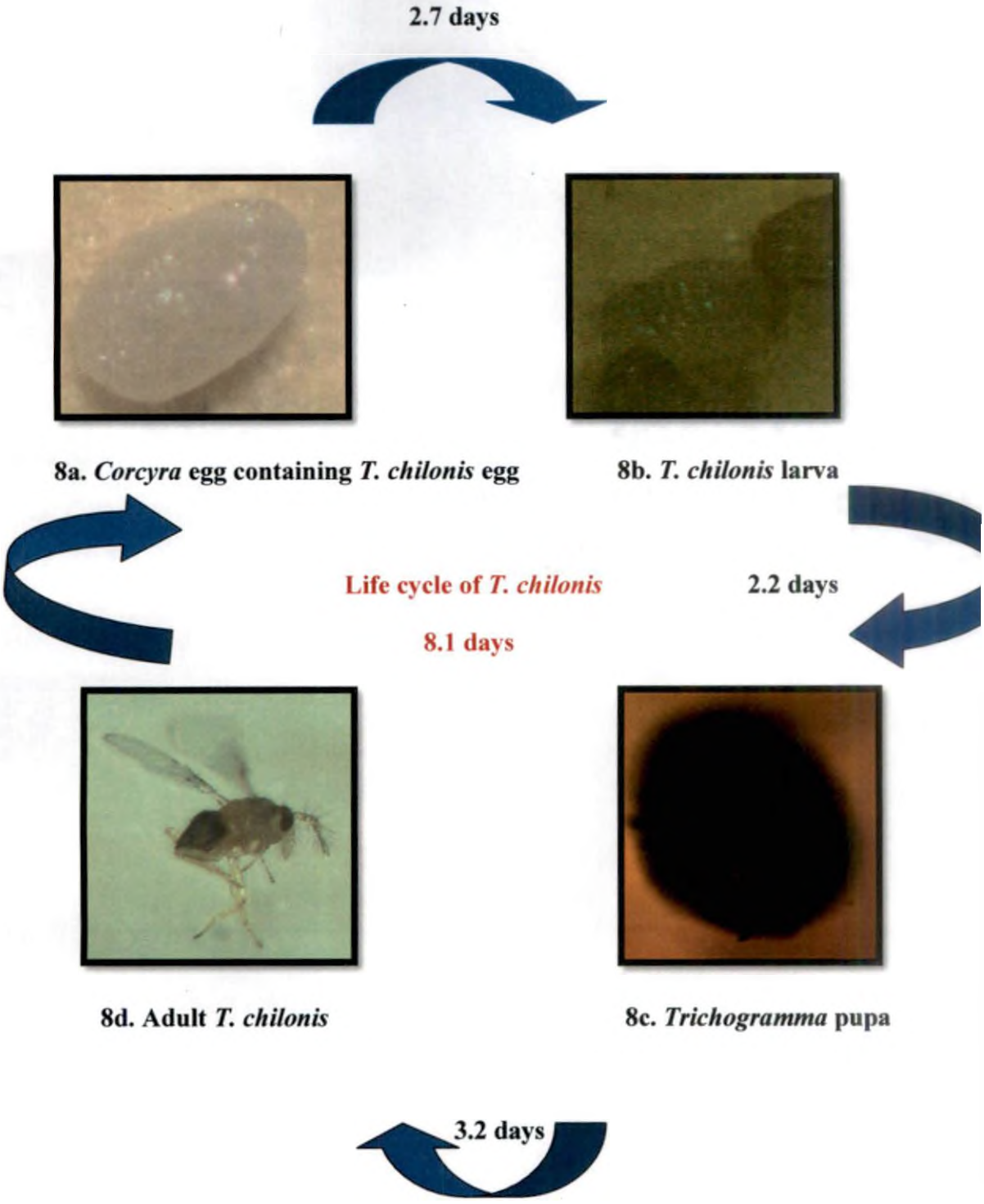


Plate 8. Life cycle of *T. chilonis*

Table 8. Developmental period of *T. chilonis*

Replication	Development period (days)			Total period (days)	Longevity (days)
	Egg	Larva	Pupa		
1	2.8	1.8	2.7	7.3	4.1
2	2.7	1.7	2.6	7.0	3.8
3	2.6	2.1	2.7	7.4	3.9
4	2.7	1.7	2.6	7.0	3.7
5	2.7	2.2	2.8	7.7	4.2
6	2.9	2.2	2.7	7.8	4.2
7	2.6	2.1	2.6	7.3	4.1
8	2.7	1.8	2.8	7.3	3.9
9	2.8	2.1	2.9	7.8	3.8
10	2.6	2.4	2.7	7.7	4.1
Mean	2.7 ± 0.09	2.2 ± 0.24	2.8 ± 0.09	7.4 ± 0.30	4.0 ± 0.18

All the tested insecticides at field recommended concentration were significantly toxic to *T. chilonis* (Table 9). The tested newer insecticides caused 50.00 to 68.42 % mortality to adults of *T. chilonis* at 24 HAT. Among the five insecticides, spinosad and thiamethoxam caused significantly higher mortality (68.42 %) to *T. chilonis* adults and they were on par in toxicity to *T. chilonis*. Chlorantraniliprole caused the lowest toxicity of 50.00% of mortality to the adults of *T. chilonis* whereas buprofezin and flubendiamide caused a mortality of 56.52 % and 55.26 %. Based on IOBC/WPRS safety scale classification, all the five tested insecticides were rated in the group of 'slightly harmful' to *T. chilonis* as they caused 50 – 79 % mortality at 24 HAT.

At 48 HAT, all the insecticides showed significant lethal effect on the adults of *T. chilonis*. The highest mortality was recorded with thiamethoxam (96.00 %) at 48 HAT followed by spinosad (94.44 %). Buprofezin caused a mortality of 93.00 %. Insecticides chlorantraniliprole and flubendiamide caused lowest mortality and were on par in causing lethal effect to the adults of *T. chilonis* at 48 HAT. Based on IOBC safety classification, all the five selected insecticides *viz.*, buprofezin, chlorantraniliprole, flubendiamide, spinosad, and thiamethoxam were rated as 'moderately harmful' (class 3) causing 80-99 % mortality to *T. chilonis*.

4.3. ESTIMATION OF LETHAL CONCENTRATION OF INSECTICIDES

4.3.1. Dosage- mortality response to *T.chilonis*

Different concentrations of insecticides were tested on the adults of *T. chilonis* to estimate the median lethal concentrations of insecticides. The median lethal concentrations of the five tested insecticides against *T. chilonis* are shown in Table 10. The LC_{50} values indicated that thiamethoxam was the most toxic insecticide (0.0011 mg a.i. l^{-1}) to the adults of *T. chilonis* followed by spinosad (0.08663 mg a.i.l^{-1}) flubendiamide (0.4731 mg a.i.l^{-1}), buprofezin (0.5623 mg a.i.l^{-1}) and chlorantraniliprole (1.3860 mg a.i.l^{-1}). Based on the LC_{50}

Table 9. Acute contact toxicity of newer insecticides to adults of *Trichogramma chilonis*

Insecticide formulation	Field recommended concentration (g a.i. ha^{-1})	24 hours after treatment (HAT)			48 hours after treatment (HAT)		
		Mortality (%)		Safety scale	Mortality (%)		Safety class
		Observed	*Corrected		Observed	*Corrected	
Buprofezin 25 SC	200	58.75	56.52 (7.5511)^{d}	2-Slightly harmful	93.75	93.06 (9.6726)^{c}	4-Moderately harmful
Chlorantraniliprole 18.5 SC	30	52.52	50.00 (7.1063)^{b}	2-Slightly harmful	92.50	91.67 (9.6005)^{b}	4-Moderately harmful
Flubendiamide 480 SC	25	57.50	55.26 (7.4672)^{c}	2-Slightly harmful	92.50	91.67 (9.6005)^{b}	4-Moderately harmful
Spinosad 2.5 SC	75	70.00	68.42 (8.3018)^{e}	2-Slightly harmful	95.00	94.44 (9.7437)^{d}	4-Moderately harmful
Thiamethoxam 25 WG	25	70.00	68.42 (8.3018)^{e}	2-Slightly harmful	96.50	96.11 (9.8290)^{e}	4-Moderately harmful
Untreated control	Distilled water	5.00	0 (0.0000)^{a}		10.00	0 (0.0000)^{a}	

*Corrections made as per Abbott's formula

Figures in parentheses are square root ($\sqrt{x} + 0.5$) transformed values

In a column, means superscripted by a common letter are not significantly different by DMRT (P=0.05)

Rating of insecticide (IOBC safety scale): 1 - Harmless (< 30 % mortality), 2 - Slightly harmful (30 – 79 % mortality), 3 - Moderately harmful (80 –99 % mortality), 4 - Harmful (> 99% mortality)

Table 10. Median lethal concentration of insecticides to *T. chilonis*

Insecticide formulation	LC_{50} (mg a.i l^{-1})	Fiducial limits		Regression equation Y = a+bx	χ^2 values	*Relative toxicity
		Lower	Upper			
Buprofezin 25 SC	0.5623	0.2187	1.4333	Y = 5.037 + 1.24x	4.33	2.46
Chlorantraniliprole 18.5 SC	1.3860	1.0577	1.8181	Y = 5.051 + 1.27 x	4.22	1
Flubendiamide 480 SC	0.4731	0.410	0.544	Y = 4.512 + 1.53x	5.96	2.93
Spinosad 2.5 SC	0.0863	0.0695	0.1786	Y= 5.0051 + 1.23x	7.52	78.75
Thiamethoxam 25 WG	0.0011	0.0010	0.0012	Y = 5.2034 + 4.36x	10.34	1260

All the log concentration probit mortality lines are a significant good fit to the data ($P< 0.05$)

* Insecticide with highest LC_{50} value (chlorantraniliprole) was taken as the unit for relative toxicit

value,thiamethoxam was the most toxic insecticide as it was 1260 times more toxic than chlorantraniliprole. The next toxic insecticide was spinosad which was 15.99 times more toxic than chlorantraniliprole. But flubendiamide and buprofezin showed only 2.93 and 2.46 times more toxicity than chlorantraniliprole. The ascending order of relative toxicity of different insecticides to *T. chilonis* is as follows:

Chlorantraniliprole < buprofezin < flubendiamide < spinosad < thiamethoxam

4.3.2. Comparision of field recommended concentration with LC_{50}, LC_{95} and LC_{99}

Based on the regression equation worked out in 4.3.1, LC_{95} and LC_{99} values were computed (Table 11) and it was found that thiamethoxam has the lowest LC_{95} value of 0.0097 mg a.i l^{-1} indicating its high toxicity to *T. chilonis* followed by spinosad (0.7552 mg a.i l^{-1}), flubendiamide (2.1544 mg a.i l^{-1}), buprofezin (3.9812 mg a.i l^{-1}), and chlorantraniliprole with highest value (5.6231 mg a.i l^{-1}). The concentration of thiamethoxam that killed 95% of *T. chilonis* was 0.0097 mg a.i l^{-1} while 50% kill was caused by 0.0011 mg a.i l^{-1}. Chlorantraniliprole killed 50% *T. chilonis* at a highest concentration of 1.3860 mg a.i l^{-1} while 95% parasitoids were killed at a concentration of 5.6231 mg a.i l^{-1}. The variability in susceptibility (range between LC_{50} and LC_{95}) was less in chloratraniliprole and flubendiamide as compared to higher value in thiamethoxam and spinosad. A concentration of about 8.7 to 8.8 times that of the LC_{50} of thiamethoxam and spinosad could kill 95% of *T. chilonis* whereas the LC_{95} values of chlorantraniliprole, flubendiamide and buprofezin were 4.06, 4.55 and 7.09 times their respective LC_{50} values. Chlorantraniliprole was rated as the safest with highest LC_{95} value followed by buprofezin < flubendiamide < spinosad < thiamethoxam.

Table 11. Comparision of LC $_{95}$ values with field recommended concentration of insecticides against *T. chilonis*

Insecticide formulation	Regression equation Y = a + bx	*FRC (mg a.i l^{-1})	LC_{50} (mg a.i l^{-1})	LC_{95} (mg a.i l^{-1})	LC_{99} (mg a.i l^{-1})	LC_{95}/LC_{50}
Buprofezin 25 SC	Y = 5.037 + 1.24x	400	0.5623	3.9812	8.3172	7.08
Chlorantraniliprole 18.5 SC	Y = 5.051 + 1.27 x	60	1.3860	5.6231	10.7122	4.06
Flubendiamide 480 SC	Y = 4.512 + 1.53x	50	0.4731	2.1544	3.9810	4.55
Spinosad 2.5 SC	Y= 5.0051 + 1.23x	150	0.0863	0.7552	0.8321	8.72
Thiamethoxam 25 WG	Y = 5.2034 + 4.36x	50	0.0011	0.0097	0.01238	8.82

All the log concentration probit mortality lines are a significant good fit to the data ($P< 0.05$)

*FRC – Field recommended concentration

LC_{95}:LC_{50} – Variability in susceptibility

4.3.3. Risk quotient of insecticides to *T. chilonis*

Insecticides were classified as harmless, slightly to moderately toxic and dangerous based on the risk quotient values calculated from the LC_{50} values at 24 HAT (mg a.i.l^{-1}) and recommended field concentration of insecticides (g a.i. ha^{-1}). Results (Table 12) indicated that thiamethoxam was dangerous to *T. chilonis* with a risk quotient value of 22727.27 whereas buprofezin and spinosad were in the category of 'slightly to moderately toxic' insecticides with risk quotient values of 355.68 and 896.06. Chlorantraniliprole was found to be safe insecticide as the risk quotient values less than 50 and hence rated as harmless insecticides. Based on the risk quotient values, insecticides can be arranged in the decreasing order of toxicity as

Thiamethoxam > Spinosad > Buprofezin >Flubendiamide > Chlorantraniliprole

22727.27 869.06 355.68 52.84 21.64

4.4. EFFECT OF INSECTICIDE ON THE PREIMAGINAL STAGES OF *T. chilonis*

Eggs, larvae and pupae of *T. chilonis* were treated separately with different insecticides and their effect on the development of *T. chilonis* was studied with respect to the rate of parasitisation (pupation rate), parasitism viability (adult emergence), developmental period and adult longevity. The impact of insecticides on second generation of *T. chilonis* in terms of parasitism and parasitism viability was also assessed. The effect of insecticides on the reduction in parasitism (RP) and reduction in parasitism viability (RPV) as compared to untreated control was worked out and accordingly the insecticides were classified as per IOBC.

Table 12. Risk quotient of insecticides to *T. chilonis*

Insecticide formulation	Field recommended concentration (g a.i. ha^{-1})	LC_{50} (mg a.i. l^{-1})	*Risk quotient	Insecticide Category (Value range)
Buprofezin 25 SC	200	0.5623	355.68	Slightly to moderately toxic (50- 2500)
Chlorantraniliprole 18.5 SC	30	1.3860	21.64	Harmless (< 50)
Flubendiamide 480 SC	25	0.4731	52.84	Harmless (< 50)
Spinosad 2.5 SC	75	0.0863	869.06	Slightly to moderately toxic (50- 2500)
Thiamethoxam 25 WG	25	0.0011	22727.27	Dangerous (>2500)

* Ratio between the field recommended dose (g a.i. ha^{-1}) and the LC_{50} of beneficial insect

4.4.1. Insecticide effect on egg stage

Insecticides were applied on host eggs containing *T. chilonis* in the egg stage and the results are given in Table 13. All the tested insecticides showed varying degrees of toxicity to the eggs of *T. chilonis* inside host eggs. All insecticides significantly reduced parasitism (pupation) when *T. chilonis* eggs were exposed to insecticides. The parasitisation ranged from 55.75 to 83.00 % in insecticide treatments as against 91.75 % in untreated control. The rate of parasitism was significantly lowest (55.75 %) in thiamethoxam. Spinosad caused 30.24 % reduction of pupation. Chlorantraniliprole reduced pupation by 9.50 % while buprofezin and flubendiamide were on par as they brought about 23.97 to 30.24 % reduction in pupation. Thiamethoxam significantly reduced parasitism by 39.23 % over control followed by spinosad with 30.24 % reduction in parasitism. Buprofezin and flubendiamide caused 23.97 % and 22.07 % reduction in parasitism and they were on par. Significantly higher parasitism (83.00%) was observed in chlorantraniliprole as it caused only 9.50 % reduction in parasitism.

The tested insecticides showed significant effect on the parasitism viability (adult emergence) also. Thiamethoxam caused lowest adult emergence that is significantly lower than all other insecticides. Adult emergence was reduced by 43.34 % in thiamethoxam while spinosad caused 34.67 % reduction as compared to control. Chlorantraniliprole showed highest adult emergence of 73.00 % with same reduction (9.50 %) in parasitism and adult emergence. Flubendiamide also showed the same reduction (22.96 %) in parasitism and adult emergence. Reduction in adult emergence was more than that of pupation in thiamethoxam and spinosad. Buprofezin caused more reduction in pupation than that of adult emergence. As per IOBC classification, thiamethoxam and spinosad were cateogarised as 'slightly harmful' (class 2, 30-79 % mortality). Buprofezin, flubendiamide and chlorantraniliprole were ranked as harmless (class 1, < 30 % mortality) for parasitism viability.

Table 13. Effect of insecticides on parasitism and parasitism viability of *T. chilonis* when treated at egg stage

Insecticide formulation	Field recommended concentration (g a.i.ha^{-1})	Parasitism (%)	*Parasitism reduction (RP %)	Parasitism viability (%)	*Emergence reduction (RPV %)	IOBC safety class
Buprofezin 25 SC	200	69.75 (0.9885)^{c}	23.97	65.75 (0.9457)^{c}	18.57	Harmless
Chlorantraniliprole 18.5 SC	30	83 (1.1463)^{b}	9.50	73.00 (1.0246)^{b}	9.50	Harmless
Flubendiamide 480 SC	25	71.5 (1.0077)^{c}	22.07	62.25 (0.9092)^{d}	22.90	Harmless
Spinosad 2.5 SC	75	64 (0.9274)^{d}	30.24	52.75 (0.8129)^{e}	34.67	Slightly harmful
Thiamethoxam 25 WG	50	55.75 (0.8431)^{e}	39.23	45.75 (0.7428)^{f}	43.34	Slightly harmful
Untreated control	Distilled water	91.75 (1.2798)^{a}	-	80.75 (1.1167)^{a}	-	

Figures in parentheses are arcsine transformed values arcsine($\sqrt{x}/100$)

In a column, means superscripted by a common letter are not significantly different by DMRT (P=0.05)

*RP – the effect (E) of insecticides on *T. chilonis* being measured as the reduction in parasitism

*RPV - the effect (E) of insecticides on *T. chilonis* being measured as the reduction in parasitism viability

IOBC safety class: Class 1 – Harmless (E > 30%), Class 2 – Slightly harmful (30% < E < 79%),

Class 3 – Moderately harmful (80 % < E < 99 %), Class 4 – Harmful (E > 99 %)

Results on the longevity of adults and the development period of immature stages emerging from insecticide treated eggs of *T. chilonis* are summarised in Table 14. Insecticide treatment on eggs indicated no significant effect on the development period from egg to larvae. Mean egg period was for 3.2 days both in treatment and control. Larval stage reached blackening stage i.e. pupal stage after 2.2 days (mean) in control whereas insecticides *viz.*, buprofezin, flubendiamide, spinosad and thiamethoxam took 4.1, 4.2, 4.5 and 4.5 days to reach pupal stage and chlorantraniliprole took only 3.1 days. Pupal period was also extended in all the treatments with respect to control. But chlorantraniliprole had no effect on pupation period. Adult emergence was observed in untreated control at 8.5 days after treatment but chlorantraniliprole and thiamethoxam had adult emergence at 9.9 and 12.9 days after treatment respectively. The longevity of emerged adults of *T. chilonis* in untreated check lasted for 4.1 days whereas all other treatments reduced the longevity from 1.7 to 2.1 days. The survival period of adults was reduced to half due to insecticide exposure.

Table 14. Developmental period and adult longevity of *T. chilonis* emerging from insecticide treated eggs

Insecticide formulation	Developmental period (days)			Total period for adult emergence	Longevity of adults (days)
	Egg to larvae	Larvae to pupae	Pupae to adult		
Buprofezin 25 SC	3.2 ± 0.29	4.1 ± 0.02	3.5 ± 0.12	10.8 ± 0.53	2.1 ± 0.06
Chlorantraniliprole 18.5 SC	3.1 ± 0.03	3.1 ± 0.51	3.2 ± 0.52	9.9 ± 0.25	2.1 ± 0.07
Flubendiamide 480 SC	3.2 ± 0.41	4.2 ± 0.02	3.8 ± 0.62	11.2 ± 0.76	1.9 ± 0.90
Spinosad 2.5 SC	3.2 ± 0.23	4.5 ± 0.29	4.1 ± 0.42	11.8 ±0.18	1.8 ± 0.60
Thiamethoxam 25 WG	3.2 ± 0.52	4.5 ± 0.47	4.9 ± 0.69	12.9 ± 0.23	1.7 ± 0.57
Untreated control	3.2 ± 0.04	2.2 ± 0.01	3.1 ± 0.01	8.5 ± 0.27	4.1 ± 0.27

4.4.2. Effect of insecticide treatment on larval stage

Larval stage of *T. chilonis* was exposed to insecticides and their impact on pupation and adult emergence was studied. All insecticide treatments to larval stage significantly reduced the rate of pupation (Table 15). Spinosad was found to be most toxic to the larval stage as indicated by lowest pupation (22.10 %) causing 75.09 % reduction in pupation. Buprofezin reduced pupation by 55.77 %, thiamethoxam (64.14 %) and flubendiamide (53.07 %). Chlorantraniliprole was found to be the safest as it caused only 22.81 % reduction in pupation.

Adult emergence was also significantly reduced due to larval exposure to insecticides (Table 15). Spinosad caused highest reduction of adult emergence (83.69 %) and was rated as the moderately harmful as per IOBC classification. Insecticides *viz,* buprofezin, flubendiamide and thiamethoxam were labelled as 'slightly harmful' (class 2) causing 30 – 79 % reduction in adult emergence. Chlorantraniliprole was the only harmless insecticide as indicated by lowest reduction in adult emergence (27.89 %). Spinosad was moderately harmful whereas other three *viz.*, buprofezin, flubendiamide and thiamethoxam were slighty harmful.

Table 15. Effect of insecticide on pupation and adult emergence of *T. chilonis* when treated at larval stage

Insecticide formulation	Field recommended concentration (g a.i.ha^{-1})	Pupation rate (%)	*Pupation reduction (E%)	Adult emergence (%)	*Emergence reduction (E%)	Safety class
Buprofezin 25 SC	200	39.25 (0.6770)^{d}	55.77	29.00 (0.5685)^{d}	63.63	Slightly harmful
Chlorantraniliprole 18.5 SC	30	68.50 (0.9749)^{b}	22.81	57.5 (0.8607)^{b}	27.89	Harmless
Flubendiamide 480 SC	25	41.65 (0.7015)^{c}	53.07	32.06 (0.6012)^{c}	59.79	Slightly harmful
Spinosad 2.5 SC	75	22.10 (0.4892)^{f}	75.09	13.00 (0.3683)^{f}	83.69	Moderately harmful
Thiamethoxam 25 WG	50	31.82 (0.5993)^{e}	64.14	25.5 (0.5293)^{e}	68.02	Slightly harmful
Untreated control	Distilled water	88.75 (1.2295)^{a}	-	79.75 (1.1043)^{a}	-	

Figures in parentheses are arcsine transformed values arcsine($\sqrt{x}/100$)

In a column, means superscripted by a common letter are not significantly different by DMRT (P=0.05)
*RP – the effect (E) of insecticides on *T. chilonis* being measured as the reduction in parasitism

*RPV - the effect (E) of insecticides on *T. chilonis* being measured as the reduction in parasitism viability

IOBC safety class: Class 1 – Harmless (E > 30%), Class 2 – Slightly harmful (30% < E < 79%),

Class 3 – Moderately harmful (80 % < E < 99 %), Class 4 – (E > 99 %)

Results on the longevity of adults emerging from insecticide treated larval stage of *T. chilonis* and development period of immature stages are presented in Table 16. The larval stage in untreated control proceeded to its darkened pupal stage at 2.1 days after treatment whereas all other treatments showed exceeded larval period. Pupal period lasted for approximately three days in all the treatments and untreated control. Adult emergence was recorded at 5.2 days after treatment in untreated control whereas all in other treatments the duration for adult emergence was increased. The survival period of adult *T. chilonis* emerging from untreated control was for 4.2 days whereas buprofezin, chlorantraniliprole and flubendiamide recorded an adult survival with 50.00 % reduction over control. Thiamethoxam and spinosad showed approximately 75.00 % adult survival reduction over control. Adults survived for only a day in spinosad and thiamethoxam.

Table 16. Development of *T. chilonis* from larva treated with insecticides

Insecticide formulation	Days taken for development of		Total days (larva to adult)	Adult longevity (days)
	Larva to pupa	Pupa to adult		
Buprofezin 25 SC	2.9 ± 0.05	3.5 ± 0.56	6.4 ± 0.23	2.1 ± 0.02
Chlorantraniliprole 18.5 SC	2.7 ± 0.01	3.2 ± 0.05	5.9 ± 0.21	2.1 ± 0.04
Flubendiamide 480 SC	3.2 ± 0.21	3.5 ± 0.21	6.7 ± 0.06	2.3 ± 0.10
Spinosad 2.5 SC	3.5 ± 0.41	3.7 ± 0.67	7.2 ± 0.12	1.5 ± 0.02
Thiamethoxam 25 WG	3.3 ± 0.51	3.8 ± 0.51	7.1 ± 0.21	1.4 ± 0.03
Untreated control	2.1 ± 0.01	3.1 ± 0.01	5.2 ± 0.02	4.2 ± 0.27

4.4.3. Insecticide treatment effect on pupal stage of *T. chilonis*

Adult emergence was significantly reduced when *T. chilonis* pupae were exposed to different insecticides (Table 17). Adult emergence was reduced from 5.55 to 50.92 % due to different insecticides applied on pupae. Thiamethoxam was most toxic to pupal stage as indicated by the lowest adult emergence (39.75 %). It caused 50.92 % reduction in adult emergence followed by spinosad (38.88 %) and buprofezin (23.75 %). Flubendiamide caused only 13.50 % reduction of adult emergence while 5.50 % reduction occurred with chlorantraniliprole. Among the five insecticides tested, thiamethoxam and spinosad were slightly harmful while the others were harmless to *T. chilonis* when pupa was exposed to insecticides.

Table 17. Effect of insecticide on adult emergence of *T. chilonis* when treated at pupal stage

Insecticide formulation	Field recommended concentration (g a.i. $ha^{-1)}$	Parasitism viability (%)	Emergence reduction (RPV %)	IOBC safety class
Buprofezin 25 SC	200	57.25 $(0.8582)^{d}$	23.75	Harmless
Chlorantraniliprole 18.5 SC	30	76.50 $(1.0647)^{b}$	5.55	Harmless
Flubendiamide 480 SC	25	70.00 $(0.9913)^{c}$	13.50	Harmless
Spinosad 2.5 SC	75	49.50 $(0.7804)^{e}$	38.88	Slightly harmful
Thiamethoxam 25 WG	25	39.75 $(0.6822)^{f}$	50.92	Slightly harmful
Untreated control	Distilled water	81 $(1.1198)^{a}$		

Figures in parenthesis are arcsine transformed values arcsine($\sqrt{x}/100$)

In a column, means superscripted by a common letter are not significantly different by DMRT (P=0.05)

*RP – the effect (E) of insecticides on *T. chilonis* being measured as the reduction in parasitism

*RPV - the effect (E) of insecticides on *T. chilonis* being measured as the reduction in parasitism viability

IOBC safety scale classification Class 1 – Harmless (E < 30%)

Class 2 – Slightly harmful (30% < E < 79%)

Class 3- Moderately harmful (80% < E < 99%)

Class 4 – Harmful (E > 99%)

Results on the pupation period and longevity of adults emerging from insecticide treated pupae of *T. chilonis* are presented in Table 18. Untreated check and all the selected insecticides except spinosad and thiamethoxam took three days for adult emergence from pupal stage. Adults emerged from all the insecticide treatments had a shorter longevity of approximately two days whereas in control adults had longevity of about four days. Thus there was a 50.00 % reduction in longevity over control in all the treatments.

Table 18. Effect of insecticides on the pupal period and adult longevity of *T. chilonis* when treated at pupal stage

Insecticide formulation	Pupal period (days)	Adult longevity (days)
Buprofezin 25 SC	3.1 ± 0.01	2.0 ± 0.03
Chlorantraniliprole 18.5 SC	3.0 ± 0.03	2.1 ± 0.02
Flubendiamide 480 SC	3.1 ± 0.01	1.9 ± 0.09
Spinosad 2.5 SC	3.9 ± 0.05	1.9 ± 0.10
Thiamethoxam 25 WG	3.9 ± 0.06	1.7 ± 0.07
Untreated control	3.1 ± 0.03	4.07 ± 0.21

4.5. PARASITIZATION EFFICIENCY OF ADULTS EMERGING FROM INSECTICIDE TREATED PRE-IMAGINAL STAGES

The parasitisation efficiency and the adult viability of adult *T. chilonis* emerging from insecticide treated eggs, larvae and pupae were further evaluated by giving them fresh *Corcyra* eggs for parasitisation.

4.5.1. Parasitization efficiency of adults emerging from insecticide treated egg stage

The results on the impact of insecticides on the rate of parasitisation (parasitism) and adult emergence (parasitism viability) of second generation of *T. chilonis* when first generation eggs were exposed to insecticides are given in Table 19. The rate of parasitisation of offsprings that emerged from insecticide treated parent eggs ranged from 76.25 to 86.00 % as against 89.75 % parasitisation in untreated control. All insecticides caused significant reduction of parasitism in second generation when the eggs of parents were exposed to insecticides. Thiamethoxam, spinosad and buprofezin caused maximum reduction in parasitisation (11 – 15 %) whereas chlorantraniliprole caused lowest reduction (4.17 %) of parasitisation in second generation. Flubendiamide caused 10.02 % reduction in parasitisation. However, thiamethoxam and spinosad were on par with regard to the reduction of parasitism in second generation. But they showed significantly different effect on adult emergence. Thiamethoxam caused highest reduction (36.13 %) in adult emergence followed by spinosad (23.05 %) and buprofezin (11.52 %). Chlorantraniliprole caused lowest reduction (2.49 %) of adult emergence.

Based on the IOBC safety classification, impact of insecticides in terms of reduction in parasitism and adult emergence (parasitism viability), as compared to untreated control, the insecticides were classified (Table 19) into different classes of safety. All the tested insecticides were found to be harmless ($E < 30$ %) except thiamethoxam as slightly harmful ($30 < E < 79$ %) to the second generation of *T. chilonis* when parental eggs were exposed to these insecticides.

Table 19. Rate of parasitism and adult emergence of second generation emerged from the insecticide treated parental eggs of *T. chilonis*

Insecticide formulation	Field recommended concentration (g a.i.ha^{-1})	Parasitism rate (%)	*Parasitism reduction (RP %)	Parasitism viability (%)	*Emergence reduction (RPV %)	IOBC safety class
Buprofezin 25 SC	200	79.5 (1.0537)^{d}	11.42	71 (1.0021)^{d}	11.52	Harmless
Chlorantraniliprole 18.5 SC	30	86.00 (1.1876)^{b}	4.17	78.25 (1.0856)^{b}	2.49	Harmless
Flubendiamide 480 SC	25	80.75 (1.1166)^{c}	10.02	74.25 (1.0386)^{c}	7.47	Harmless
Spinosad 2.5 SC	75	76.75 (1.0680)^{e}	14.48	61.75 (0.9040)^{e}	23.05	Harmless
Thiamethoxam 25 WG	50	76.25 (1.0622)^{e}	15.04	51.25 (0.7979)^{f}	36.13	Slightly harmful
Untreated control	Distilled water	89.75 (1.2452)^{a}		80.25 (1.1104)^{a}		

Figures in parentheses are arcsine transformed values arcsine ($\sqrt{x}/100$)

In a column, means superscripted by a common letter are not significantly different by DMRT (P=0.05)

*RP – the effect (E) of insecticides on *T. chilonis* being measured as the reduction in parasitism

*RPV - the effect (E) of insecticides on *T. chilonis* being measured as the reduction in parasitism viability

IOBC safety class: Class 1 – Harmless (E > 30%), Class 2 – Slightly harmful (30% < E < 79%),

Class 3 – Moderately harmful (80 % < E < 99 %), Class 4 – (E > 99 %)

The longevity of adult parasitoids emerging from insecticide treated eggs of *T. chionis* is summarised in Table 20. There was no much significant difference in the longevity of second generation *T. chilonis* adults that had emerged from insecticide treated eggs. Longevity of adults emerging from buprofezin, chlorantraniliprole, flubendiamide and spinosad treatment were approximately four days whereas thiamethoxam treatment has reduced the longevity of second generation adults of *T. chilonis* to 2.3 days.

Table 20. Longevity of second generation *T. chilonis* emerging from insecticide treated parental eggs

Insecticide formulations	Longevity in days
Buprofezin 25 SC	3.53 ± 0.07
Chlorantraniliprole 18.5 SC	3.71 ± 0.02
Flubendiamide 480 SC	3.58 ± 0.05
Spinosad 2.5 SC	3.49 ± 0.09
Thiamethoxam 25 WG	2.32 ± 0.25
Untreated control	3.78 ± 0.58

4.5.2. Effect on second generation when parental larval stage was treated with insecticides

The results on rate of pupation and adult emergence of second generation consequent to insecticide exposure in the larval stage of parents are summarized in Table 21. All the insecticide treatments significantly reduced the pupation of *T. chilonis* adults. The rate of pupation of adults ranged from 39.50 % to 62.50 % in different insecticide treatments as against 76.50 % in untreated control. Thiamethoxam was found to be most toxic to the second generation when larval stage of parents was given the exposure as it showed 39.50 % pupation and 34.25 % of adult emergence. It was followed by spinosad, buprofezin, flubendiamide and chlorantraniliprole. Chlorantraniliprole was found to be the safest with 62.00 % pupation and 18.30 % reduction in pupation over control. All insecticides showed significantly different effects on pupation.

The rate of adult emergence of second generation adults emerging from insecticide treated larval stages of parents is summarized in Table 21. Highest percentage of adult emergence was recorded in chlorantraniliprole (56.00%) followed by flubendiamide (53.25%), buprofezin (44.25 %), spinosad (40.50%) and thiamethoxam (34.25%). Thiamethoxam caused lowest adult emergence (56.08 % reduction). Based on IOBC safety classification, chlorantraniliprole and flubendiamide were rated as 'harmless'. Buprofezin, spinosad and thiamethoxam were found to be 'slightly harmful' to second generation also when first generation larvae were exposed to insecticides.

The longevity of adult *T. chilonis* emerging from insecticide treated larva is summarised in Table 22. The mean longevity of adults emerging from untreated control was 3.78 days whereas the longevity of adults emerging from treatment with buprofezin, chlorantraniliprole and flubendiamide was approximately three days. Spinosad and thiamethoxam reduced longevity to 2.78 and 2.61 days.

Table 21. Rate of pupation and adult emergence of the second generation of *T. chilonis* emerged from the insecticide treated larval stage of parents

Insecticide formulation	Field recommended concentration (g a.i.ha^{-1})	Parasitism rate (%)	*Parasitism reduction (RP %)	Parasitism viability (%)	*Emergence reduction (RPV %)	IOBC Safety class
Buprofezin 25 SC	200	55.69 (0.8425)^{d}	27.20	44.25 (0.7278)^{d}	43.26	Slightly harmful
Chlorantraniliprole 18.5 SC	30	62.50 (0.9117)^{b}	18.30	56.00 (0.8456)^{b}	28.20	Harmless
Flubendiamide 480 SC	25	59.75 (0.8835)^{c}	21.88	53.25 (0.8355)^{c}	31.73	Slightly harmful
Spinosad 2.5 SC	75	48.25 (0.7679)^{e}	36.92	40.50 (0.6898)^{e}	48.07	Slightly harmful
Thiamethoxam 25 WG	50	39.50 (0.6796)^{f}	48.36	34.25 (0.6251)^{f}	56.08	Slightly harmful
Untreated control	Distilled water	76.50 (1.0648)^{a}		78 (1.0827)^{a}		

Figures in parentheses are arcsine transformed values arcsine($\sqrt{x}/100$)

In a column, means superscripted by a common letter are not significantly different by DMRT (P=0.05)

*RP – the effect (E) of insecticides on *T. chilonis* being measured as the reduction in parasitism

*RPV - the effect (E) of insecticides on *T. chilonis* being measured as the reduction in parasitism viability

IOBC safety class: Class 1 – Harmless (E > 30%), Class 2 – Slightly harmful (30% < E < 79%),

Class 3 – Moderately harmful (80 % < E < 99 %), Class 4 – (E > 99 %)

Table 22. Longevity of second generation adults emerging from insecticide treated larvae of *T. chilonis*

Insecticide formulation	Adult longevity (days)
Buprofezin 25 SC	3.15 ±0.29
Chlorantraniliprole 18.5 SC	3.51±0.02
Flubendiamide 480 SC	3.22 ± 0.39
Spinosad 2.5 SC	2.78 ± 0.06
Thiamethoxam 25 WG	2.61±0.01
Untreated control	3.78± 0.58

4.5.3. Effect on second generation when pupal stage was treated with insecticides

Results on the rate of pupation and adult emergence of the second generation when the pupal stage of *T. chilonis* was exposed to insecticides are summarized in Table 23. There was no significant difference between the treatments in the rate of pupation and all the treatments on par with the control indicating no effect on pupation. The reduction in pupation over control ranged between 0.33% to 2.36%.

Adult emergence showed reduction with respect to control ranging from 1.80 % to 3.00 %. All the tested insecticides buprofezin, chlorantraniliprole, flubendiamide, spinosad and thiamethoxam were on par and were rated as harmless. Thiamethoxam and spinosad exhibited highest reduction in adult emergence (3.00%) and were rated as harmless indicating that all the tested insecticides were harmless to second generation.

Table.23. Rate of pupation and adult emergence of the second generation of *T. chilonis* emerged from the insecticide treated pupal stage of parents

Insecticides	Field recommended concentration (g a.i.ha^{-1})	Parasitism rate (%)	*Parasitism reduction (RP%)	Parasitism viability (%)	*Emergence reduction (RPV %)	IOBC Safety class
Buprofezin 25 SC	200	73.25 (1.0247)^{a}	1.00	80.25 (1.0979)^{b}	2.30	Harmless
Chlorantraniliprole 18.5 SC	30	73.75 (1.0334)^{a}	0.33	80.75 (1.1169)^{b}	1.80	Harmless
Flubendiamide 480 SC	25	73.25 (1.0273)^{a}	1.00	80.75 (1.1167)^{b}	1.80	Harmless
Spinosad 2.5 SC	75	72.50 (1.0188)^{a}	2.00	79.75 (1.1041)^{b}	3.00	Harmless
Thiamethoxam 25 WG	25	72.25 (1.0160)^{a}	2.36	79.75 (1.1041)^{b}	3.00	Harmless
Untreated control	Distilled water	74 (1.0358)^{a}		82.25 (1.1363)^{a}		

Figures in parentheses are arcsine transformed values arcsine($\sqrt{x}/100$)

In a column, means superscripted by a common letter are not significantly different by DMRT (P=0.05)

*RP – the effect (E) of insecticides on *T. chilonis* being measured as the reduction in parasitism

*RPV - the effect (E) of insecticides on *T. chilonis* being measured as the reduction in parasitism viability

IOBC Safety class: Class 1 – Harmless (E > 30%), Class 2 – Slightly harmful (30% < E < 79 %)

Class 3 - Moderately harmful (80% < E < 99%), Class 4 – Harmful (E > 99%)

The longevity of adult parasitoids emerging from insecticide treated pupae is summarised in Table 24. The results highlight the fact that there is no much difference in the longevity of adults due to various treatments with respect to control.

Table 24. Longevity of second generation adults emerging from insecticide treated pupae of *T. chilonis*

Insecticide formulation	Adult longevity (days)
Buprofezin 25 SC	3.59 ± 0.15
Chlorantraniliprole 18.5 SC	3.67 ± 0.06
Flubendiamide 480 SC	3.48 ± 0.21
Spinosad 2.5 SC	3.39 ± 0.21
Thiamethoxam 25 WG	3.16 ± 0.14
Untreated control	3.78 ± 0.58

4.6. PARASITISATION OF *T. chilonis* TO INSECTICIDE TREATED *Corcyra* EGGS

The results are summarised in Table 25. All the treatments were significantly different in parasitism from control. Insecticide caused significant difference in parasitism and adult emergence of *T. chilonis* when the factitious host eggs were exposed. Parasitisation was highest (80.50 %) on *Corcyra* eggs without any insecticide treatment followed by chlorantraniliprole (60.75%), buprofezin (54.50%), flubendiamide (50.50 %), spinosad (34.25 %) and thiamethoxam (27.75 %). Chlorantraniliprole caused lowest reduction of parasitism (24.53 %) and adult emergence (33.88 %). Parasitism and adult emergence were most affected by thiamethoxam as it caused highest reduction in parasitism (65.52 %) and adult emergence (72.20 %) followed by spinosad. Insecticide treatment of *Corcyra* eggs caused a higher reduction in adult emergence than that in parasitism.

Table 25. Parasitisation of *T. chilonis* on insecticide treated *Corcyra* eggs

Insecticide formulation	Field recommended concentration (g a.i.ha^{-1})	Parasitism rate (%)	*Parasitism reduction (RP %)	Parasitism viability (%)	*Emergence reduction (RPV %)	IOBC Safety class
Buprofezin 25 SC	200	54.50 (0.5764)^{c}	32.29	46.75 (0.4865)^{c}	48.05	Slightly harmful
Chlorantraniliprole 18.5 SC	30	60.75 (0.6529)^{b}	24.53	59.50 (0.6373)^{b}	33.88	Slightly harmful
Flubendiamide 480 SC	25	50.50 (0.5294)^{d}	37.26	48.56 (0.5064)^{c}	46.04	Slightly harmful
Spinosad 2.5 SC	75	34.25 (0.3498)^{e}	57.45	33.00 (0.3363)^{d}	63.33	Slightly harmful
Thiamethoxam 25 WG	50	27.75 (0.2812)^{f}	65.52	25 (0.2527)^{e}	72.22	Slightly harmful
Untreated control	Distilled water	80.50 (0.9359)^{a}	-	90 (1.12)^{a}		

Figures in parentheses are arcsine transformed values arcsine($\sqrt{x}/100$)

In a column, means superscripted by a common letter are not significantly different by DMRT (P=0.05)

*RP – the effect (E) of insecticides on *T. chilonis* being measured as the reduction in parasitism

*RPV - the effect (E) of insecticides on *T. chilonis* being measured as the reduction in parasitism viability

IOBC Safety class: Class 1 – Harmless (E > 30%), Class 2 – Slightly harmful (30% < E < 79 %)

Class 3 - Moderately harmful (80% < E < 99%), Class 4 – Harmful (E > 99%)

Insecticides showed significant reduction in adult emergence over control. Adult emergence was significantly lower in thiamethoxam (25.00 %) followed by spinosad (33.00 %) and chlorantraniliprole (59.50 %). Buprofezin and flubendiamide were on par. Based on IOBC classification all the tested insecticides were rated as slightly harmful with 30 to 79 % mortality. All the tested insecticides were 'slightly harmful' to parasitism and parasitism viability of *T. chilonis* when host eggs were exposed to insecticides as they caused 30 -79 % reduction in parasitism and adult emergence.

4.6. RESIDUAL TOXICITY OF INSECTICIDES TO *T. chilonis*

4.6.1. Persistent toxicity of insecticides against *T. chilonis*

The results on the persistent toxicity of new generation inscecticides *viz.*, buprofezin, chlorantraniliprole, flubendiamide, spinosad and thiamethoxam to *T. chilonis* are given in Table 26. Thiamethoxam showed highest persistence upto 21 days after treatment with a PT value of 927.36 followed by spinosad and flubendiamide with a PT value of 647.5 and 373.33. Spinosad and thiamethoxam caused 5 % mortality even at 21 days after treatment. The lowest PT value (93.33) was shown by chlorantraniliprole showing the least persistence. The lowest average toxicity was indicated by chlorantraniliprole (13.33) and period (7 days) of toxicity persistence. The persistent toxicity of insecticides based on their PT index value can be ranked as

Thiamethoxam > Spinosad > Flubendiamide > Buprofezin > Chlorantraniliprole

(927.36) (647.5) (373.33) (280) (93.33)

Results regarding the effects of insecticide persistency on *T. chilonis* are summarised in Table 27. The residual toxicity of insecticides was assessed by studying the interval of time during which their residues caused the maximum level of 30 % mortality to adults of *T. chilonis.* Accordingly, the insecticides were rated as short lived (<5 days), slightly persistent (5 – 15 days), moderately persistent (16-30 days) and persistent (> 30 days).

Buprofezin residues were short lived as it caused 30 % mortality within one to three days and hence buprofezin was labelled for short lived persistence. Chlorantraniliprole and flubendiamide were also rated as short lived as chlorantraniliprole caused maximum 30 % mortality between one and three days after application. Flubendiamide residues caused 30 % mortality at five days after treatment. Spinosad caused 30 % minimum mortality at five to seven days and hence it was labelled as slightly persistent. Thiamethoxam caused 30 % mortality at 21 DAT and hence it was rated as moderately persistent.

Table 26. Mortality of *T.chilonis* exposed to insecticide treated test tubes after 1, 3, 5, 7, 14 and 21 days

Insecticide formulation	Percentage mortality at different days after treatment(DAT)						P	T	PT	ORE
	1	3	5	7	14	21				
Buprofezin 25 SC	50	30	20	15	5	0	14	20	280	4
Chlorantraniliprole 18.5 SC	45	20	10	5	0	0	7	13.33	93.33	5
Flubendiamide 480 SC	65	40	30	15	10	0	14	26.66	373.33	3
Spinosad 2.5 SC	60	55	35	20	10	5	21	30.8	647.5	2
Thiamethoxam 25 WG	50	75	65	40	30	5	21	44.16	927.36	1
Untreated control	0	0	0	0	0	0	0	0		

P – Period upto which the toxicity is persisted (days)

T – Average toxicity

PT –Persistent toxicity index

ORE – Order of relative efficacy

Table 27. Mortality (%) of *T. chilonis* adults after exposure to 1, 2, 5, 7, 14, 21 and 30 days old insecticide residues in laboratory conditions

Insecticide formulation	Percentage mortality at different days after treatment(DAT)							Class
	1	3	5	7	14	21	30	
Buprofezin 25 SC	50	30	20	15	5	0	0	A
Chlorantraniliprole 18.5 SC	45	20	10	5	0	0	0	A
Flubendiamide 480 SC	65	40	30	15	10	0	0	A
Spinosad 2.5 SC	60	55	35	20	10	5	0	B
Thiamethoxam 25 WG	80	75	65	40	35	30	5	C
Untreated control	0	0	0	0	0	0	0	

IOBC persistency ranking;

A - Short lived (< 5 days)

B – Slightly persistent (5 – 15 days)

C – Moderately persistent (16 – 30 days)

D – Persistent (> 30 days)

DISCUSSION

5. DISCUSSION

The new generation insecticides with a high potency to kill target insect pests may also destroy the hymenopteran Trichogrammatid parasitoid *Trichogramma chilonis* because of their common physiology. Therefore, five newer insecticides *viz.*, buprofezin 25 SC, chlorantraniliprole 18.5 SC, flubendiamide 480 SC, spinosad 2.5 SC and thiamethoxam 25 WG were evaluated for their acute contact toxicity and sub-lethal effects on *T. chilonis*. The results obtained on the parasitoid biology under the ambient conditions, acute contact toxicity of insecticides to adult parasitoids, dosage – mortality response, effect of insecticides on pre-imaginal stages, parasitism as influenced by insecticide exposure on the factitious host, side-effects of insecticide treatments and persistent toxicity of insecticides to *T. chilonis* are discussed hereunder to elucidate the observations and findings.

5.1. BIOLOGY OF *T. chilonis*

The results (Table 8) on the life cycle of *T. chilonis* revealed that *T. chilonis* completed its development within a mean period of 7.3 days at an ambient temperature of 32 ^{0} C and 75 % relative humidity in the laboratory. The parasitoid eggs hatched out in about 2.7 days and larvae developed through three instars within 2.2 days. Larvae then transformed to the inactive pupal stage within 2.8 days. After a mean period of 2.8 days, the adult wasps emerged from the pupae by chewing a circular hole in the egg shell of *Corcyra* egg. During the 3rd instar (three to four days after the host egg was parasitized) dark melanin granules were seen deposited on the inner surface of the egg chorion, causing the host egg to turn black. The longevity of emerging adults was for four days. These results are in agreement with the findings of Strand (1986) and Pinto *et al.* (1994) who reported 2.5 days for egg period, 3.5 days for larval period and 4.5 days for pupal period at 24 ^{0}C and 70 % relative humidity. At 68 to 81^{0}F temperature and relative humidity of 60 %, *Trichogramma* species were reported to have an egg period of two days, larval period lasted for ten days and pupal period for 2.5 days

with an overall adult longevity of 7.75 days. Total life span may be for 7-7.5 days depending upon temperature, relative humidity and species of host parasitized. At 20^0C and 70 % relative humidity, *Trichogramma* had longevity of 11 days with a developmental period of 17.3 days to complete its life cycle whereas at 35^0C and 70 % relative humidity a life span of seven days and longevity of 2.3 days were observed (Nadeem *et al.*, 2010).

The adult *Trichogramma* wasp uses chemical and visual cues to locate a host. The chemical cues, called kairomones, are on the host moth scales left near the egg by the female moth during oviposition (Nordlund *et al.*, 1981). Egg shape and colour also serve as visual cues to the wasp (Ruberson *et al.*, 1993). Once an adult wasp locates the egg, it drills a hole through the chorion (egg shell) and inserts two to three eggs into the host egg. The internal pressure of the host egg forces a small drop of yolk out of the oviposition hole. Females feed on this yolk, which increases their longevity. Under laboratory conditions, an adult wasp parasitizes one to ten host eggs per day or from 10 to 190 during the life. Adults provided with honey and fresh host eggs to feed survived for 11 days, while females receiving only honey lived for three days (Ruberson and Kring, 1993). According to our results, the longevity of adults of *T. chilonis* was four days with honey. But Suh *et al.* (2000) reported an average adult life span of 24 days. The yolk and embryo of the parasitized host egg are digested before the *Trichogramma* egg hatches. Venom injected by the female at the time of oviposition is believed to cause the predigestion of the egg's contents. The black layer inside the chorion and the exit hole are evidence of parasitism by *Trichogramma.*

5.2. ACUTE CONTACT TOXICITY OF INSECTICIDES TO ADULTS OF *T. chilonis*

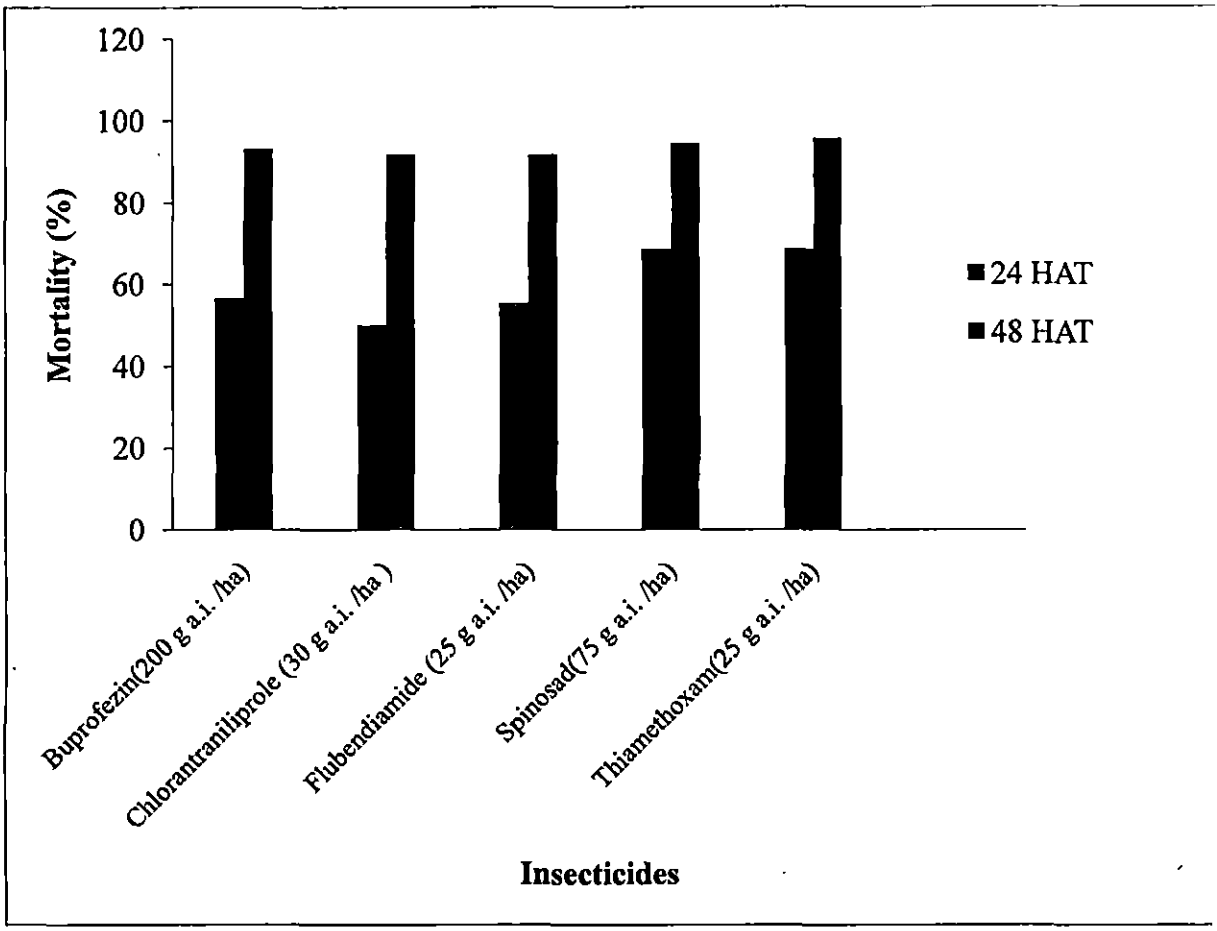
The direct contact toxicity of five insecticides *viz.* buprofezin 25 SC, chlorantraniliprole 18.5 SC, flubendiamide 480 SC, spinosad 2.5 SC and thiamethoxam 25 WG manifested as lethal effects to the adults of *T. chilonis* were

evaluated at field recommended concentrations by residue film bioassay. Results (Table 9, Fig.1) indicated that all the tested insecticides at field recommended concentrations were significantly toxic to *T. chilonis* by causing 50.00% to 68.42% mortality to adults at 24 HAT. This finding corroborates with Suh *et al.* (2000) who confirmed a higher susceptibility of *Trichogramma* adults to insecticides. Among the five insecticides evaluated for acute contact toxicity to *T. chilonis* adults, chlorantraniliprole was the safest one as it caused lowest mortality (50.00 %) to adults at 24 HAT. The present finding is in conformity with Preetha *et al.* (2009) who reported least susceptibility of *T. chilonis* to chlorantraniliprole at field recommended concentration and hence it can be successfully included in IPM programme. Results of the study also revealed that both thiamethoxam and spinosad were equal in toxicity by causing significantly higher mortality of 68.42% to *T. chilonis* adults at 24 HAT. All the tested insecticides caused significant mortality of 50 – 68.42 % at 24 hours after treatment and were thus rated as 'slightly harmful' as per International Organisation for Biological Control (IOBC) safety scale classification (Hassan, 1992). But at 48 hours after treatment all the tested insecticides caused significant mortality of 80-99 % and were thus rated as 'moderately harmful'

Neonicotinoid insecticides were introduced in the early 1990's and are one of the most important newer chemical groups used to control sucking pests (Nasreen *et al.*, 2001). Recommended concentration of thiamethoxam was found to be 'moderately harmful' to *T. chilonis* (Nasreen *et al.*, 2004). Thiamethoxam was reported to have high acute contact and oral toxicity to the egg parasitoid *Anagrus nilaparvatae* (Pang et Wang) of rice plant hopper (Wang *et al.*, 2008). Thiamethoxam was comparatively harmful than other neonicotinoids to *T. japonicum* (Zhou *et al.*, 2012).

Spinosad caused significantly higher mortality of 68.42 % at 24 HAT and 94.44 % at 48 HAT to the adults of *T. chilonis*. The present finding of significantly higher mortality to *T. chilonis* by spinosad is in conformity with Saljoqi *et al.* (2012) and Suh *et al.* (2000) who reported higher toxicity of

Fig. 1. Acute contact toxicity of insecticides to *T. chilonis* adults at 24 and 48 HAT



spinosad to adults of *T. exiguam*. Spinosad, a mixture of A and D spinosyns is a macrocyclic lactone produced by actinomycete *Saccharopolyspora spinosa*. This insecticide acts primarily as a stomach poison with some contact activity (Bret *et al.*, 1997). Although it is classified as an environmentally and toxicologically reduced risk insecticide and less harmful to predators but hymenopteran parasitoids are significantly more susceptible to its effects (William *et al.*, 2003). Although the negative effects of spinosad have been reported against *T. pretiosum* (Rubersan and Tillman, 1999) and other parasitoids, its mode of action on parasitoid physiology remains unclear (Carmo *et al.*, 2010).

Chlorantraniliprole exhibited a lowest mortality of 50.00 % at 24 HAT. This result is in line with the findings of Preetha *et al.* (2009) who reported that *T. chilonis* was least susceptible to the field recommended concentration of chlorantraniliprole and thus can be successfully implemented in integrated pest managememnt (IPM) programmes.

The anti-moulting insecticide buprofezin at field recommended concentration showed lower acute contact toxicity (56.52 % mortality) to *T. chilonis* at 24 HAT. The lower toxicity of buprofezin to *Trichogramma* has been documented by Garrido *et al.* (1985), Smith and Papacek (1990), Gerling and Sinai (1994), Hoddle *et al.* (2001) and Wang *et al.* (2008). Insect growth regulators (IGRs) were reported to be highly toxic to target pest, but relatively safe to natural enemies. Buprofezin showed minimal impact on hymenopteran parasitoid (Zhou *et al.*, 2003). The IGR chemicals inhibit chitin synthesis and kill target insects slowly by disturbing exoskeleton formation after moulting (Reynolds, 1987). Acting at specific point in the course of moulting, IGR chemical may show some selectivity since the hormones that trigger this physiological process differ across insect taxonomic orders. Since pests and their natural enemies commonly differ in their taxonomic orders, the IGRs are generally able to specifically kill the target pest while preserving the beneficial counterpart (Carmo *et al.*, 2010).

5.3. DOSAGE-MORTALITY RESPONSE OF INSECTICIDES TO *T. chilonis*

Results (Table 10) on the dosage - mortality response of insecticides revealed that thiamethoxam had the lowest LC_{50} value of 0.0011 mg a.i. l^{-1} to the adults of *T. chilonis* indicating its highest toxicity whereas chlorantraniliprole showed lowest toxicity with the highest LC_{50} value of 1.3860 mg a.i. l^{-1}.

The present finding of high toxicity of thiamethoxam to *T. chilonis* is in close agreement with the findings of Preetha *et al.* (2009) who reported a lowest LC_{50} value of 0.0014 mg a.i. l^{-1} for thiamethoxam against *T. chilonis*. The high susceptibility of *Trichogramma* species to thiamethoxam has been demonstrated in earlier studies as to *T. pretiosum* Riley (William and Price, 2004), *T. exiguam* Pinto and Plater (Lee, 2006) and *T. platneri* Nagarkatti (Brunner *et al.*, 2001).

The safety of chlorantraniliprole to *T. chilonis* has been documented by Sharma (2005) and Preetha *et al.* (2009) who concluded that chlorantraniliprole is the least toxic chemical to *T. chilonis* with LC_{50} value of 1.9530 mg a.i. l^{-1}. According to the reports of Sharma (2005), chlorantraniliprole caused minimal impact (0 – 30 % mortality) to *T. chilonis*.

Relative toxicity of insecticides was worked out taking the LC_{50} of chlorantraniliprole as unity (Table 10). Thiamethoxam was found to be 1260 times more toxic than chlorantraniliprole indicating its highest toxicity. Preetha *et al.* (2009) reported that thiamethoxam was 1395 times more toxic than chlorantraniliprole.

Risk quotient is used to assess the risk of insecticides sprayed to parasitoids. It is based on hazard ratio (Feltan *et al.*, 1986) and was later termed as risk quotient (Hassan *et al.*, 1998). Risk quotient is the ratio between the field recommended dose and the LC_{50} of insecticides to the beneficial insect. The field recommended concentration also has to be considered while studying the safety of insecticides to parasitoids as they are also subjected to the same concentration under field conditions. This is the concentration which the parasitoid also gets under field concentration. Risk quotient was worked out for the five insecticides

and the results (Table 12) indicated that thiamethoxam of neonicotinoid group was the only 'dangerous' insecticide to *T. chilonis*. whereas chlorantraniliprole and flubendiamide (anthranilic diamide and phthalic acid diamide groups) were harmless. Thiamethoxam showed highest risk quotient value (22727.27) whereas spinosad and buprofezin were moderately toxic to *T. chilonis*. The present result is in agreement with Preetha *et al.* (2009) who also observed thiamethoxam as 'dangerous' and chlorantraniliprole as harmless to *T. chilonis* based on risk-quotient categorisation. Spinosad was found to be lower in toxicity order than thiamethoxam to *T. chilonis*. This finding is in agreement with William and Price (2003) who reported spinosad to be lower in toxicity than thiamethoxam to *T. pretiosum*.

A comparison of LC_{95} values with that of field recommended concentration of insecticides (Table 11) showed that the field recommended concentration of thiamethoxam is 5154.6 times higher than the dose required to kill 95 % population of *T. chilonis* adults indicating the high susceptibility to egg parasitoids. However, in chlorantraniliprole the field recommended concentration is 10 times higher than the LC_{95} values indicating its comparative safety to *T. chilonis*. This finding is in agreement with the reports of Preetha *et al.* (2009) who observed chlorantraniliprole as harmless to *T. chilonis* by comparing its LC_{95} value with its field recommended concentration.

5.4. EFFECT OF INSECTICIDES ON THE PRE- IMAGINAL STAGES OF *T. chilonis*

Insecticides were applied on the pre-imaginal stages (egg, larva and pupa) of *T. chilonis* and studied the detrimental effect of insecticides on the development of the immature stages of the parasitoid.

5.4.1. Insecticide effect on egg stage of *T. chilonis*

All the insecticides showed varying degrees of toxicity to the development of *T. chilonis* when eggs were exposed to insecticides (Table 13). The insecticides significantly affected the pupation (parasitism) and adult emergence of *T. chilonis* as illustrated in Fig. 2. and Fig. 3. Thiamethoxam recorded highest reduction in parasitism (39.23 %) followed by spinosad (30.24 %), buprofezin (23.97 %), flubendiamide (22.07 %) and chlorantraniliprole (9.50 %). Adult emergence was also significantly reduced when *T. chilonis* eggs were exposed to insecticides. Thiamethoxam significantly reduced adult emergence by 43.43 %. Flubendiamide reduced both parasitism and adult emergence by 22.00 %. Chlorantraniliprole showed the same lowest reduction in pupation as well as adult emergence (9.50%). Thiamethoxam caused 34.67 % reduction in adult emergence while it was only 30.24% in pupation. But chlorantraniliprole and flubendiamide caused the same magnitude of reduction in both pupation and emergence. The effect of buprofezin was more on pupation reduction (23.97 %) than on adult emergence reduction (18. 75 %). Buprofezin, chlorantraniliprole and flubendiamide were thus rated as harmless insecticides to *T. chilonis* eggs.

Spinosad recorded 64.00 % pupation rate indicating 30.20 % reduction in pupation and 34.67 % emergence reduction and was thus rated as 'slightly harmful' as per IOBC protocols (Hassan, 1992). The present finding of the adverse effect of spinosad on adult emergence might be due to the spinosad residue remaining on the egg chorion which might have affected the adult emergence while chewing exit holes through the egg chorion. The present results are in agreement with Suh *et al.* (2000). Spinosad was reported to have a significant adverse effect on the emergence of *T. chilonis* when exposed to all immature stages (Hussain *et al.*, 2010). Certain insecticides did not penetrate the host egg chorion and *T. chilonis* were affected only upon emergence from the egg due to chewing of exit holes (Plewka *et al.*, 1975). The exposure of egg stage of *T. chilonis* to spinosad resulted in low adult emergence while the highest emergence was observed in chlorantraniliprole treatment. The present results corroborates

Fig. 2. Reduction in parasitism over control due to insecticide treatment at egg stage

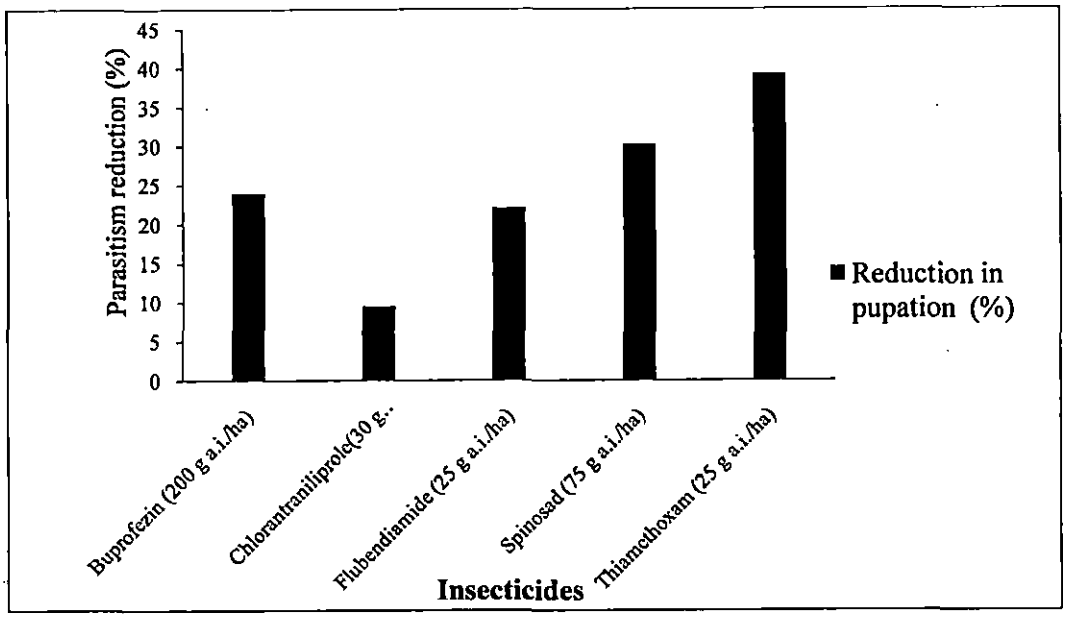
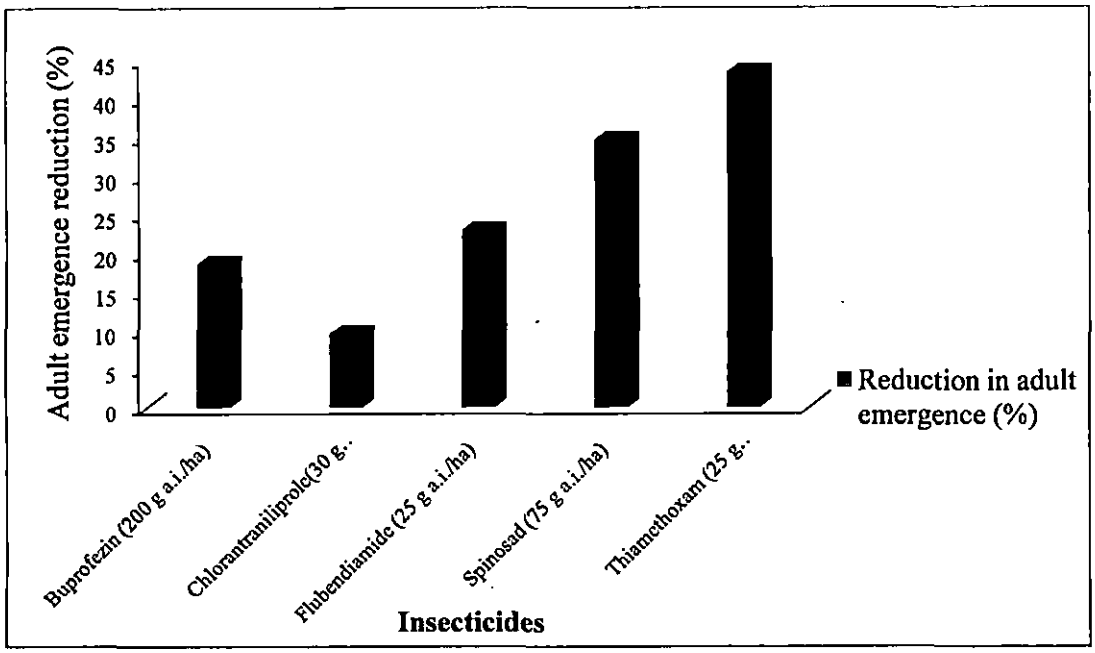


Fig. 3. Reduction in adult emergence over control due to insecticide treatment at the egg stage



with the findings of Ughade *et al.* (2002) and Hussain *et al.* (2012) who reported chlorantraniliprole as safe insecticide than spinosad to *T. chilonis.* The studies by Willams *et al.* (2004) revealed an order of toxicity of insecticides to *T. chilonis* as thiamethoxam > spinosad > oxamyl and is in conformity with our findings.

Buprofezin caused less impact on the emerging adults as evident from 65.75% of adult emergence from a pupation rate of 69.75 % and was rated as harmless to the eggs of *T. chilonis.* Buprofezin, an insect growth regulator acts on insect by disrupting the exoskeleton formation and moulting process (Reynolds, 1987). Adult emergence occurs after moulting which might be interfered by the hormone triggering physiological process that might have caused a reduction in adult emergence. This indicates that beneficial insects particularly *Trichogramma* spp. are less susceptible to buprofezin as compared to other chemical groups even though some negative side effects have been reported (Santos *et al.*, 2006). Another insect growth regulator triflumuron was rated as 'moderately harmful' (class 3) to eggs of *T. pretiosum* (Bueno *et al.*, 2008a) which might be due to the difference in chemical structure of triflumeron and buprofezin. Also the difference in species of *Trichogramma* may alter the selectivity of insecticides and the atmospheric and climatic parameters are also other determining factors.

Rill *et al.* (2008) evaluated the impact of buprofezin on the percentage pupation and adult emergence of *Aphytis melinus* Debach (Hymenoptera: Aphelinidae) an ectoparasitoid and it was reported to have minimal or no impact on the percentage of pupation whereas adult emergence was affected causing 46.60 % emergence from treatment at the egg stage. This is in contrary to our findings which showed 23.97 % reduction in parasitism over control whereas adult emergence was found to be 65.75%. This may be substantiated by the facts that taxonomic differences among the test insect will add to the response to same factor.

According to the reports by Torres *et al.* (2003) there was no direct effect on the rate of pupation and adult emergence of aphid egg parasitoid *Aphelinus*

gossypii Glover, (Hymenoptera: Aphelinidae) treated at egg stage due to thiamethoxam treatment which was inconsistent with our findings. They, however, opined that indirect effects may be observed when host mortality takes place before the parasitoid develops. Spinosad is regarded to have low toxicity to parasitoids and predators (Hendrix *et al.*, 1997, Murray and Lloyd, 1997). But in the present study, spinosad was found to have detrimental effect on adult emergence which is in line with the findings of Suh *et al.* (2000).

Flubendiamide caused 62.25 % adult emergence indicating a reduction of 22.90 % over control. Based on IOBC safety scale (Hassan, 1992), flubendiamide was rated as harmless which is in agreement with the results of Carvalho *et al.* (2005b) and Rezende *et al.* (2005) for *T. pretiosum* and *T. atopovirilia.*

The chemical properties and mode of action of insecticides are important determinants of toxicity to different insect taxonomic groupings and life stages such as larvae, pupae, and adults (Oliver *et al.*, 2006).

According to Busvine (1957), egg stages were found usually resistant to conventional contact poisons. Buprofezin, chlorantraniliprole and flubendiamide showed only 24 %, 9.5 % and 22 % respective reduction in pupation over control and 18.57 %, 9.50 % and 22.90 % reduction in adult emergence over control. Thus these insecticides were rated as 'harmless' to *T. chilonis*. It might be due to the fact that these insecticides might not have penetrated the host egg chorion and *T. chilonis* was affected only upon the emergence of adults from eggs (Plewka *et al.*, 1975).

Parasitoids are grouped in two categories as idiobiont and koinobiont based on nutritional strategy as reported by Haeselbarth (1979), Askew and Shaw (1986). Parasitoids categorized as idiobiont are those attacking egg and pupal stages of host and thus paralyzing or killing the hosts by venom preceding oviposition. Thus the parasitoid *T. chilonis* develops in non-growing hosts (*C. cephalonica*) and utilize the host resource existed at the time of parasitization for growth and development. Venoms have characteristics to paralyse or kill the

hosts and contain many kinds of enzymes to digest most of host tissues (Moreau and Guillot, 2005). Venoms (female-derived factor or acid gland) are injected into the host eggs along with parasitoid egg and arrest the host development. Jarjees and Merritt, (2004) suggested that venom was responsible for host death and degeneration of host tissues. Egg parasitoid ingests the host contents like yolk at once after hatching for growth and development (Takada *et al.*, 2000) and consumes the contents of the host killed or decomposed by venom as nutritional resource for growth and development in the host. Rapid ingestion of the host yolk leads to entry of insecticides in the egg shell and thus disturbs the growth and development of the parasitoid. The difference in susceptibility of egg parasitoids to insecticides may be attributed from direct effect on the larval and pupal stages from the residual effect of insecticides outside of egg-shell disturbing the emergence from host egg. Earlier reports highlighted that the conventional insecticides like organophosphates or carbamates have adverse affect on the pre-imaginal stages. Slight to moderate effects were exhibited by pyrethroids, whereas insect growth regulators and other selective compounds generally appeared to have no effect on emergence. The lethal and disruptive effect of insecticides on *T. brasiliensis* Ashmead emergence decreased as parasitoids advanced in development as reported by Varma and Singh (1987). The host size and the number of conspecifics emerging from it are determined by the quality and overall fitness of *Trichogramma* (Bai *et al.* 1992). This was observed in *T. exiguam* and a relatively large natural host egg (*Helicoverpa zea*), one of the other studies (Consoli *et al.*1998) used smaller factitious eggs (e.g. *Ephestia kuehniella*) as host and different species of *Trichogramma.*

The developmental period of *T. chilonis* when treated at egg stage showed that life cycle was extended to four days by thiamethoxam (Table 14). The egg period was not affected as it was for approximately three days in all the treatments and control. But, the larval period in all the insecticide treatments was increased as compared to control (2.2 days). Buprofezin, flubendiamide, spinosad and thiamethoxam showed about 50 % increased larval period over control. Pupal

period for treatment with chlorantraniliprole and control was not significantly different. The longest pupal period was reported in thiamethoxam (4.9 days). Longevity is used as an index of wasp quality (Marston and Ertle, 1973; Waage and Ming, 1984). Longevity of all the emerging adults was reduced due to insecticide treatment in the egg stage of *T. chilonis*. The present finding is in conformity with Charles *et al.* (2000), Nasreen *et al.* (2004) and Saljoqi *et al.* (2012). But Shoeb (2010) reported that longevity of adults emerged from spinosad treated eggs of *T. chilonis* did not differ significantly from that of the control which is contrary to our findings.

5.4.2. Impact of insecticide exposure to the larval stage of *T. chilonis*

When the larval stage of *T. chilonis was* exposed to insecticides, spinosad was found to cause the highest deleterious effect by causing 75 % reduction in parasitism rate and 83.69 % reduction in adult emergence (Fig.4 and Fig.5). Spinosad was found to be 'moderately harmful' to larval stage of *T. chilonis* . This indicates the high susceptibility of larval stage of *T. chilonis* to spinosad. Chlorantraniliprole was found to be the safest as it caused only 22.81% reduction in pupation over control whereas adult emergence was reduced by 27. 89%. A higher degree of spinosad toxicity to the larval stage of *T. chilonis* was reported by Consoli *et al.* (2008). But spinosad was observed to be slightly harmful to the larval stage of *T. chilonis* with 38 % reduction in adult emergence(Sattar *et al.* 2011). Hussain *et al.* (2012) reported a comparative safety of spinosad and chlorantraniliprole to *T. chilonis.*

In the present study, buprofezin reduced the rate of parasitisation by 55.77% due to insecticide treatment at larval stage. As per IOBC (Hassan, 1992) norms buprofezin was thus rated as 'slightly harmful' to the larval stage of *T. chilonis* and the finding is in conformity with Sattar *et al.* (2011). Insect growth regulators have sub lethal effects on the larval stage of *Trichogramma* and thus reduced the parasitisation capacity as reported by Consoli *et al.* (1998). The effect of buprofezin, an insect growth regulator, was studied by Rill *et al.* (2008) on the

Fig. 4. Reduction in pupation over control due to insecticide treatment at larval stage

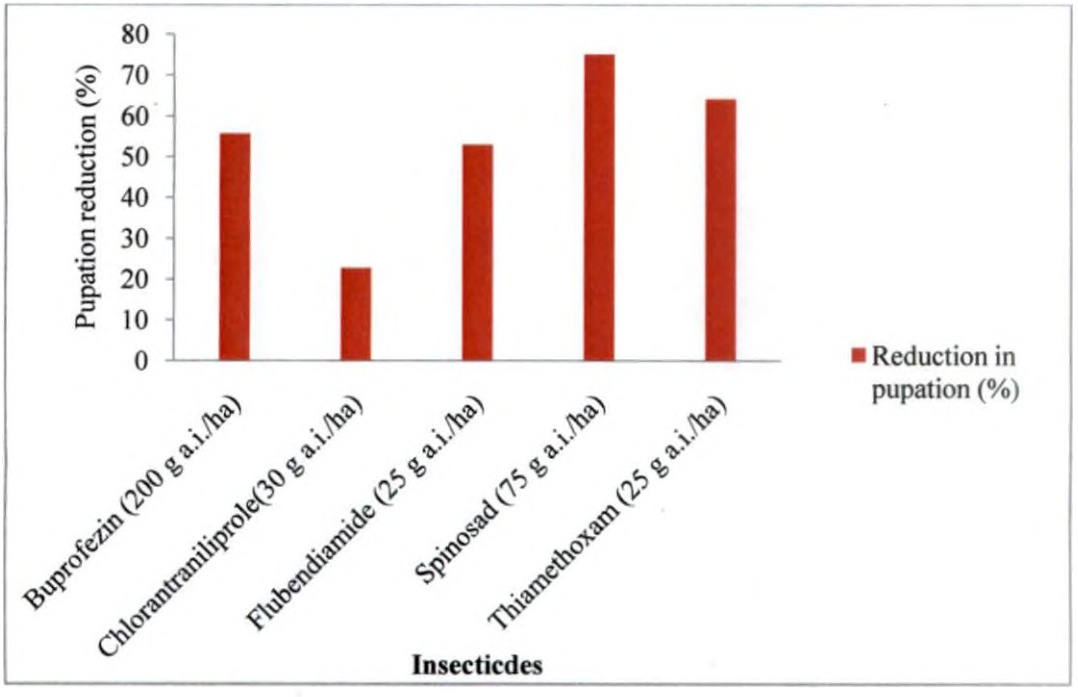
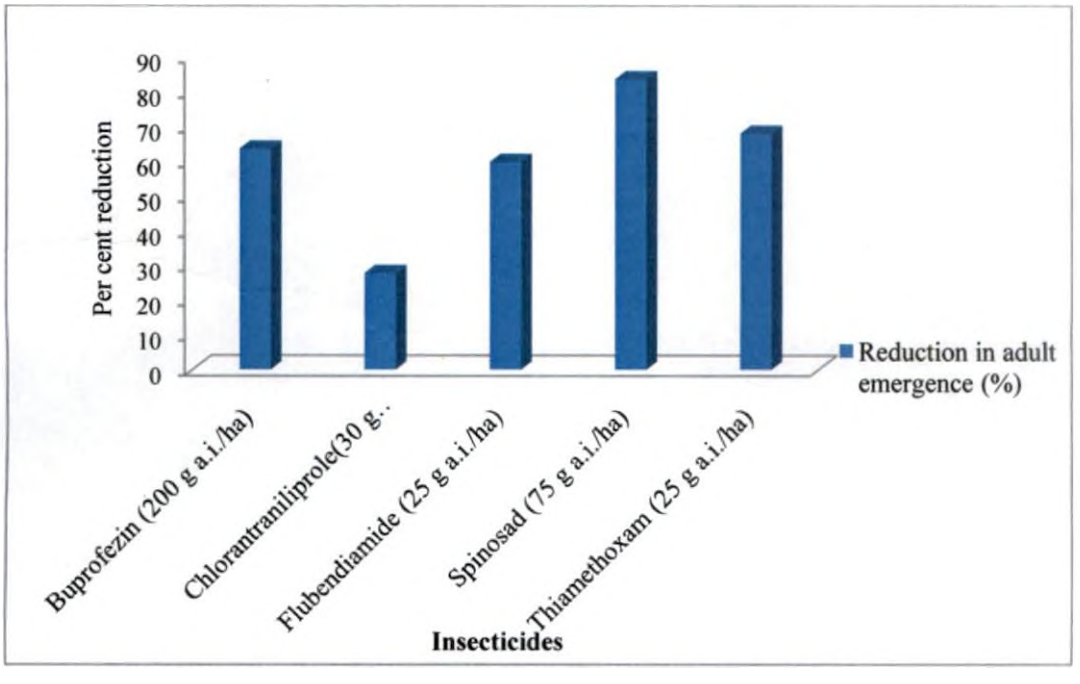


Fig. 5. Reduction in adult emergence over control due to insecticide treatment at larval stage



rate of pupation and adult emergence of an ectoparasitoid, *Aphytis melinus* (Hymenoptera : Aphelinidae) and their results stated that there was no toxic effect on the biological parameters when the larval stage was exposed to insecticides. Our studies indicated a significant reduction in rate of pupation and adult emergence which might be attributed to the difference in the family of test insect being evaluated.

Torres *et al.* (2003) reported the insignificant effect of thiamethoxam on the rate of pupation and adult emergence of the aphid egg parasitoid *Aphelinus gossypii*, (Hymenoptera: Aphelinidae) when treated at the larval stage which was contrary to our findings. They, however, opined that indirect effects might be observed when host mortality takes place before the parasitoid develops.

Sattar *et al.* (2011) demonstrated that flubendiamide was 'harmless' to the larval stage of *T. chilonis* with 26.00 % reduction in adult emergence over control. This finding is contrary with our results wherein flubendiamide caused adult emergence reduction of 59.79 % over control.

The developmental period of insecticide treated larvae of *T. chilonis* differed significantly from control (Table 16). Larval period was increased in all insecticides treatments as compared to untreated control. Similar trend was observed in pupal period also. The total development period from larva to adult was extended due to insecticide treatments.

Different insecticides reduced the longevity of adults that had emerged from insecticide treated larvae of *T.chilonis*. Adults survived only for about one day with spinosad and thiamethoxam treatment whereas they survived for about two days in buprofezin, chlorantraniliprole and flubendiamide treatments indicating high reduction in adult life span due to insecticides. The significant reduction in the longevity of adults emerging from spinosad treatment at larval stage was reported earlier by Suh *et al.* (2000) which is in line with our results.

5.4.3. Impact of insecticide exposure on the pupal stage of *T. chilonis*

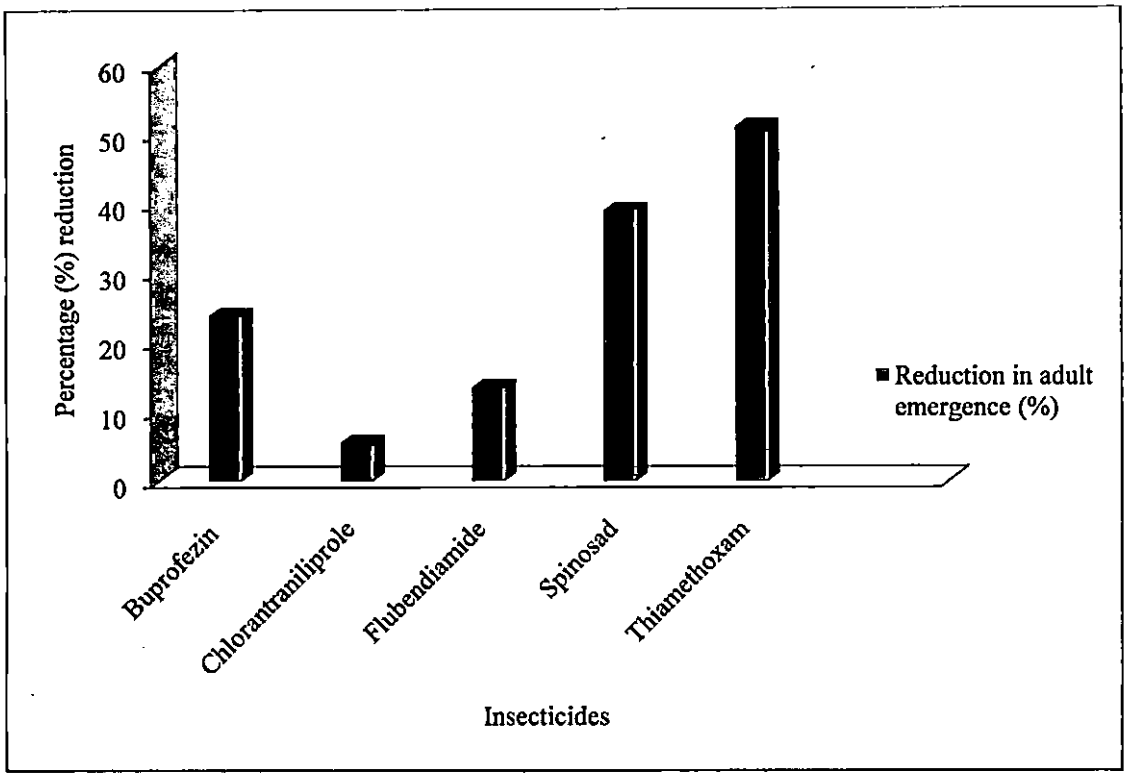
The results on the rate of adult emergence as influenced by insecticide treatment on the pupal stage of *T. chilonis* are summarised in Table 17 and illustrated in Fig.6. Insecticides caused 5.55 to 50.92 % reduction of adult emergence when pupae were exposed. Thiamethoxam was the most toxic insecticide to *T. chilonis* pupae causing 50.92 % reduction in adult emergence whereas chlorantraniliprole was the safest with 76.50% adult emergence. Thiamethoxam was followed by spinosad with 38.88 % reduction in adult emergence. Based on IOBC protocol (Hassan, 1992); both thiamethoxam and spinosad were rated as 'slightly harmful' whereas buprofezin, chlorantraniliprole and flubendiamide were 'harmless' to the pupal stage of *T. chilonis*.

Hussain *et al.* (2012) reported high lethality of chlorantraniliprole to the pupal stage of *T. chilonis* which is inconsistent with our findings where as spinosad was found to be toxic and flubendiamide was comparatively safer. This corroborates with our results.

The impact of an insect growth regulator, buprofezin on the rate of pupation and adult emergence of an ectoparasitoid, *Aphytis melinus* (Hymenoptera: Aphelinidae) was studied by Rill *et al.* (2008). They stated that there was no toxic effect on the biological parameters when the pupal stage was exposed to insecticides. Our studies indicated a significant reduction in rate of pupation and adult emergence due to buprofezin. This might be attributed to the difference in the systematic position of the tested insect.

The toxicity of thiamethoxam to the pupal stage of egg parasitoid *Aphelinus gossypii*, (Hymenoptera: Aphelinidae) was studied by Torres *et al.* (2003) and they reported a reduction in rate of pupation and adult emergence. Our results are in conformity with the finding. It was also reported that thiamethoxam caused no alteration in time taken for the adult emergence which was inconsistent with our finding. It might be explained by the quick action of thiamethoxam (Nicholson *et al.* (1995) and Maiensfisch *et al.* (1999).

Fig. 6. Reduction in adult emergence over control due to insecticide treatment at pupal stage



Our conclusions regarding the safety of the chemicals to the pupal stage of the *T. chilonis* are also in concurrence with that of Cristina *et al.* (2006). The harmlessness of flubendiamide to the pupal stage of *Trichogramma* spp. was confirmed by similar reports presented by Carvalho *et al.* (2005b) and Rezende *et al.* (2005) of *T. pretiosum* and *T. atopovirila* respectively.

The high sensitivity of *T. chilonis* to spinosad and thiamethoxam was observed by its longer pupal period which lasted for about four days and shorter adult longevity of 1.7 - 1.9 days (Table 18). Buprofezin, chlorantraniliprole, and flubendiamide resulted in a pupal period of about three days and adult longevity for two days which was 50 % lower than that in control. The reduction in longevity of adults emerging from spinosad treated pupae of *T. chilonis* is in agreement with the findings of Suh *et al.* (2000) who reported a lower longevity of *T. exiguam* due to spinosad treatment at pupal stage.

5.5. PARASITIZATION EFFICIENCY OF ADULTS EMERGING FROM INSECTICIDE TREATED PRE-IMAGINAL STAGES

5.5.1. Effect on second generation when parental egg stage was treated with insecticides

The results on the rate of parasitisation and adult emergence of the descendent generation emerging from insecticide treated eggs of *T. chilonis* are presented in Table 19 as illustrated in Fig. 7 and Fig. 8. A significant reduction of parasitisation in the second generation was observed when parental egg stage was treated with insecticides. Parasitism reduction varied from 4.17 to 15.04 %. Chlorantraniliprole was found to be least deleterious to the second generation of *T. chilonis* that emerged from insecticide treated eggs as there was only a reduction of 2.49 % in adult emergence over control. Thiamethoxam exhibited highest reduction (36.13 %) in adult emergence. Vianna *et al.* (2009) reported that the adults emerging from thiamethoxam treated eggs were not able to parasitize the factitious host which is contrary to our finding. According to our results 76.25 % parasitisation was observed from the descendent generation emerging

Fig. 7. Reduction in pupation of second generation over control due to insecticide treatment to the parental eggs

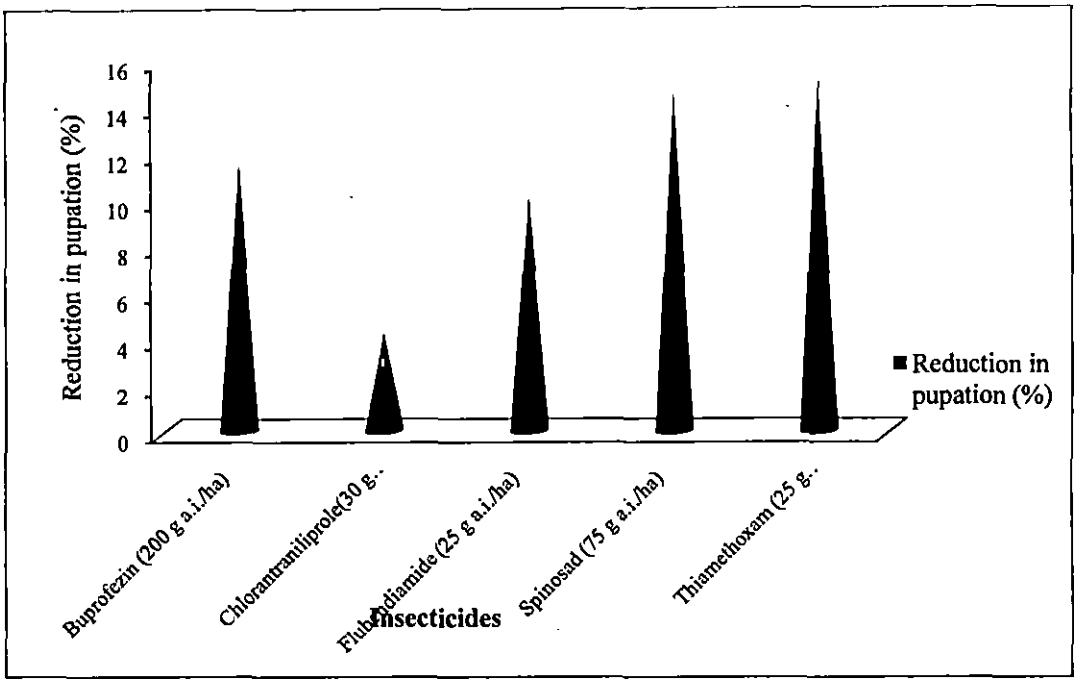
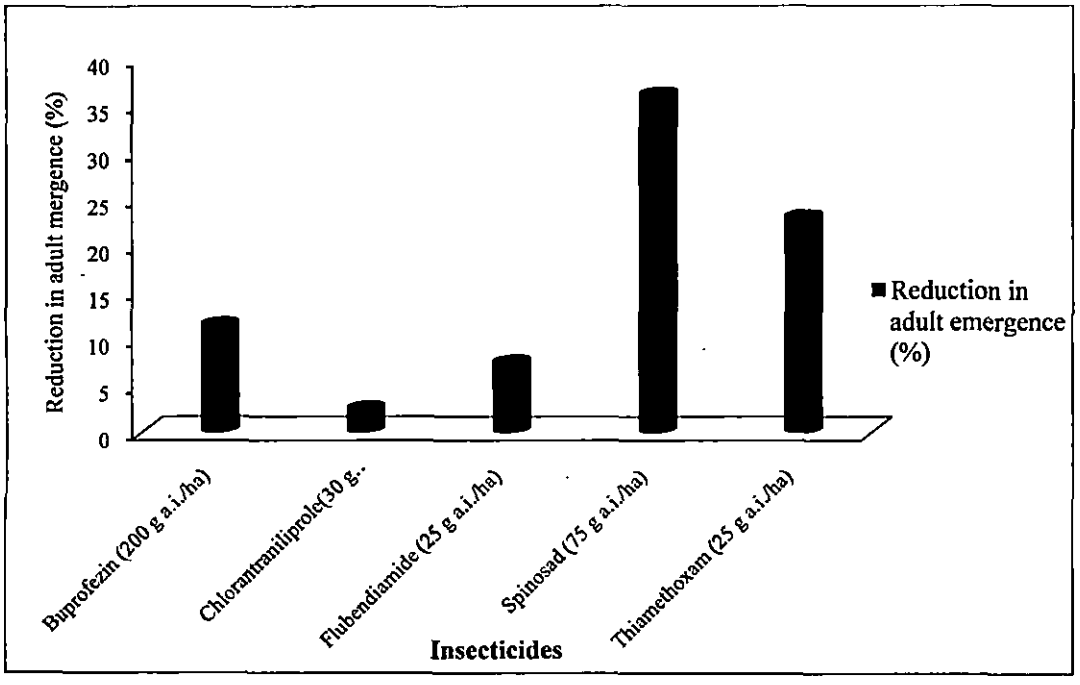


Fig. 8. Reduction in adult emergence of second generation over control due to insecticide treatment to the parental eggs



from thiamethoxam treated eggs. The parasitisation efficiency of second generation adults emerging from buprofezin treatment was reduced by 11.42 % with respect to control (Vianna *et al.*, 2009).

5.5.2. Effect on descendent generation when parental larval stage was treated with insecticides

The findings of the impact on the rate of pupation and adult emergence by the offsprings emerging from insecticide treated larvae of *T. chilonis* is summarised in Table 21 and illustrated in Fig. 9 and Fig. 10. The lowest pupation was recorded in thiamethoxam (39.50 %) whereas chlorantraniliprole exhibited highest rate of pupation (62.50 %) and an adult emergence of 56.00 %. The impact of insecticides on the second generation was drastically lowered than the impact on insecticide treated parental generation. This indicates the degradation of insecticide residue and its transfer to the next generation and the results are in close agreement with the findings of Vianna *et al.* (2009).

5.5.3. Effect on descendent generation when parental pupal stage was treated with insecticides

Insecticide treatment in the pupal stage was found to cause least toxic effect on the rate of pupation and adult emergence in the second generation of *T. chilonis* (Table 23, Fig. 11 and Fig. 12). A reduction of only 1.80 to 3.00 % was reported in the adult emergence and all the insecticides were 'harmless' to second generation. The present finding corroborates with Vianna *et al.* (2000), wherein it was reported that pupal stage was very tolerant to insecticides and the second generation adults of *T. pretiosum* showed no significant difference in parasitisation and adult emergence from treatment.

Fig. 9. Reduction in pupation of second generation over control due to insecticide treatment to the parental larvae

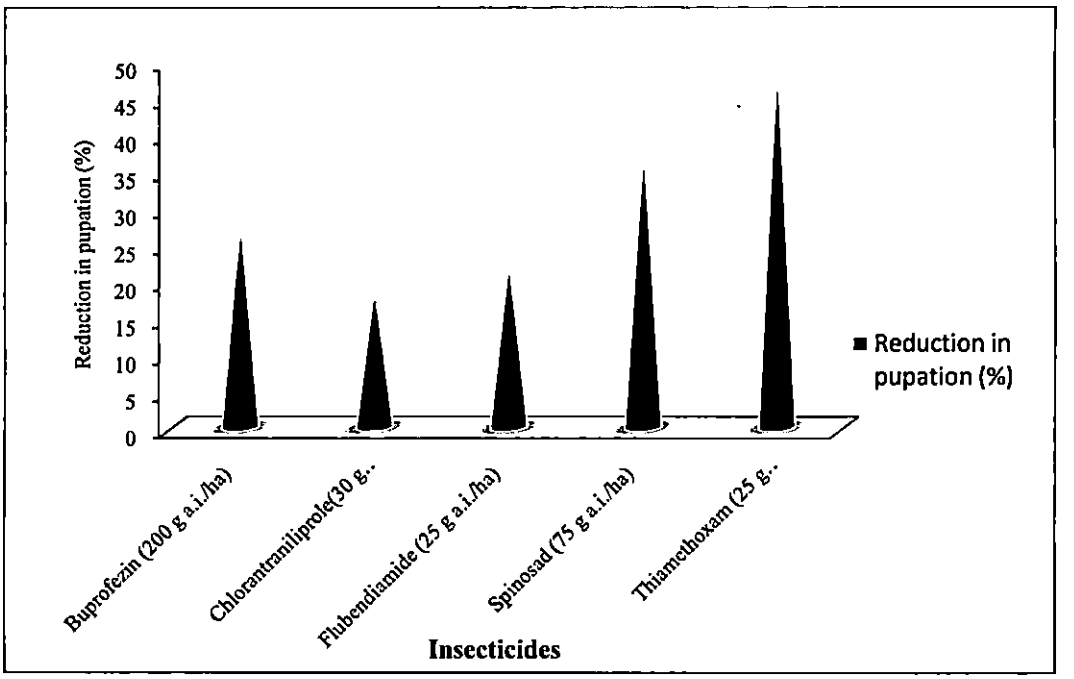


Fig. 10. Reduction in adult emergence of second generation over control due to insecticide treatment to the parental larvae

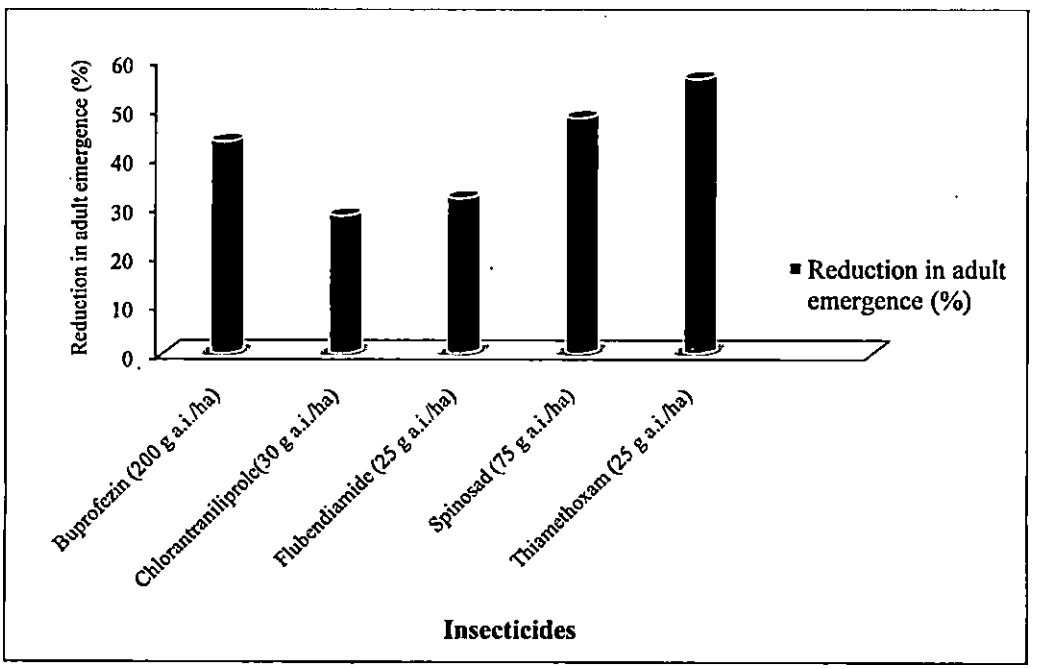


Fig. 11. Reduction in pupation of second generation over control due to insecticide treatment to the parental pupae

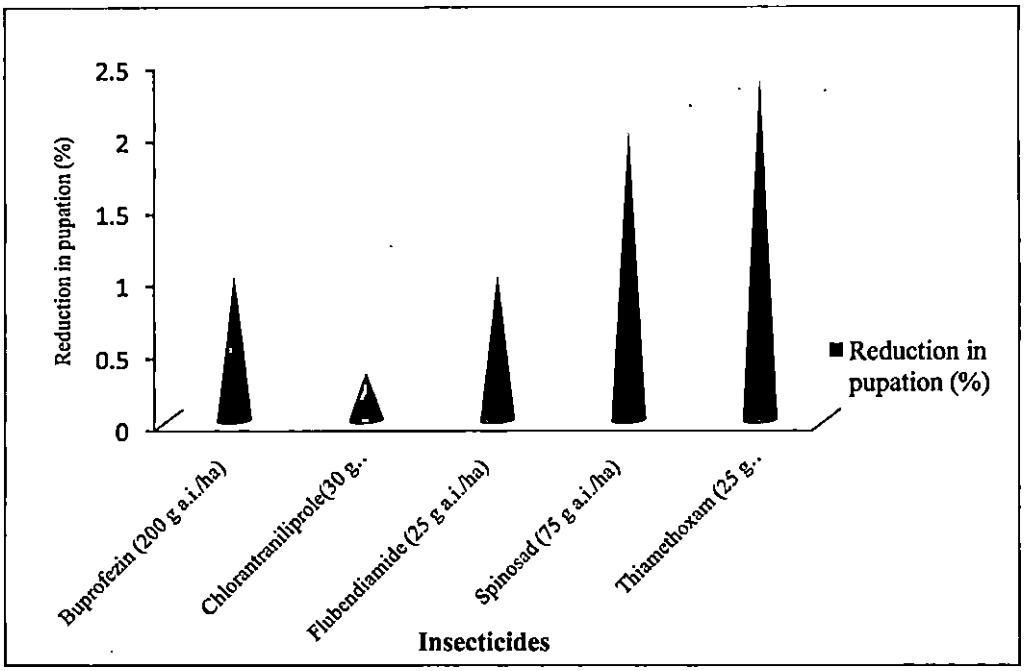
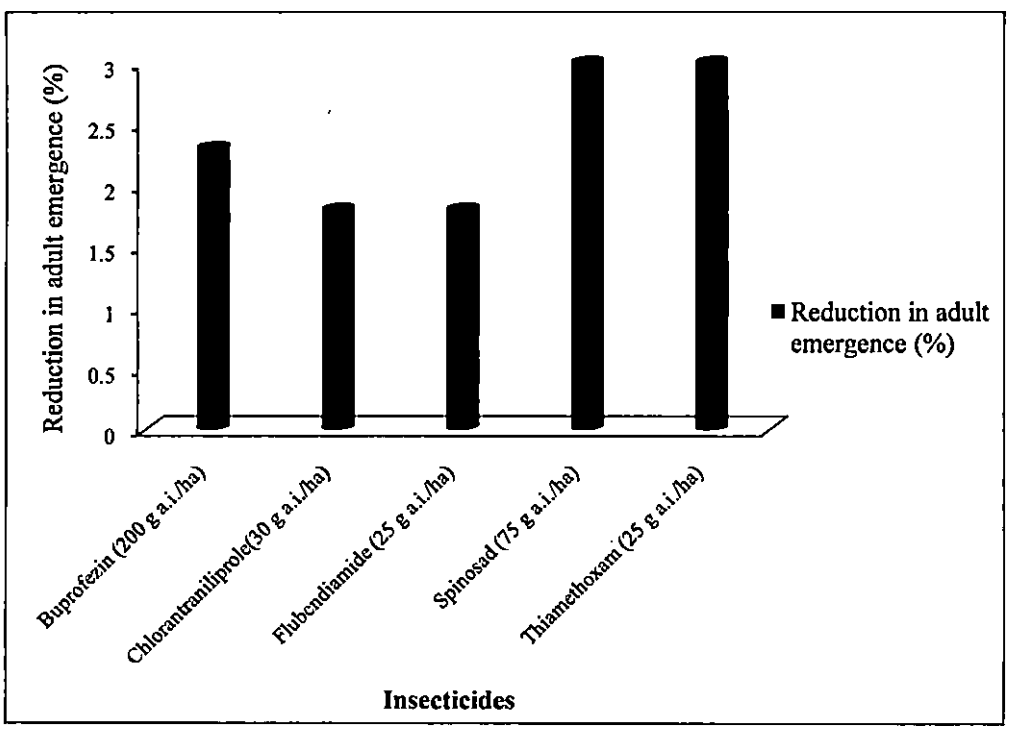


Fig. 12. Reduction in adult emergence of second generation over control due to insecticide treatment to the parental pupae



5.6. INSECTICIDE TREATMENT ON FACTITIOUS HOST EGGS

The results on the effect of insecticide treatment on factitious host (*Corcyra* eggs) of *T. chilonis* are depicted in Fig. 13 and Fig. 14. Thiamethoxam showed highest adverse effect with only 27.75 % pupation and 25.00 % adult emergence whereas chlorantraniliprole was the safest with 60.75 % pupation and 33.88 % reduction in adult emergence over control. As per IOBC safety scale classification, all the insecticides were rated as 'slightly harmful'. Spinosad showed 57.45 % reduction in parasitism over control and 63.33 % reduction in adult emergence and was thus rated as 'slightly harmful'. Our findings are in line with the reports of Saljoqi *et al.* (2012) who revealed the toxic impact of spinosad on the oviposition preference to *T. chilonis*.

5.7. PERSISTENT TOXICITY

The new generation inscecticides were evaluated for persistent toxicity under laboratory conditions to *T. chilonis*. Thiamethoxam showed highest persistence upto 21 days after treatment with a PT value of 927.36 followed by spinosad and flubendiamide with a PT value of 647.5 and 373.33 (Table 26). Chlorantraniliprole showed the least persistence (7 days) and the lowest average toxicity was also indicated by chlorantraniliprole (13.33).

The residual toxicity of insecticides was also assessed in the laboratory by studying the interval of time during which their residues caused the minimum level of 30 % mortality to adults of *T. chilonis* and the insecticides were rated as short lived (<5 days), slightly persistent (5 – 15 days), moderately persistent (16-30 days) and persistent (> 30 days) (Table 27, Fig. 15). Buprofezin, chlorantraniliprole and flubendiamide residues were short lived as they caused minimum 30 % mortality between 1 to 3 days after application whereas the residual toxicity of spinosad was slightly persistent.

Hendrix *et al.* (1997) reported that dried spray residues of spinosad was toxic to the adult wasps of *Trichogramma*. The residues might have been absorbed by the adult parasitoid through contact as it walked on the treated

Fig. 13. Reduction in parasitism by *T. chilonis* exposed to insecticide treated *Corcyra* eggs

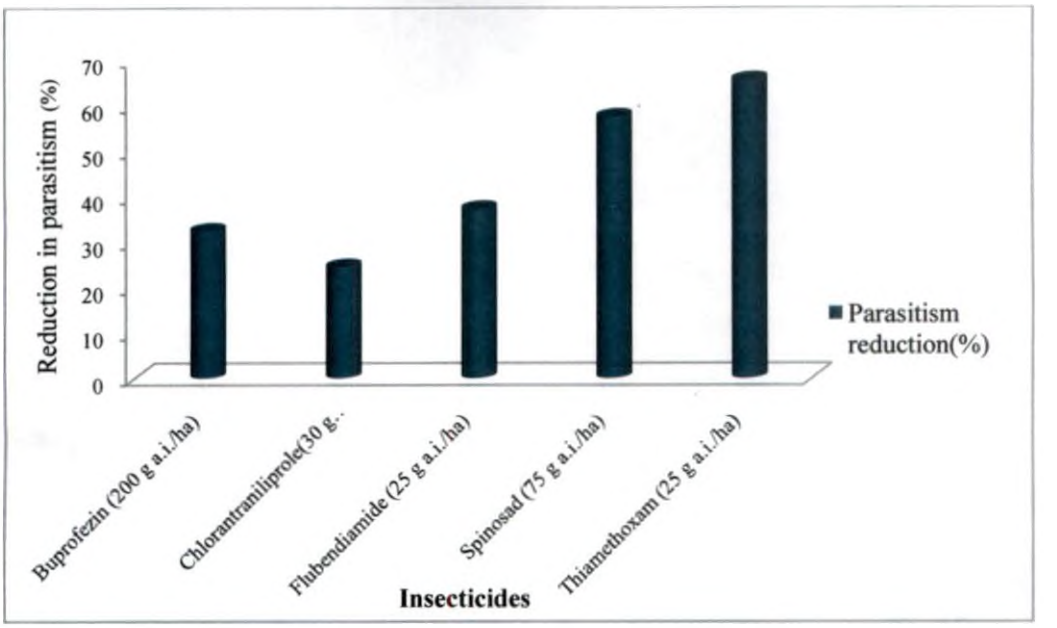


Fig. 14. Reduction in adult emergence by *T. chilonis* exposed to insecticide treated *Corcyra* eggs

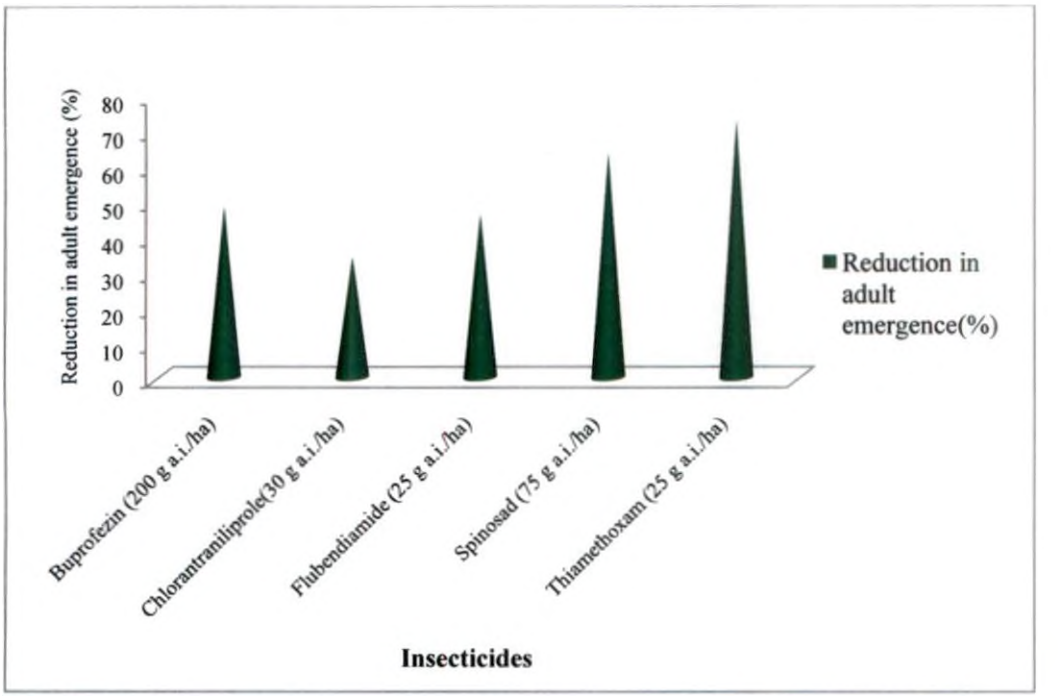
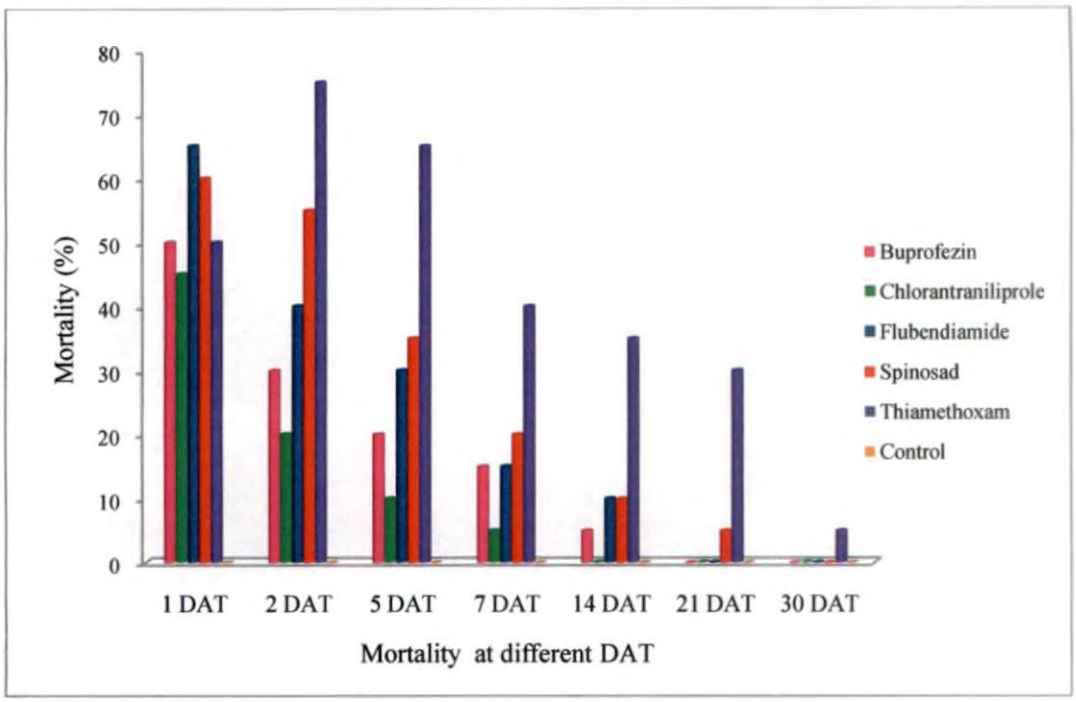


Fig. 15. Persistent toxicity of insecticides to *T. chilonis* at different days after treatment



surface. Insecticide treatment to the hymenopterans can lead to sub lethal effects such as alternations in their host finding ability and parasitizing behaviour (Rogers and Potter 2003). The short time interval between the substrate treatment and adult introduction may have exposed wasps to higher pesticide levels than normally found in the field environment under natural conditions. Under field conditions, pesticide dissipation is influenced by factors such as the substrate treated, the chemical and physical properties of the pesticide and environmental conditions such as rainfall, temperature, and solar incidence (Montgomery, 1997 and Vittum, 1999). But the laboratory conditions eliminated or reduced some dissipation factors such as rainfall and photolytic depletion. Also pesticides that dissipate quickly by vaporizing would reduce residues on substrate (Harris, 1982 and Elliott, 1988). In addition to the amount of pesticide residue on the substrate, a more important determinant under natural conditions may be the amount of residue that dislodges from the treated substrate. The toxicity of insecticides to hymenopterans such as honey bees and a solitary parasitic wasp was directly proportional to the amount of dislodged residues (Bellows *et al.*, 1993 and Chukwudebe *et al.*, 1997). The substrate surface and the type of pesticide can alter the amount of dislodgeable residue (Antonious and Snyder, 1995 and Chukwudebe *et al.*, 1997). Therefore, methods that reduce pesticide residues on leaf surfaces such as post treatment irrigation or subsurface pesticide placement, when compatible with the requirements for effective pest control, may enhance conservation of parasitoids.

Conclusion

The impact of five newer insecticides on different biological parameters of *T. chilonis* is summarised and concluded hereunder. Among the five insecticides evaluated for its toxicity on various biological parameters (Table 28), only flubendiamide was found to be harmless to all the pre-imaginal stages (egg, larvae and pupa) of *T. chilonis* for parasitism and adult emergence. Buprofezin and chlorantraniliprole were found to be harmless to egg stage treatment with respect to pupation and harmless to egg and pupal stage for adult emergence due

to insecticide treatment whereas these insecticides were slightly harmful to larval stage with respect to pupation and adult emergence. Spinosad and thiamthoxam were found to be slightly harmful when the egg stage was treated with the recommended concentration of insecticides with respect to parasitism and its viability whereas larval stage was reported to be 'moderately harmful' to the larval stage treatment of *T. chilonis* by spinosad in parasitism and adult emergence. Thiamethoxam was also moderately harmful to the adult emergence due to treatment of larval stage with insrcticide. Insecticides *viz.*, buprofezin, chlorantraniliprole and flubendiamide caused minimal impact on adult emergence due to insecticide treatment at the pupal stage wheras spinosad and thiamethoxam imparted slightly toxic effect on adult emergence.

It needs to be emphasized that the present experiments were carried out under laboratory conditions, where *T. chilonis* was subjected to the highest possible insecticide pressure. Under the field conditions, however, the extend of negative impact of the insecticides may be reduced because the parasitoids can benefit from refuge areas or may avoid insecticide treated areas. Moreover, the degradation of insecticide residues due to abiotic factors in the field condition may also decrease the magnitude of insecticide impact on the parasitoid. Therefore, after the laboratory assays, the insecticides are needed to be tested in semi-field condition to evaluate the residue persistence and therefore field testing has to be undertaken to study the environmental degradations, shelter and other factors that might influence the insecticide impact. Only after such studies, the negative impact of insecticides on *T. chilonis* can be fully assessed.

Table 28. Effect of insecticides on different biological parameters of *T. chilonis*

Insecticide formulation	Parasitism	Adult emergence	Mortality 24 HAT	IOBC persistence ranking
Buprofezin 25 SC	HL (egg), SH (larvae)	HL (egg), SH (larvae), HL (pupa)	SH (adult)	Short lived
Chlorantraniliprole 18.5 SC	HL (egg) , SH (larvae)	HL (egg), SH (larvae), HL (pupa)	SH (adult)	Short lived
Flubendiamide 480 SC	HL (egg), HL (larvae)	HL (egg), SH (larvae), HL (pupa)	SH (adult)	Short lived
Spinosad 2.5 SC	SH (egg), MH (larvae)	HL (egg), SH (larvae), SH (pupa)	SH (adult)	Slightly persistent
Thiamethoxam 25 WG	SH(egg), SH (larvae)	HL (egg), SH (larvae), SH (pupa)	SH (adult)	Moderately persistent

HL – Harmless (< 30 % mortality)

SH – Slightly harmful (50 – 79 % mortality)

MH – Moderately harmful (80 – 90 % mortality)

H – Harmful (> 90 % mortality)

SUMMARY

6. SUMMARY

The study entitled 'Impact of insecticides on *Trichogramma chilonis* Ishii (*Hymenoptera : Trichogrammatidae*)' was carried out in the Department of Agricultural Entomology, College of Horticulture, Vellanikkara, Thrissur during 2012-13. Five newer generation insecticides *viz.*, buprofezin, chlorantraniliprole, flubendiamide, spinosad and thiamethoxam were evaluated for their lethal and sub-lethal effects on the biological parameters of the egg parasitoid *T. chilonis* by adopting internationally approved and standardised protocols developed by the Pesticide and Beneficial Organisms Working Group of the International Organization for Biological Control (IOBC/WPRS).

- **Lethal effects**

The lethal effects of the selected new insecticides on adults of *T. chilonis are* summarized below.

Insecticide formulations	Adult mortality 24 HAT	LC_{50} (mg a.i. l^{-1})	Risk quotient categorization
Buprofezin 25 SC @ 200 g a.i. ha^{-1}	Slightly harmful (56.52 %)	0.5623	Slightly to moderately toxic (50 - 2500)
Chlorantraniliprole 18.5 SC @ 30 g a.i. ha^{-1}	Slightly harmful (50.00 %)	1.3860	Harmless (< 50)
Flubendiamide 480 SC @ 25 g a.i. ha^{-1}	Slightly harmful (55.26 %)	0.4731	Slightly to moderately toxic (50 - 2500)
Spinosad 2.5 SC @ 75 g a.i. ha^{-1}	Slightly harmful (68.42 %)	0.0863	Slightly to moderately toxic (50 - 2500)
Thiamethoxam 25 WG @ 25 g a.i. ha^{-1}	Slightly harmful (68.42 %)	0.0011	Dangerous (> 2500)

All the tested five insecticides at field recommended concentrations indicated significant toxicity by causing 50.00 to 68.42 % mortality to adults of

T. chilonis at 24 HAT. Among the five insecticides, spinosad and thiamethoxam caused significantly higher mortality (68.42 %) and they were on par in toxicity to *T. chilonis*. Chlorantraniliprole caused the lowest toxicity of 50.00% of mortality whereas buprofezin and flubendiamide caused a mortality of 56.52 % and 55.26 %. However, based on IOBC/WPRS safety scale classification, all the five insecticides were rated as 'slightly harmful' to *T. chilonis* as the mortality was in the range of 50 – 79 %.

Based on the estimated LC_{50} values, thiamethoxam showed highest toxicity with lowest LC_{50} value of 0.0011 mg a.i. l^{-1} whereas chlorantraniliprole was the safest (LC_{50} value 1.3860 mg a.i. l^{-1}). By the risk quotient analysis, thiamethoxam was found to be 'dangerous' while spinosad, buprofezin and flubendiamide were 'slightly to moderately toxic' to *T. chilonis*. Chlorantraniliprole was the only 'harmless' insecticide to *T. chilonis*.

- **Sub-lethal effects of insecticides on pre-imaginal stages of first generation**

Safety of insecticides to the pre-imaginal stages of *T. chilonis* with respect to parasitism viability (adult emergence) is given below:

Insecticide formulations	Egg stage	Larval stage	Pupal stage
Buprofezin 25 SC @ 200 g a.i. ha^{-1}	Harmless	Slightly harmful	Harmless
Chlorantraniliprole18.5 SC @ 30 g a.i. ha^{-1}	Harmless	Harmless	Harmless
Flubendiamide 480 SC @ 25 g a.i. ha^{-1}	Harmless	Slightly harmful	Harmless
Spinosad 2.5 SC @ 75 g a.i. ha^{-1}	Slightly harmful	Moderately harmful	Slightly harmful
Thiamethoxam 25 WG @ 25 g a.i. ha^{-1}	Slightly harmful	Slightly harmful	Slightly harmful

The safety of insecticides to different immature stages was assessed in terms of adult parasitoid emergence. Chlorantraniliprole was found 'harmless' to all the three immature stages of egg, larvae and pupael of *T. chilonis*. But buprofezin and flubendiamide were 'harmless' to egg and pupal stages whereas it was 'slightly harmful' to the larval stage. Spinosad was 'moderately harmful' to the larval stage while it was 'slightly harmful' to the egg and larval stages whereas thiamethoxam was 'slightly harmful' to all the immature life stages of *T. chilonis*. Larval stage of the parasitoid was found to be most susceptible while eggs and pupae were tolerant to all insecticides.

- **Sub-lethal effect on developmental period and longevity due to insecticide treatment at pre-imaginal stages**

The impact of insecticide treatment on the developmental period and adult longevity of *T. chilonis*, when the pre-imaginal stages were treated is given below:

Insecticide formulations	Egg stage		Larval stage		Pupal stage	
	Days for adult		Days for adult		Days for adult	
	Emergence	Survival	Emergence	Survival	Emergence	Survival
Buprofezin 25 SC @ 200 g a.i. ha^{-1}	10.8	2.1	6.4	2.1	3.1	2.0
Chlorantraniliprole18.5 SC @ 30 g a.i. ha^{-1}	9.9	2.1	5.9	2.1	3.0	2.1
Flubendiamide 480 SC @ 25 g a.i. ha^{-1}	11.2	1.9	6.7	2.3	3.1	1.9
Spinosad 2.5 SC @ 75 g a.i. ha^{-1}	11.8	1.8	7.2	1.5	3.9	1.9
Thiamethoxam 25 WG @ 25 g a.i. ha^{-1}	12.9	1.7	7.1	1.4	3.9	1.7
Untreated control	8.5	4.1	5.2	4.2	3.1	4.07

It is indicated that the developmental period has shown significant increase in all the treatments as compared to control. Adult emergence was observed after 11-12 days when the parental eggs were treated with buprofezin, flubendiamide, spinosad and thiamethoxam as compared to 8.5 days in control. Larval stage was most susceptible to spinosad treatment with highest developmental period. Only spinosad and thiamethoxam marked escalated developmental period when the pupal stage of *T. chilonis* was treated.

The longevity of emerging adults has shown significant reduction with respect to control in all the treatments. Longevity of parasitoid has reduced to about half when the pre-imaginal stages were exposed to insecticides.

- **Sub-lethal effects of insecticides on second generation**

The parasitisation capacity and adult emergence of second generation adults emerging from insecticide treated eggs, larvae and pupae were further evaluated. Safety to second generation adults emerging from insecticide treated immature stages is summarized below.

Insecticide formulations	Egg stage	Larval stage	Pupal stage
Buprofezin 25 SC @ 200 g a.i. ha^{-1}	Harmless	Slightly harmful	Harmless
Chlorantraniliprole 18.5 SC @ 30 g a.i. ha^{-1}	Harmless	Harmless	Harmless
Flubendiamide 480 SC @ 25 g a.i. ha^{-1}	Harmless	Slightly harmful	Harmless
Spinosad 2.5 SC @ 75 g a.i. ha^{-1}	Harmless	Slightly harmful	Harmless
Thiamethoxam 25 WG @ 25 g a.i. ha^{-1}	Slightly harmful	Slightly harmful	Harmless

Thiamethoxam was 'slightly harmful' even to second generation when the parental eggs were exposed to it. Buprofezin, chlorantraniliprole, flubendiamide and spinosad were harmless to second generation adults when parental eggs were exposed to them. But when the parental larval stage was exposed to insecticides, all of the tested insecticides except chlorantraniliprole were 'slightly harmful' to second generation adults. However, all insecticides were 'harmless' to adults of second generation when pupae of first generation were exposed to insecticides indicating the tolerance of pupae to insecticides.

- **Sub-lethal effect : longevity of second generation adults emerging from insecticide treated pre-imaginal stages of *T. chilonis***

The longevity of second generation adults emerging from insecticide treated pre- imaginal stages of the egg parasitoid is summarised below:

Insecticide formulations	Longevity of adults (days) when treated at		
	Egg stage	Larval stage	Pupal stage
Buprofezin 25 SC @ 200 g a.i. ha^{-1}	3.53	3.15	3.59
Chlorantraniliprole18.5 SC @ 30 g a.i. ha^{-1}	3.71	3.51	3.67
Flubendiamide 480 SC @ 25 g a.i. ha^{-1}	3.58	3.22	3.48
Spinosad 2.5 SC @ 75 g a.i. ha^{-1}	3.49	2.78	3.39
Thiamethoxam 25 WG @ 25 g a.i. ha^{-1}	2.32	2.61	3.16
Untreated control	3.78	3.78	3.78

It is observed that longevity of second generation adults emerging from insecticide treated pre-imaginal stages of *T. chilonis* is in close approximation with the untreated control. Thiamethoxam treatment in the egg stage has reduced the longevity of second generation adults to 2.32 days whereas in larval stage both

spinosad and thiamethoxam has reduced the longevity of *T. chilonis* adults. Second generation emerging from insecticide treated pupal stage was most tolerant with longevity more than three days in treatments and control.

- **Persistent toxicity**

Persistence of the insecticides, to *T. chilonis* was evaluated and mortality at different days after treatment was observed. It was indicated that chlorantraniliprole had the lowest persistence (7 days) whereas buprofezin and flubendiamide persisted for two weeks (14 days). The longest persistence period was observed in spinosad and thiamethoxam (21 days) indicating the longest residual action.

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*Originals not seen

Impact of novel insecticides on *Trichogramma chilonis* Ishii (Hymenoptera : Trichogrammatidae)

by

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(2011-11-113)

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement

for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University, Thrissur

Department of Agricultural Entomology

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

2013

ABSTRACT

The egg parasitoid *Trichogramma chilonis* Ishii is widely distributed in many crop ecosystems throughout the country. Its inundative field releases have achieved an appreciable success in the suppression of the population density of rice leaf folder. But the potential of *T. chilonis* can be severely curtailed by the application of insecticides and hence it is essential to identify the insecticides that are compatible with this species. In this context, the present study entitled 'Impact of novel insecticides on *Trichogramma chilonis* Ishii (Hymenoptera : Trichogrammatidae)' was carried out in the Department of Agricultural Entomology, College of Horticulture during 2012-13 to evaluate the lethal effects of insecticides on the egg parasitoid.

Five new generation insecticides *viz.*, buprofezin 25 SC, chlorantraniliprole 18.5 SC, flubendiamide 480 SC, spinosad 2.5 SC and thiamethoxam 25 WG were tested for their toxicity to *T. chilonis* in the laboratory by adopting the standardised protocols developed by the International Organisation for Biological Control of Noxious Plants and Animals / West Palaeractic Region Section (IOBC/WPRS). The selected newer insecticides are being used in rice ecosystem and have replaced the old conventional insecticides. Hence the acute contact toxicity to adults, dosage-mortality response, detrimental effects to pre-imaginal stages of the parasitoid inside the host eggs and persistent toxicity of the five selected insecticides at field recommended concentrations were investigated.

All the insecticides tested revealed different degrees of acute contact toxicity to the adults of *T. chilonis*. The recommended concentration of thiamethoxam and spinosad showed highest toxicity by causing 68.42 % mortality at 24 hours after treatment to adult parasitoid. Chlorantraniliprole indicated lowest mortality (50.00%) to the adult wasps.

Median lethal concentration (LC_{50}) of each insecticide was determined. Thiamethoxam indicated highest toxicity to *T. chilonis* requiring the lowest concentration to cause 50% mortality (0.0011 mg a.i. l^{-1}) to the parasitoid. Chlorantraniliprole was safe to *T. chilonis* with the highest LC_{50} value of (1.3860 mg a.i. l^{-1}). Based on risk quotient (the ratio between field recommended concentration and LC_{50} of the beneficial insects) only chlorantraniliprole was found to be 'harmless' to *T. chilonis* while thiamethoxam was 'dangerous' whereas buprofezin, flubendiamide and spinosad were 'slightly to moderately toxic' to *T. chilonis*.

The effect of insecticides on the immature stages egg, larva and pupa along with the impact on the second generation of *T. chilonis* was also studied. All insecticides significantly reduced parasitisation when *T. chilonis* eggs were exposed to insecticides. Thiamethoxam caused highest reduction (39.23%) in parasitism while chlorantraniliprole reduced parasitism by 9.50%. Adult parasitoid emergence was also reduced when eggs were exposed to insecticides. Thiamethoxam and spinosad were significantly 'slightly harmful' while buprofezin, flubendiamide and chlorantraniliprole were 'harmless' to the eggs of *T. chilonis*.

Spinosad was found to be 'moderately harmful' to the larval stage while other insecticides were 'slightly harmful' to *T. chilonis* except chlorantraniliprole which caused 27.89% reduction in adult emergence over control and was thus 'harmless'.

Exposure of *T. chilonis* pupae to insecticides caused a significant reduction of adult emergence. Thiamethoxam was most toxic to pupal stage followed by spinosad and buprofezin. Buprofezin, chlorantraniliprole and flubendiamide were 'harmless' to pupae of *T. chilonis*.

Parasitisation efficiency and adult emergence of adult parasitoids emerging from insecticide treated egg, larva and pupa of *T. chilonis* were studied. The adults emerging from thiamethoxam treated eggs caused 15.00% reduction in parasitism while adult emergence was reduced by 36.13%. Thus thiamethoxam was found to be

'slightly harmful' whereas all other insecticide treatments (buprofezin, chlorantraniliprole, flubendiamide and spinosad) were 'harmless'. The progenies emerging from chlorantraniliprole treated larvae of *T. chilonis* resulted in highest rate of parasitisation (62.50%) and were harmless whereas all the other tested insecticides were 'slightly harmful'. All the insecticide treatments were found to be 'harmless' to the second generation when the pupae were exposed to insecticides.

All the tested insecticides were 'slightly harmful' for adult emergence when the factitious host eggs were exposed to insecticides.

With respect to persistent toxicity of insecticides to *T. chilonis*, it was observed that thiamethoxam had the longest persistence up to 21 days and was rated as 'moderately persistent'according to IOBC ranking whereas buprofezin, chlorantraniliprole and flubendiamide were rated as 'short lived'.

Among the five insecticides tested, chlorantraniliprole was the safest and thiamethoxam was most toxic to *T. chilonis* at field recommended concentration. Therefore, potential toxicity of insecticides to parasitoids are be considered before application in rice ecosystem. Let us hope that chemical control and biocontrol will join hands to make Integrated Pest Management a successful venture.