

**ASSESSMENT OF THE LETHAL AND SUBLETHAL EFFECTS
OF SOME NOVEL PESTICIDES ON *Trichogramma chilonis* (Ishii)
(TRICHOGRAMMATIDAE: HYMENOPTERA)**



PhD Dissertation

By

Muhammad Ashraf Khan

**DEPARTMENT OF ENVIRONMENTAL SCIENCES
UNIVERSITY OF PESHAWAR, PAKISTAN.**

(Session 2007-2008)

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UNIVERSITY OF PESHAWAR, PAKISTAN.**

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Muhammad Ashraf Khan

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DEDICATION

I would like to dedicate this humble work to my loving parent; my wife, Ulfat Sikandri, and my young children. Their good wishes have contributed to my success.

This modern world has deep reliance on science and technology, and such reliance have brought prosperity to human being along misery. The total submission to Almighty Allah, supremacy of law, honesty, love, devotion, mutual respect, patriotism, unity, discipline, commitment, patience, determination, courage, sympathy, dedication, sincerity, modesty, truthfulness, hospitality, fairness, and sacrifice for other have become the stories of our forefathers. All these elements of the good society have been lost due to our selfishness.

The story of my success is like the voyage in a sea without a ship.

I faced the tides in the sea with strong determination, commitment, patience, courage, and dedication, and these were the unforgettable days of my life.

Muhammad Ashraf Khan,

PhD scholar

APPROVAL SHEET

It is recommended that this dissertation prepared by **Mr. Muhammad Ashraf Khan** entitled “**Assessment of the lethal and sublethal effects of some novel pesticides on *Trichogramma chilonis* (Ishii)**” be accepted as fulfilling this part of the requirements for the degree of “**DOCTOR OF PHILOSOPHY IN ENVIRONMENTAL SCIENCES**”.

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ABSTRACT

Effects of some novel pesticides on emergence of the *Trichogramma chilonis* (Ishii) from *Sitotroga cerealella* Olivier eggs based on field dose demonstrated fipronil as harmful in the egg, but was moderately harmful and harmless in the larval and pupal stage treatments, respectively. Acetamiprid and spinetoram were slightly and moderately harmful, respectively, in all three immature stages. Abamectin was moderately harmful in egg and larval stages but was slightly harmful in the pupal stage.

The pesticides effect on parasitism by *T. chilonis* emerged from the egg treated at field dose showed that both acetamiprid and fipronil when treated against larvae and pupae, were slightly harmful; however, acetamiprid was harmful in the egg stage treatment. While abamectin was moderately harmful when treated against pupae.

Parasitism of treated host eggs by *T. chilonis* (no-choice test) based on field dose showed that acetamiprid, spinetoram, fipronil, abamectin and haloxyfop-p-methyl were slightly harmful, while spiromesifen was moderately harmful for parasitism.

The corrected mortality (%) for control of adult *T. chilonis* at field dose demonstrated that acetamiprid, spinetoram, fipronil, and abamectin were highly toxic for adult survival of *T. chilonis* exposed to 1-, 5-, 10-, and 15-day-old pesticide residues. While spiromesifen, haloxyfop-p-methyl, bispyribac sodium, pyraclostrobin + metiram and trifloxystrobin + tebuconazole (also showed moderate toxicity in 15-day treatment) were moderately toxic after 24 hours (1-day-residue). However, haloxyfop-p-methyl, bispyribac sodium and pyraclostrobin + metiram were moderately toxic wh-

en adults were exposed to the treated surface 5 days after treatment. HaNPV and chlorantraniliprole exhibited low toxicity in all four residual treatments.

Female *T. chilonis* exposure to the treated glass surface of 1-, 5-, 10- and 15-day-old pesticide residues at field dose assessed for parasitism showed that acetamiprid, spinetoram and abamectin were moderately harmful when exposed to all four residues. However, fipronil and pyraclostrobin + metiram were moderately harmful and harmless in the 1-day and 15-day residual treatment, respectively, but both were slightly harmful in their remaining treatments. Spirotetramat, bispyribac sodium and nicosulfuron were harmless except for being slightly harmful in the 1-day treatment.

HaNPV, spirotetramat, chlorantraniliprole, spiromesifen, haloxyfop-p-methyl, bispyribac sodium, nicosulfuron, myclobutanil, mixture of chlorothalonil + procymidone, pyraclostrobin + metiram and trifloxystrobin + tebuconazole were statistically at par with respective controls regarding emergence from immature stages treated at field dose. HaNPV, spirotetramat, chlorantraniliprole, spiromesifen and pyraclostrobin + metiram were significantly not different from respective controls at field dose regarding parasitism by females emerged from all immature treated stages. The field doses of HaNPV, bispyribac sodium, nicosulfuron, chlorothalonil + procymidone and pyraclostrobin + metiram were statistically similar with respective controls regarding parasitism of previously treated eggs (no-choice test). All four residual treatments of HaNPV, chlorantraniliprole, acetamiprid, spinetoram, fipronil and abamectin were statistically at par with each other at field dose regarding adult mortality. HaNPV, spiromesifen, myclobutanil were statistically similar in all the four residual treatments with respective controls at field dose regarding parasitism based on residual toxicity.

It is concluded that the above mentioned chemicals found statistically at par

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Chapter 1

INTRODUCTION

One of the most significant challenges of the 21st century will be feeding the expanding global population particularly in developing countries (FAO, 2009; Niyaki *et al.*, 2010). The global population of 7 billion has been increasing by 70 million per annum, at 30% increase, that will reach to 9.2 billion by 2050 (Popp *et al.*, 2013). The increase in population growth led to increase in demand for food production by 70%, this can be correlated with changed dietary habits in developing countries towards high quality food, e.g. greater consumption of meat and milk products, and to the increasing use of grains for livestock feed, supplemented by the limited availability of additional agricultural land (Popp *et al.*, 2013).

To address this great challenge, greatly improved management of soils, water and energy will be required, and a clear focus must be made on devising ways and means to increase sustainability of modern agriculture. Modern agriculture including arable farming has developed a deep reliance on agrochemicals including pesticides to maintain maximum production, despite the considerable efforts made for the last few decades to minimize pesticide use (Nash and Hoffmann, 2012).

1.1) Brief History of Pesticides Application and Development of IPM

The brief history of pesticides trends in application in the last about one and half centuries against the variety of pests in agro-ecosystems outlined below.

a) The inorganic and natural products era (1870-1944)

Prior to the development of the synthetic pesticide dichloro-diphenyl-trichloroethane (DDT), the early pesticides used to control agricultural pests were derived from the inorganic heavy metals (e.g., lead arsenate) or were derived from plant toxins (e.g. nicotine and rotenone). Generally, such pesticides were moderately effective against the pests (Croft, 1990). The researchers of that period (1880s) faced the problem of occasional resurgence of pests related to chemical control (Ripper, 1956; Bartlett, 1956).

b) The synthetic-organic pesticides era (1945-1960)

The modern era of insect pest control began with the first development and use of the synthetic pesticide DDT in 1939 and its release for pest control in agriculture during 1945 (Croft, 1990). The emergence of chlorinated hydrocarbons, including DDT, dieldren, aldrin and benzenhexachloride (BHC), subsequently led to synthesis of the organophosphates, including parathion and summithion, etc. (Croft, 1990).

By the late 1950s, carbamates such as carbaryl were being synthesized and these synthetic insecticides were all being widely used (Croft, 1990). The wide use of synthetic pesticides was due to their effective suppression of many pests (Hajek, 2004). During this period, pesticides were almost exclusively evaluated for toxicity to pests, and generally the effects on the natural enemies were ignored (Croft, 1990).

The over-reliance on synthetic pesticides from late 1940s to mid 1960s has been called “the dark ages” of pest control (Peshin and Dhawan, 2009). The continuous use of synthetic pesticides contributed to resistance in pests (Soranjani, 1998), and led to mass appearance of secondary pests (Norris *et al.*, 2003), adverse impacts on human health and environmental quality (Ripper, 1956), pest resurgences, adverse impacts on non-target organisms including beneficial species, and increased costs of

production (Robert *et al.*, 1985). These alarming problems emerged toward the end of this era and contributed to the development of the concept of integrated pest management, IPM (Croft, 1990).

c) The IPM era (1961-1980)

Initially, IPM was considered integration of chemical and biological control (Bartlett, 1956; Pickett *et al.*, 1958; Stern *et al.*, 1959; Perkins, 1982) and was focused on localized pest elimination. The integrated approach was based on the premise that pesticides could have a minimum impact on the natural enemies of the pest if applied at the correct time and under correct conditions (Peshin and Dhawan, 2009). However, in the 1960s, the concept of “pest management” broadened and included other suppressive tactics such as semiochemicals, host plant resistance, cultural control, and use of microbes (Peshin and Dhawan, 2009).

Use of DDT was widely banned due to environmental risks in the 1970s and in 1972; the microbial insecticide *Bacillus thuringiensis* Berliner (Bt) was introduced to control caterpillar pests (Peshin and Dhawan, 2009). Thus, the concept of pest eradication of IPM was shifted to and became based more on ecological principles (Croft, 1990). The determination of the adverse impacts of the pesticides on natural enemies were greatly emphasised (Croft, 1990), and sublethal effects on beneficials also began to be studied (Adam, 1960). While development of pesticide resistance in natural enemies also began to be examined (Croft, 1990).

d) Current era (1981-present)

During the current era, selectivity and ecological impacts of pesticides, pest resistance and resistance in natural enemies are being evaluated and utilized in designing pest management programs (Croft, 1990). During the 1980s, the emphasis in IPM

was shifted from chemical control to more active use of additional control tactics, including expanded use of cultural controls, resistant varieties and biological control (Peshin and Dhawan, 2009).

One of the revolutionary changes that has occurred in IPM since the introduction of pesticides is the introduction of transgenic pest-resistant crops in 1996 (Peshin and Dhawan, 2009). Moreover, major studies have been conducted to assess the impact of lethal as well as sublethal effects of different pesticides on biological control agents, allowing integration of biological and chemical control that is based on ecological principles.

1.2) Brief History and Concept of IPM, and Use of Chemical Control

The concept of integrated pest management (IPM) was developed during the late 1950s by entomologists at the University of California (Stern *et al.*, 1959), USA, in response to two major problems resulted by application of insecticides: the development of resistance in pest species, and the destruction of insect natural enemies (Peshin and Dhawan, 2009). The term “Integrated Pest Management” was used for the first time by Smith and van dan Bosch (1967) and in 1969 this term was formally recognized by the US National Academy of Sciences (Peshin and Dhawan, 2009).

This concept consisted mainly of integrated use of insecticides with biological control of insect pests (Norris *et al.*, 2003) - and brought applied ecologists and bio-control experts together (Perkins, 2002). A decade later this concept had evolved and the concept of “integrated control” which combined and integrated biological and chemical control based on economic threshold concepts was put forward (Stern *et al.*, 1959). Rachel Carson (1962) wrote the book “Silent Spring” that made the public and the scientists aware of the serious problems caused by the use of pesticides. During

the last 30 years, IPM has played a critical role in managing and suppressing pest populations below economic threshold levels (Baloch and Haseeb, 1996).

IPM has been defined in many ways. A panel of experts on integrated pest control at the UN Food and Agricultural Organization (FAO), Rome, has defined IPM as: A pest management system that in the context of the associated environment and the population dynamics of the pest species, utilizes all suitable techniques and methods in as compatible a manner as possible and maintains the pest population at levels below those causing economic injury (FAO, 1968). However, United States Department of Agriculture in 1996 defined IPM (Stoddard *et al.*, 2010) as, “sustainable approach to integrate biological, cultural, physical, and chemical tactics in a manner that minimizes risk to economy, health, and environment”. The word “pests”, in this definition, includes pathogens and weeds as well as animals.

One of the goals of effective IPM is to integrate chemical and biological control. Therefore, it is desirable to select the chemicals that have not only the least negative effects on natural enemies’ key performance, but also provide suppression of the target pests (Desneux *et al.*, 2006). It emphasizes the need-based use of selective pesticides instead of using traditional calendar or other uninformed approaches to application timing and contributes to reduced use of insecticides with safety concerns to environment and human health (Peshin and Dhawan, 2009).

The use of pesticides in IPM is a major effective tactic due to ease in application, affordability of use, and efficacy and consistency in reducing pest populations (Endo and Tsurumachi, 2001; Zhao, 2000). During the last five decades, pesticides

contributed significantly in the traditional crop protection that resulted in high productivity of crops (Amano and Haseeb, 2001).

1.3) Natural Enemies and Pesticides in IPM

Biological control agents or natural enemies play a key role in agricultural production and can help to minimize the use of synthetic chemicals (Petersen, 1993; Thomson and Hoffmann, 2010). They constitute a key component of many integrated pest management programs (De Bach and Rosen, 1991; Youn *et al.*, 2003), and include predators (feeds on prey), parasitoids (parasitize and kill host) and pathogens (disease-causing agents).

Successful biological control is based on availability or efficient establishment of natural enemies, maintenance of appropriate natural enemy numbers in the field, and effective manipulation of enemy behavior, if needed, to manage pests. Natural enemies should exhibit a strong functional response, have strong foraging and reproductive capacities, persist in pest patches, and prioritize their feeding/reproduction on target pests (Waage, 1983).

The adverse impacts of pesticides on natural enemies have highlighted the need of research and establishment of pest control tactics besides pesticides, including biological control (Jervis, 2005). As long-term survival of natural enemies within agro-ecosystem is affected through sublethal impacts of pesticides on their ability to manage the density of pests (Amano and Haseeb, 2001). The use of biological control is in progress due to adverse impacts of many conventional pesticides on the environment and food, and their failure based on the development of pesticide resistance in the pest insects (Dent, 1993).

Pesticides impact on biological control agents could be reduced by continuous releases of natural enemies and the number to be released should be adapted to the increase in level of resistance to pesticides in pests (Liang *et al.*, 2015). Effective management of population density of the pests requires inundative release of natural enemies along with selected insecticides, which is harmless/low toxic for the biological control agents (Preetha *et al.*, 2009). *Trichogramma* inundative releases in the fields are generally recommended during the pupal or adult stages of the parasitoids (Smith, 1996). Therefore, the combination of biological and chemical control has been an efficient strategy to prevent or slowing emergence of evolution of resistance in pests to pesticides (Khan *et al.*, 2015; Liang *et al.*, 2015).

1.4) Biological Control

The safe, effective and sustainable management of insect pests demands use of strategies that encourage biological control (Lou *et al.*, 2013). Biologically based pest management includes use of natural enemies for management of pests. It also includes several other approaches, including conservation of natural enemies, the sterile male method, host plant resistance and sex attractants. Biological control with beneficials (natural enemies) is an integral part of IPM and is an effective control tactic for reducing pest densities and adverse effects of pesticides (Debach, 1964). This tactic is utilized around the world at low cost and with very little adverse impact on the environment or on humans (Cunningham *et al.*, 2005). This tends to be delayed in action but have long-term effectiveness compared with chemical control and has general advantages, including reduced exposure of growers and workers to harmful pesticides, no residual activity by the marketed product, and extremely low environmental pollution (Yang *et al.*, 2014).

1.5) Principles of Integration: Pesticides and Biological Control for Effective IPM

Integration of natural enemies with pesticides for more sustainable management of pests is one of the major historical focuses of IPM (Khan *et al.*, 2015). The integration of chemical and biological control measures under the umbrella of IPM can lead to effective management of pest problems. The principles of effective integration of chemical and biological control measures are briefly given below.

a) Use of pesticides compatible with natural enemies

More effective integration of biological and chemical control tactics is required to fulfill the growing demand of reduced input of pesticides in agricultural systems (Khan *et al.*, 2015). In IPM, the key component is to maintain the normal functioning (efficacy) of natural enemies against pests. Successful implementation of biological control in crop systems often depends in part on the compatibility of natural enemies with pesticide use in the targeted agricultural system (Biondi *et al.*, 2012) as beneficial arthropods, especially parasitoids, are typically more severely adversely affected by pesticides compared to the target pest species (Croft and Brown, 1975). Therefore, such differential susceptibility can result in serious compatibility problems for the integration of both chemical and biological control tactics in IPM programs (Khan *et al.*, 2015).

The compatibility of pesticides with natural enemies depends on several factors, i.e. (1) type of natural enemy (parasitoid or predator: parasitoids tend to be more susceptible to pesticides than predators), 2) natural enemy species within a specific group, 3) stage of development of the natural enemy, 4) rate of application, (5) timing of application, (6) type of chemical, and (7) its mode of action (Cloyd, 2005).

For successful integration, laboratory and field bioassays are needed to evaluate the compatibility of pesticides with beneficial arthropods (Croft, 1990; Hassan, 1989 and 1992; Vierre *et al.*, 1996). The pesticides tested and found compatible to the specific natural enemy in the laboratory will likely also have no adverse impacts on the same beneficial arthropod in the field, because of the extreme exposures typically used in the laboratory (EPPO/OEPP, 1990), and usually no further testing is required under semi-field and field conditions (Amano and Hasseeb, 2001). However, further testing is recommended when a pesticide is found to be harmful in the initial toxicity test in the laboratory (Amano and Hasseeb, 2001). Moreover, Hassan *et al.* (1994) suggested field tests for residual toxicity and evaluation of pesticide effects on plants and soil fauna.

b) Adoption of novel approaches

The adverse impacts of broad spectrum pesticides are commonly responsible for high mortality and/or adverse effects on the efficacy of natural enemies as biological control agents (Ruberson *et al.*, 1998). Use of chemicals that are effective against target pests but induced little or no negative effects on their natural enemies is ideal (Preetha *et al.*, 2010; Bastos *et al.*, 2006; Khan *et al.*, 2015). Thus, an intense effort has been carried out to assess a wide range of insecticides, and a number have been determined to be safe to biological control agents worldwide, including to genus *Trichogramma* (Tiwari and Khan, 2001; Paul and Agarwal, 1989).

Replacement of broad-spectrum insecticides by the use of novel approaches, including use of 1) selective/newer pesticides, and 2) biorational pesticides in IPM technology for controlling key lepidopteran pests is very important for maintenance and smooth functioning of select parasitoids (Grzywacz *et al.*, 2010). The broad-

spectrum insecticides were replaced with other novel classes, including neonicotinoids, diamides, benzoylureas, spinosyns and tetroneic acid derivatives etc. These chemicals exert variable risk effects on natural enemies (Bostanian *et al.*, 2009).

Newer pesticides tend to be more specific/selective and more compatible with biological control agents than broad range synthetic chemicals (Croft, 1990), and have helped to manage resistance that insect pests developed against the broad-spectrum chemicals (Gentz *et al.*, 2010). As, Rodrigues *et al.* (2015) described that the developments of novel insecticides are based on the need to overcome pesticide resistance. Thus, insecticides with novel chemistries and novel modes of action provide greater potential to conserve biological control agents in agro-ecosystems, while at the same time providing effective suppression of the target pests (Brunner *et al.*, 2001). Furthermore, the outbreaks of secondary pests could be reduced by integrating selected pesticides with natural enemies (Nasreen *et al.*, 2004).

Use of environmental friendly biorational pesticides such as botanicals (Neem seed kernel extract against sucking insect pests as well as bollworms), *Bacillus thuringiensis* and HaNPV against caterpillar pests contribute towards conservation of native as well as augmented biological control agents, and reduction in insecticide use.

c) Adoption of extra practices

The undesired impacts of pesticides on biological control agents can be reduced by modifying the pesticide use including adoption of such practices if applicable, as 1) mode of application (spraying or seed treatment) and coverage, 2) reducing dose concentration, or 3) number of applications, and 4) the compatible timing of spraying (Way, 1986; Martinson *et al.*, 2001). Furthermore, the integration of classical (importation) and augmentative biological controls with existing pesticides relies on several

options: 1) to develop pesticide-resistant strain of natural enemies, and alternately 2) timing release of natural enemies in order to avoid or decrease adverse impacts of pesticides on natural enemies, if pesticide impacts on target natural enemies are known (Udayagiri *et al.*, 2000). The first approach has some drawbacks including requiring extensive time to select for resistance, and success mostly limited to predatory mites.

1.6) Methods in Biological Control

Beneficial arthropods are typically present along with the crop pests. If the natural enemies are not present in an area or their numbers are insufficient to suppress the insect pests, human intervention can introduce, augment or conserve natural enemies (if they are available) for biological control. The three approaches in biological control (DeBach, 1964; Waage and Greathead, 1986; Van Driesche and Bellows, 1996; Knutson, 1998) are briefly described as below.

a) Importation

Importation or classical biological control is the importing of specific biological control agents from the region of origin of a typically exotic invasive pest species, and to introduce these exotic natural enemies into the concerned region, in order to establish permanent populations of the natural enemy throughout the infested region to provide long-term suppression of the target pest. Different species of *Trichogramma* were also imported; e.g., *T. euproctidis* was imported from Russia, and released in cotton in Georgia in 1975 (Johnson *et al.*, 1986). Despite numerous successes worldwide, importation programs more often meet with failure due to the inability of the novel natural enemy to establish in the target region, or the candidate natural enemies

have been found to pose significant risks to non-target organisms, or their impact after establishment is trivial (Wajinberg *et al.*, 2008).

b) Augmentation

Augmentation is the periodic release of biological control agents in an area, where the natural enemy does not exist in adequate numbers in nature to keep pest populations below economic threshold (Knutson, 2005). Successful augmentation biological control programs require not only knowledge of number of biological control agents to be released relative to pest abundance, but also the spatial release pattern in a given crop and habitat for mass release. The cost/benefit ratio for natural enemy production, utilization and crop yield/quality outcomes are key considerations in using mass releases compared to other tactics, particularly use of pesticides. The release is accomplished either by inundative or inoculative method.

Inundative - release is the single or multiple mass releases of the reared natural enemies to keep pest outbreaks under control. Such releases are helpful in preventing the active damage by the mass population of pests. The goal of inundation is direct and rapid mortality of the pests by biological control agents: playing the role of biological insecticides (Stinner, 1977). Effective inundative release is based on: 1) having large numbers of appropriate natural enemies for release, 2) proper timing of release (presence of target hosts or prey), and 3) favorable weather and cropping conditions (Knutson, 2005). *Trichogramma* have been used in inundative releases more than any other natural enemy (Stinner, 1977).

Inoculative - releases include one or several releases of relatively low natural enemy numbers to initiate a natural enemy population of sufficient size locally to suppress pest populations, as they begin to increase (Knutson, 2005). The population of

biological control agents steadily increases on the target pest, or on an alternate host to a level to prevent the pest from causing damage later in the season. For example, in China, *Trichogramma* are inoculatively released in spring to establish populations in gardens, which search out hosts in the adjacent cotton fields in the late season (Li, 1994).

c) Conservation

Conservation biological control (CBC) is the modification of the agro-environment, or existing management tactics to conserve and enhance specific biological control agent to manage the pests (Debach, 1974; Eilenberg *et al.*, 2001). Thus, CBC is the artificial alteration of the agro-ecosystems to improve survival, adjustment and behavioral functioning of biological control agents, and to enhance their pest management capability (Barbosa, 1998; Landis *et al.*, 2000).

Applying conservation techniques include such practices as use of selective chemicals such as *Bacillus thuriangiensis* Berliner (Bt) insecticide, and growing strip crops in and around fields to provide sources of food and habitat for enhancement of biological control agents. For example, interplanting of rye grass in seed corn fields decreased soil temperatures, and this fosters survival of released *Trichogramma* (Orr *et al.*, 1997). Moreover, successful conservation can involve use of compatible pesticides with the release of natural enemies well before, or well after insecticide application (Thomson *et al.*, 2000). The application of selective insecticides can be justified by two reasons: 1) It is based on the principles of conservation of natural enemies in the agro-ecosystem (Carvalho *et al.*, 2003), 2) selective insecticides may be valuable to effectively overcome increasing pesticide resistance (Nabil and Wakeil, 2013). Furthermore, care must be taken to ensure conservation of generalist natural enemies

in cropping systems as this will enhance sustainability of cropping systems where biological control is integrated with compatible pesticides (Khan *et al.*, 2015).

Conservation approaches require knowledge of the agro-environment and insect species in the field (Croft, 1990), as well as an understanding of how agronomic practices interact with the biotic environment to influence pest and natural enemy populations. The growers apply conservation techniques and use natural enemies as one of the most important and potentially cost-effective biological control practices (Niu *et al.*, 2014).

The type of biological control strategy - i.e. importation, augmentation and/or conservation-determines the implications of pesticide impacts on natural enemies. The determination of adverse impacts of pesticide residues on natural enemies' survival and efficacy is required well before application to decide whether or not to integrate inundative release with the use of pesticides in agricultural systems more easily than programs involving inoculative or classical biological control (Udayagiri *et al.*, 2000).

1.7) *Trichogramma* Species: Important Natural Enemies Globally

Parasitic wasps of the genus *Trichogramma* Westwood are tiny egg parasitoids of mean size between 0.2 and 1.5mm (Bastos *et al.*, 2006) belonging to the family Trichogrammatidae in the order Hymenoptera. These wasps are distributed world wide (Hussain *et al.*, 2010). There are around 210 described species of *Trichogramma* worldwide, but even more, most certainly, based on the prevalence of cryptic species in this taxon (Pinto, 2006).

Trichogramma spp. parasitize more than 400 species of arthropods from 24 families belonging to Lepidoptera, Hymenoptera, Coleoptera, Diptera, Neuroptera,

etc. (Bao and Chen, 1989; Guo, 1998, Sattar *et al.*, 2011, Khan *et al.*, 2015). They commonly parasitize lepidopterous pests, including sugarcane borer *Chilo sacchariphagus* (Bojer), maize stem borer *Chilo partellus*, American bollworm *Helicoverpa armigera* (Hübner) and corn earworm *Helicoverpa zea* (Boddie). *Trichogramma* even have been used to suppress stored grain caterpillar pests; e.g., *Ephestia kuehniella* (Zeller) and *Ephestia elutella* are parasitized by *Trichogramma evanescens* Westwood and *Trichogramma embryophaga* (Scholler *et al.*, 1996). Furthermore, Adarkwah, *et al.* (2015) concluded that *Trichogramma evanescens* has the potential ability to locate host and parasitize eggs of rice moth *Corcyra cephalonica* both on paper and jute bags, and could be successfully used for the biological control of moth pests in bagged stored products.

Some of the common examples of pest control worldwide by the *Trichogramma* are: control of *Ostrinia* spp., *Heliothis* spp. and *Cnophalocis* spp. on grain crop in China (Li, 1984, Shoeb, 2010), control of *Helicoverpa* eggs on corn in Brazil (De Sa and Parra, 1994), control of the noctuids *Brusseola fasca* and *Jesamina calamistis*, pyraloids *Chilopartellus orichalociliellus* and *Eladana saccharins*, which attack maize in east Africa (Bonhof *et al.*, 1997), control of *Ostrinia nubilalis* on corn fields in Germany and Switzerland (Shoeb, 2010), *Chilo agamenon* on sugar-cane and rice, *Anarsia lineatella* on peach and apricot, *Lopezia botrana* and *Ephestia* spp on date palms in Egypt (Abbas, 2004, Shoeb, 2010) and control of the cranberry fruitworm, *Acrobasis vaccinii*, in the U.S.A (Simser, 1994).

Each major continent has been dominated by one or two *Trichogramma* species in both field studies and in commercial application: *T. chilonis* (Ishii) in Asia, *T. dendrolimi* Matsumura in China, *T. evanescens* in Europe and the former USSR, and

T. pretiosum Riley in both North and South America (Hassan, 1993; Smith, 1996; Lenteren, 2000; Parra and Zucchi, 2004).

There have been great improvements in the mass production technology and release technique of *Trichogramma* in recent years, which led to more practical and efficient parasitoid production and field application (Wang *et al.*, 2014). The mass rearing programs have been developed for only 19 species, which are used for augmentative releases worldwide (Li, 1994), including species most commonly collected from crops and orchards: *T. atopovirilia*, *T. brevicapillum*, *T. deion*, *T. exiguum*, *T. fuentesi*, *T. minutum*, *T. nubilale*, *T. platneri*, *T. pretiosum*, and *T. thalense* (Olkowski and Zhang, 1990). These species have been used throughout the world as natural enemies of insect pests (Smith, 1996).

Trichogramma species have played a significant role in successfully managing pest problems in many agro-ecosystems (Smith, 1996; Sorokina, 1999; Hussain *et al.*, 2010), and are the most widely reared of the egg parasitoids for biological control programs. The wide use of *Trichogramma* in biological control is based on two main reasons, including 1) easy and inexpensive mass rearing/production, and 2) they attack and effectively parasitize eggs of many important crop pests and can withstand broad environmental conditions (Nadeem and Hamed, 2008; Nadeem *et al.*, 2009, 2010; Nadeem and Hamed, 2011).

Development of an efficient biological control strategy of insect pests by parasitoids depends primarily on knowing about population dynamics of parasitoid–host interactions (Maalouly, *et al.*, 2015). *Trichogramma* releases under optimal conditions can provide results equivalent to insecticide treatments, but efficacy of this parasitic wasp is not consistent because of poor adaptation to local environment

including pesticide stress (Ballal *et al.*, 2009). *Trichogramma* wasps can develop between 18 and 30°C (Butler Júnior and López, 1980; Harrison *et al.*, 1985; Pak and Heiningen, 1985; Pratissoli, *et al.*, 2005). However, mortality rate increase with higher temperatures (Gross, 1988; Cabello and Vargas, 1989; Pratissoli, *et al.*, 2005).

1.8) Origin of Biological Control with *Trichogramma*

One of the first cited examples of biological control is the manipulation of predatory ants *Oecophylla smaragdina* F. (Hym., Formicidae) by Chinese citrus growers, dating back to ancient times. They used predatory ant nests in mandarin orange trees to help control leaf feeding insect pests and used bamboo bridges to help the ants cross between trees (DeBach, 1964).

Active biological control with *Trichogramma* originated in 1900 with the introduction of two exotic species from Austria into the USA for control of the Lepidopteran *Euproctis chrysorrhoea* L. (Luck and Forster, 2003). About this same period, mass rearing of *Trichogramma minutum* was started on *E. chrysorrhoea* and parasitoids were stored at low temperatures for release at the appropriate time, when host eggs would be available. Nevertheless, biocontrol of *E. chrysorrhoea* with *Trichogramma* was unsuccessful.

The first attempts at using *Trichogramma* in applied biological control programs failed due to lack of knowledge regarding (1) appropriate release rates for the parasitoids in relation to host density, (2) the best strains or species of *Trichogramma* for control of the specific target, (3) quality control maintenance for laboratory-reared insects, (4) releasing devices and methodologies, (5) pest population dynamics, plant phenology and competition with resident natural enemies, and (6) adverse impacts of

broad spectrum pesticides used simultaneously with parasitoid release (Cônsoli *et al.*, 2010).

1.9) Adverse Effects of Pesticides on *Trichogramma* Species: Lethal and sublethal Effects

The natural enemies have three modes of uptake of pesticides: 1) direct contact, 2) residual contact, and 3) food chain uptake and transfer (Croft, 1990). Understanding of both lethal and sublethal effects is needed to develop successful integration of biological and chemical control measures (Croft, 1990; Staple *et al.*, 2000; Hamed *et al.*, 2010; Sohrabi *et al.*, 2013). The lethal effect of pesticides is acute mortality of beneficial species, while the sublethal effect is the decrease in the biocontrol capacity of beneficial through alterations in behavior (foraging e.g., feeding behavior modified by pesticides acting as repellents, inhibitors, or olfaction disruptors), reduced fertility (parasitism efficiency), altered sex ratio, protracted development and reduced lifespan (Elzen, 1990; Ruberson *et al.*, 1998, Cloyd, 2005).

Trichogramma are affected by pesticides through all three means of uptake. Persistent chemicals can intensify the adverse impacts (lethal and sublethal) on *Trichogramma* due to residual toxicity for a longer duration post spray (Hewa- Kapuge *et al.*, 2003). Pesticides may exert lethal effects on the parasitic wasps in immature stages inside the host eggs or in the adult stage. Sublethal effects on the tiny parasitoid include decreased parasitism efficiency and longevity, altered foraging behavior and sex ratio, etc. Less effort has been carried out for the assessment of sublethal effects of pesticides than lethal effects on *Trichogramma* spp. and most of these only related to parasitism (Hagley and Laing, 1989; Brar *et al.*, 1991).

1.10) Interactions of Pesticides and *Trichogramma*: Why?

There is great value in describing the nature of pesticide effects on natural enemies in general as well as specific to *Trichogramma*.

a) Nature of pesticide effects in general

Natural enemies are generally more susceptible to the pesticides than their plant feeding hosts or prey (Khan *et al.*, 2015). Similarly, parasitoids are typically more susceptible to pesticides as compared to predators (Croft, 1990; Hassan, 1989 and 1992). Two different species of biological control agents may experience different levels of toxicity with the same chemical; similarly, the same species of natural enemy may be affected differently by the same chemical in different life stages (Stark and Banken, 1999) and/or across different geographic races or biotypes (Croft, 1990; Bostanian *et al.*, 2000).

Pesticide toxicity to predators and parasitoids is generally highest for insecticides, followed by herbicides, acaricides and fungicides. However, even within the same classes of chemicals, there can be considerable variability in effects on biocontrol agents. Within insecticides, generally speaking, toxicity level increases from the early inorganics to the synthetic pyrethroids; moreover, more selectivity and less adverse effects are exerted by microbial insecticides and insect growth regulators (Theiling and Croft, 1988).

b) Adverse effects of pesticides specifically on *Trichogramma*

Parasitoids play key roles in regulating density of insect pests; however, they are adversely affected by various insecticides (Delpeuch *et al.*, 2013). The synthetic pesticides adversely affect *Trichogramma* generally in the same way as other egg parasitoids belonging to the family Trichogrammatidae. Therefore, successful IPM requires an integration of biological and chemical control.

In the light of the aforementioned facts, it is very important to determine the possible adverse effects of pesticides on *Trichogramma*, based on pesticide interaction with the parasitoid, and as a result of an intense effort, a range of insecticides has been determined to be safe to the beneficials worldwide, with focus on *Trichogramma* (Tiwari and Khan, 2001; Paul and Agarwal, 1989), in order to successfully integrate *Trichogramma* with chemical control for effective management of pests.

1.11) *Trichogramma chilonis* (Ishii): Biological Control Agent

Trichogramma chilonis (Ishii) is widely distributed throughout the Indian sub-continent, and has been used to successfully manage lepidopteran and heteropteran insect pests in various agro-ecosystems (Manjunath *et al.*, 1985, Ananthakrishnan, *et al.*, 1991), including *Chilo* spp. in sugarcane, maize and *Helicoverpa armigera* in cotton, tomato and lady's finger in India (Singh, 2001). This parasitoid is also widely distributed in Nepal, China, Malaysia, Indonesia, Philippines, Japan, South Africa, West Indies, Europe and Hawaii (USA), and has a high biocontrol potential against lepidopteran larvae (Rabb *et al.*, 1968; Lingathurai *et al.*, 2015). It has been successfully used in inundative and inoculative biological control programmes worldwide (Pinto *et al.*, 1994; Lingathurai *et al.*, 2015)

T. chilonis is an important egg parasitoid of lepidopteran pests in Pakistan (Sattar *et al.*, 2011). Some of the common hosts in Pakistan-i.e., sugarcane borer (*Chilo sacchariphagus*) in sugar cane, diamondback moth (*Plutella xylostella*) in cabbage and other vegetables, and cotton bollworms (*Helicoverpa armigera*) in cotton and corn-are successfully managed by *T. chilonis* (Rasool *et al.*, 2002). American bollworm *Heliothis armigera* is controlled mainly by *T. chilonis* in grain crops (Manjunth *et al.*, 1985, Ananthakrishnan *et al.*, 1991). It is also an important biological control agent

for control of leaf-folders and management of pests in rice (Preetha *et al.*, 2009), as Sagheer *et al.* (2008) also described *T. chilonis* as effective parasitoid of rice leaf folder *Canphalocrocis medinalis* (Guenée) in Pakistan.

In Pakistan, this minute parasitoid is produced commercially by several organizations including the Pakistan Agriculture Research Council (PARK), Center for Agriculture and Biosciences International (CABI), provincial research stations and sugar mills, etc. at different locations throughout the country.

1.12) Dissertation Objectives

The current studies deal with determination of lethal and sublethal effects of 15 selected/novel pesticides against *T. chilonis*. The studies were conducted at Nuclear Institute of Food and Agriculture, Peshawar, Pakistan. The pesticides tested against *T. chilonis* in the laboratory were **(i) 6 insecticides**: 1) chlorantraniliprole, 2) fipronil, 3) acetamiprid, 4) spirotetramat, 5) spinetoram, and 6) *Helicoverpa* nuclear polyhedrovirus (HaNPV), **(ii) 2 miticides**: 1) spiromesifen and 2) abamectin, **(iii) 3 herbicides**: 1) bispyribac sodium + polyethoxylated fatty alcohol (adjuvant), 2) nicosulfuron, 3) haloxyfop-p-methyl, and **(iv) 4 fungicides**: 1) pyraclostrobin + metiram, 2) myclobutanil, 3) trifloxystrobin + tebuconazole, and 4) chlorothalonil + procymidone.

The pesticides were tested against *T. chilonis* to determine: 1) lethal effects on the immature stages, in addition to the sublethal effects: effect on subsequent parasitism by the female wasp emerged from the treated host eggs, 2) mortality of adult parasitoids exposed to treated glass surface and subsequent parasitism by the exposed female during 24 h period, and 3) effects on parasitism of treated host eggs (no-choice design) by the minute parasitoid.

T. chilonis was selected as a parasitoid for the current studies based on the fact 1) parasitic wasps of the genus *Trichogramma* are distributed world wide, and the most widely reared of the egg parasitoids for biological control programs, 2) because of its key role in management of hundreds of lepidopteran and other insect pests in variety of crops, vegetable and orchard, and 3) moreover, *T. chilonis* is widely distributed in the Indian subcontinent region compared to other parasitoids. Almost all chemicals selected for the current studies were widely used worldwide.

The assessment of the current laboratory work was carried out in order to acquire to supplement information to decide how best to integrate both biological and chemical control strategies for effective control of lepidopteran pests by *Trichogramma*.

Chapter 2

LITERATURE REVIEW

Bull and House (1983) evaluated the adverse impacts of different insecticides on parasitism by *T. pretiosum*. Three doses of each pesticide (at g/ha), including methomyl (70, 140, 280), permethrin (56, 112, 224), methyl parathion (560, 1121, 1681), chlordimeform (70, 140, 280) and thiodicarb (280, 560, 840) were sprayed on the eggs of tobacco budworm, *Heliothis virescens* (F.), in the laboratory and greenhouse, either before or following exposure to *T. pretiosum*, to evaluate the effect of different insecticides on parasitism of the host eggs. Methomyl, permethrin and methyl parathion severely inhibited parasitism by 70-100%, while chlordimeform moderately decreased parasitism by 30%. Thiodicarb caused no effect on parasitism by the parasitoid. More than 90% of emergence from the parasitized eggs was observed when the eggs

were exposed before treating with thiodicarb. The residual toxicity of methomyl, chlorimeform and thiodicarb lasted seven days after treatment.

Schuld and Schmuck (2000) conducted experiments to evaluate the spray effect and determined that thiacloprid 480SC (0.025%) had no significant negative impacts on the immature stages of the egg parasitoid *Trichogramma cacoeciae* Marchal. However, the successful emergence of the parasitoids was significantly decreased when the treated host eggs were stored under greenhouse conditions optimized for the egg parasitoid *T. cacoeciae*, i.e. $25/15 \pm 3^{\circ}\text{C}$, 40- 60% air humidity, 16:8 L: D cycle. This is assumed to occur due to ingestion of thiacloprid residues from the host egg cuticle during emergence.

The thiacloprid-treated host eggs subjected to field conditions before parasitoid emergence (at temperatures 2 m above ground: min. -1.6 - 19.2°C , max. 7.5 - 32.2°C ; air humidity: min. 33-67%, max 77-99%; max. precipitation rates: 12.9-45.2 mm; max. sunshine: 10.8-14.7 hr; max. energy input: 1.674 - 3.052 kJ/cm^2 ; max. wind speed: 7.9 - 19.4 m/sec) did not cause statistically significant reduction in emergence of *T. cacoeciae*. Moreover, the fitness (parasitism efficiency) of parasitoids emerging from host eggs treated at larval and pupal stages of parasitoid was evaluated by providing parasitoids daily with 50 fresh host eggs of *S. cerealella* over their entire life-time and was found not significantly different from the water-treated control.

Suh et al., 2000 tested different insecticides (at rate in g a.i/ha) including lambda-cyhalothrin (45), cypermethrin (113), thiodicarb (840), profenophos (1121), spinosad (70), methoxyfenozide (393) and tebufenozide (280) to assess their toxicity on emergence, adult survival, sex ratio and longevity of *Trichogramma exiguum* Pinto &

Platner. Only methoxyfenozide and tebufenozide adversely affected parasitoid emergence from *Helicoverpa zea* (Boddie) host eggs when exposed at different immature stages (larvae, prepupae, or pupae).

None of the tested chemicals significantly affected sex ratio of the emerged adults regardless of the developmental stage treated. However, different chemical treatments, and also the immature stage treated, affected significantly the mean longevity of emerged *T. exiguum* females. Based on LC₅₀ values, spinosad and profenofos were found to be the most harmful insecticides to female *T. exiguum*, followed by lambda cyhalothrin, cypermethrin and thiodicarb. Insecticides field-weathered for four to six days on cotton leaves showed no residual toxicity against females of *T. exiguum* regardless of the chemical.

Brunner et al., 2001 conducted experiments to assess the impacts of different pesticides on two parasitoid species, *Colpoclypeus florus* (Walker) and *Trichogramma platneri* Nagarkatti. Organophosphate and carbamate insecticides induced high toxicity to both parasitoids in topical applications, but foliar residues of some products were non toxic 7 days after treatments.

When applied topically, abamectin and the organophosphate, including azinphosmethyl, chlorpyrifos, diazinon, dimethoate, methyl parathion, methidathion, phosmet were highly toxic to *C. florus*, but were moderately to highly toxic to *T. platneri* at 10% of the recommended. The exception was methyl parathion, which was nontoxic to *T. platneri* at same dose. Carbamate insecticides (carbaryl, formetanate hydrochloride, oxamyl) showed high toxicity *T. platneri* at 50%, pyrethroids esfenvalerate and permethrin were moderate in toxicity at 10%, imidacloprid was highly toxic at 100%,

insect growth regulators (fenoxycarb, tebufenozide, diflubenzuron) were nontoxic, low, or moderate in toxicity at 10%, 50% and 100%, respectively, insecticidal soap and *B. thuringiensis* products were highly toxic at 100%, while endosulfan caused low toxicity to *T. platneri* at 10 and 50% of the recommended rate.

The topical applications with ppm/100 gallons of water based on both imidacloprid (48) and abamectin (7) were highly toxic to adult survival of *T. platneri*, but the same chemicals showed no residual toxicity after 24 h to the minute wasp. Insect growth regulators including tebufenozide (150) and methoxyfenozide (150) did not show toxicity either as topical applications or residues to the tiny wasp. The topical application of biorational pesticides including soap (1 gal/100 gal), oil and *B. thuringiensis* (4 oz/100 gal) products physically immobilized the *T. platneri*.

The residual effects demonstrated that organophosphate insecticides: methyl parathion, azinphosmethyl, and chlorpyrifos were highly toxic to adult survival of *C. florus*, when the adults were exposed to the treated leaves of “Oregon Spur” apple trees for 48 h at 1 through 21 days after treatments. Spinosad residues were highly toxic through 7 d but declined in toxicity at 14 d. Abamectin, imidacloprid, fenoxycarb, insecticidal soap, *B. thuringiensis* products, and horticultural mineral oil were non-toxic to *C. florus* at 1d.

Field-aged residues of the organophosphate insecticides, azinphosmethyl and chlorpyrifos were highly toxic to adult *T. platneri* exposed for 48 h at 1 through 21 d. The carbamate insecticide oxamyl and the pyrethroid esfenvalerate were highly toxic at 1 through 21 d. Field-aged residues of endosulfan were highly toxic at 1 and 3 d, moderately toxic at 7 and 14 d, and low in toxicity at 21 d. Abamectin residues were

moderately toxic at 1 and 3 d and nontoxic thereafter. Insecticidal soap residues were non-toxic to *T. platneri* at 1 d.

Nasreen et al., 2004 sprayed nine insecticides under semifield conditions to evaluate their effects on *T. chilonis* in host eggs (near emergence). Out of nine insecticides tested (at gm/ha a.i), the *B. thuringiensis* (39.6), spinosad (7.2), thiodicarb (800) and indoxacarb (62.6) were found relatively harmless for parasitoid emergence, while insecticides like profenofos (1250), chlorfenapyr (297), cypermethrin (62.5), abamectin (18) and endosulfan (1050) were found toxic to *T. chilonis*.

Williams and Price (2004) completed experiments on the assessment of contact residual toxicity of pesticides to the egg parasitoids *Anaphes iole* Girault and *T. pretiosum* by fumigation technique. The insecticides were tested (dose, kg a.i/ha), including thiamethoxam (0.1), cyfluthrin (0.045), spinosad (0.1), fipronil (0.043), oxamyl (0.28), lambda-cyhalothrin (0.034) and acephate (0.28). The experiments demonstrated differential susceptibility of adults of both parasitoids to spinosad, thiamethoxam, and oxamyl. Spinosad was more toxic to *A. iole*, followed by thiamethoxam and oxamyl. While thiamethoxam was more toxic to adult *T. pretiosum*, followed by spinosad and oxamyl.

Gandhi et al., 2005 carried out experiments in the laboratory to compare the relative toxicity of biopesticides, including *Pseudomonas fluorescens* strain pfi (3×10^8 cfu/g) and neem oil (azadirachtin-a, 3%), at 12 ml/l and 20 ml/l, respectively. The other insecticides tested (at gm a.i/l) were imidacloprid (350), quinalphos (250) and endosulfan (350) against *T. chilonis* and predatory green lacewing *Chrysoperla carnea* (Stephens). Biopesticides were found safer than chemical insecticides regarding development and behavior of these two natural enemies. The host eggs treated with *P.*

fluorescens experienced high parasitism of approximately 73% by tiny parasitoid and development of egg of *T. chilonis* and *C. carnea* approximately 72% and 75%, respectively. Neem oil induced parasitoid emergence, parasitism, and egg hatchability of 58.9, 59.3, and 63.1%, respectively. Other chemicals were found highly toxic to parasitism by tiny wasp and egg development of both natural enemies

Bastos et al., 2006 tested several chemicals, including insecticides, fungicides, herbicides and plant growth regulators against adult and pupae stages of *Trichogramma pretiosum* Riley, reared in the laboratory on two different hosts, the Angoumois grain moth *Sitotroga cerealella* Olivier and the Mediterranean flour moth *Ephestia kuehniella* (Zeller). The host eggs were directly sprayed when the parasitoid was in the pupal stage, while the adults of the parasitoid were exposed to the pesticide-treated eggs exposed for parasitism.

The chemicals were tested at doses of gm/300 liters (a.i) including alpha-cypermethrin (30), carbosulfan (120), deltamethrin (10.20), endosulfan (875), profenofos (510) and zeta-cypermethrin (108) significantly reduced both the percentage of emergence of and parasitism by *T. pretiosum* of treated *E. kuehniella* or *S. cerealella* eggs, while lufenuron (51) and metamidophos (900) caused reduction only in percentage of adult emergence for both types of host. Novaluron only affected wasp emergence from *E. kuehniella* eggs. Chlorfluazuron (12.75), diafenthiuron (400), diflubenzuron (15), fentin hydroxide (75), mepiquat chloride (51), novaluron (12), thiacloprid (48.96) and triflumuron (14.40) did not affect *T. pretiosum* emergence from the host eggs of *S. cerealella* treated while at pupae stage. The pesticides azoxystrobin (105), carbendazin + thiram (72+168), mepiquat chloride (51) and novaluron (12) were fou-

nd harmless for parasitism of *E. kuehniella* eggs by *T. chilonis*. The difference in the pesticides in degree of toxicity to parasitoids is based on the type of the treated host.

Moura et al., 2006 determined the impacts of several pesticides in the laboratory at g a.i/l, including acetamiprid (0.05), abamectin (0.018), cartap (1.25) and chlorpyrifos (2.40) on the larvae, pupae, and adults of the egg parasitoid *T. pretiosum*. Cartap and chlorpyrifos were the most toxic insecticides in reducing emergence of parasitoid from as well as parasitism of host eggs of *Anagasta kuehniella* (Zeller), where abamectin was found harmful to adult wasps (residue test on glass plates), slightly harmful to larvae, and moderately harmful to pupae in the same host eggs. Acetamiprid was moderately harmful to adults, harmless to larvae, and slightly harmful to pupae; cartap was harmful to adults, moderately harmful to larvae and harmful to pupae; chlorpyrifos was harmful to adults and pupae, but harmless to larvae of *T. pretiosum*.

Bueno et al., 2008 tested several pesticides against eggs, larvae and pupae of *T. pretiosum* (at doses, g a.i./200 liters), and found variability in impacts of pesticides on *T. pretiosum*. Esfenvalerate (7.5), and spinosad (24.0) were classified (IOBC/ WP-RS,1994): harmful (class 4), while chlorfluazuron (10.0), methoxyfenozide (19.2), lactofen (165.0), fomesafen (250.0), fluazifop (125.0), glyphosate (960), azoxystrobin + ciproconazol (60.0+24.0, respectively), azoxystrobin (50.0) and myclobutanil (125.0), were ranked as harmless to all immature stages of *T. pretiosum*.

Nevarez et al., 2009 tested the adverse impacts of three insecticides (at gm a.i/l dosage): deltamethrin, 2.8% EC (0.0125); lambda-cyhalothrin, 5.5% EC (0.025); *B. thuringiensis* variety Kurstaki 16000 U.I. (25×10^9 spores/l) and one fungicide, basic copper sulphate, 20% SP (25) by direct application to parasitized host eggs, being

cold-stored diapausing prepupae of *Trichogramma cordubensis* Vargas and Cabello after cold storage (at 3°C) for three different periods (60, 120 and 180 days).

Irrespective of the period of cold storage, both pyrethroids (deltamethrin and lambda-cyhalothrin) caused decreased emergence rates of *T. cordubensis* by 25% compared to the control. Lambda-cyhalothrin also negatively affected the longevity and fecundity of parasitoids cold stored for 60 days. Emergence rate was generally 80%, while longevity and fecundity of *T. cordubensis* are little or not adversely affected by the Kurstaki strain of *B. thuringiensis* and basic copper sulphate.

Preetha et al., 2009 tested nine insecticides: imidacloprid, thiamethoxam, chlorantraniliprole, clothianidin, pymetrozine, ethofenprox, BPMC, endosulfan, acephate and the product Virtako® (Syngenta; chlorantraniliprole 20% + thiamethoxam 20%) against adult *T. chilonis* by exposure of the adults to the insecticide-coated vials (scintillation) to assess their toxicity to the adult parasitoid. The highest toxicity to *T. chilonis* was shown by thiamethoxam, LC₅₀ at 0.0014 mg a.i/l, followed by imidacloprid (0.0027 mg a.i/l). The LC₅₀ values of acephate and endosulfan were 4.4703 and 1.8501 mg a.i/l, respectively, and both demonstrated low toxicity, when compared with other used insecticides. Thiamethoxam was found to be 3.2, 1.4 and 1.34 times more toxic than acephate, chlorantraniliprole and endosulfan, respectively. Based on risk quotient, only chlorantraniliprole was found to be harmless to adult parasitoid. The insecticides thiamethoxam, imidacloprid, Virtako®, ethofenprox and BPMC were found toxic to adult *T. chilonis*.

Ballal et al., 2009 performed experiments to assess the biocontrol capacity of an endosulfan tolerant strain of *T. chilonis* against *Helicoverpa armigera* eggs on cotton plants in a net house. The strain was tolerant to endosulfan (0.07%) at FRC (350

gm/ha) and was more successful in parasitizing the host eggs as compared to the susceptible one under the stress of endosulfan. The tolerant strain exhibited significantly higher adult emergence in the treatment compared to the susceptible strain, when parasitoids were released within 3 days of endosulfan spraying. The release of the tolerant strain of *T. chilonis* simultaneous with endosulfan spray (integration of biological and chemical tactics) at field rate resulted in significantly higher pest mortality in comparison to either endosulfan treatment alone or release of *T. chilonis* alone.

Vianna et al., 2009 conducted research to assess the effects of 8 insecticides (at dose g a.i/l), including abamectin (0.018), betacyfluthrin (50), betacyfluthrin (125), esfenvalerate (25), lufenuron (50), methoxyfenozide (240), tebufenozide (240), triflumuron (250) and *B. thuringiensis* (25,000 IU/mg) on parasitism by females from two populations of *T. pretiosum* from Brazil of eggs of *A. kuehniella* previously immersed in different insecticide solutions. *B. thuringiensis*, lufenuron and triflumuron had lowest negative impacts on parasitism by and viability of emerged individuals of the parasitoid in the populations; however, considerable reduction in parasitism rate was reported for abamectin, and pyrethroids betacyfluthrin and esfenvalerate at doses 50 and 125 g/l, respectively. The parasitoids that emerged from the esfenvalerate-treated host eggs failed to parasitize the untreated host eggs of *A. kuehniella*.

Hohmann et al., 2010 studied the effects of an aqueous neem seed extract (ANSE) at 15, 3 and 1.5%, and emulsifiable concentrate neem oil (ECNO) at 2.5, 0.5 and 0.25% on life history parameters of *T. pretiosum* and *Trichogrammatoidea annulata* De Santis by application of chemicals to host eggs of *A. kuehniella* before or after parasitization (one, three or five days post parasitism). ANSE had more negative effects on both parasitoid species than ECNO. Less deleterious effects were observed on

wasp emergence, especially for *T. annulata* from host eggs treated with neem oil before parasitism. Pre-treatments (24 h) of the host eggs with ECNO at concentrations varying from 0.5% to 0.25% had no effect on *T. pretiosum* longevity, but 2.5% reduced *T. annulata* survival. Feeding wasps with honey mixed with 0.25% ECNO negatively affected *T. annulata* adult survival.

Shoeb (2010) conducted experiments in the laboratory to assess the effect of five insecticides, including a mixture of *Spodoptera littoralis* NPV (2%) and *B. thuringiensis* (6%), mineral oil group (applied at 1.5 %), lambda-cyhalothrin 478.2 mg/ml, (applied at 0.19%), spinosad 0.04% and fenitrothion 0.37% on the immature stages of the first and the second generations of the egg parasitoid *Trichogramma evanescens* under $25 \pm 1^{\circ}\text{C}$, $65 \pm 5\%$ R.H. and 12:12 L:D conditions. The chemicals were applied one, two, four and eight days after parasitism.

The result demonstrated, that the chemicals affected *T. evanescens*: 1) longevity of the emerged parasitoid: ranged from 12 hrs to 7 days (for females) and from 12 h to 4 days (for males). The host eggs treated with insecticides 4 days and 8 days post parasitism reduced the longevity of the emerged adults for only a few hours relative to the controls, 2) the number of host eggs turned to black (indicate that most of the parasitoid larvae developed to pupae) depend on timing of treatment, 3) adult emergence rate was based on the used insecticide, the parasitoid stage and the generation, 4) the treatment of host eggs 1, 2 or 4 days following parasitism with the said chemicals led to complete death of *T. evanescens* in the host eggs. However, treatment of host eggs within 24 h before parasitoid emergence led to very low emergence and 5) the treated chemicals also decreased slightly female percentage in the emerged adults.

Hussain et al., 2010 evaluated the adverse effects of ten insecticides with single doses [emamectin benzoate 1.9EC (40 ppm), indoxacarb 150SC (260 ppm), lufenuron 50EC (1000 ppm), bifenthrin 10EC (250 ppm), spinosad 240SC (200 ppm), carbosulfan 20EC (500 ppm), chlorpyrifos 40EC (3200 ppm), triflumuron 20SC (400 ppm), imidacloprid 200SL (500 ppm) and abamectin 1.8EC (40 ppm)] on the imature development and adult survival of *T. chilonis*. Emergence of parasitoids was significantly adversely affected by all the chemicals except emamectin benzoate, lufenuron, triflumuron and imidacloprid, when host eggs of *S. cerealella* were treated containing eggs, larvae, pre-pupae, early pupae and pupae of *T. chilonis*. The exposure of adult *Trichogramma* to the insecticides imidacloprid, abamectin, triflumuron, emamectin benzoate, indoxacarb and lufenuron resulted in 70.02, 32.19, 27.62, 25.98, 21.45 and 18.48 % survival, respectively, after 4 hours, but none of the insecticides was found compatible with *T. chilonis* adults after 24 hours of exposure.

Sattar et al., 2011 tested the field recommended concentrations (FRC) of six insecticides (mg active ingredient/l) under laboratory conditions, including emamectin benzoate (3.2), lufenuron (83.3), flubendiamide (80.0), spinosad (120.0), indoxacarb (186.7) and neem oil (1500 ppm) initially against all life stages of *T. chilonis*. The result demonstrated that flubendiamide was the most compatible of all the tested chemicals related to development, survival and fecundity of the tiny parasitoid. Both emamectin benzoate and spinosad severely decreased adult survival, fecundity, and also affected development of immature stages. Indoxacarb caused slight toxicity to all stages except the egg, while lufenuron caused significantly higher mortality to larval stages of this tiny wasp. Neem oil adversely affected only larval development and female fecundity. The study also revealed that flubendiamide and lufenuron were “short lived”,

indoxacarb was “slightly persistent” and spinosad and emamectin benzoate were categorised as “moderately persistent”.

Saber (2011) studied both lethal and sublethal effects of two pesticides, imidacloprid and fenpyroximate on *T. cacoeciae*. The adults were exposed to imidacloprid and fenpyroximate sprayed at rate of ppm /100 ml on glass plates with 6 concentrations, including 21, 15, 10, 7, 5 and 4 ppm for imidacloprid and 3,000; 2,600; 2,300; 2,000; 1,700 and 1,500 ppm for fenpyroximate. Imidacloprid and fenpyroximate caused 100 and 32% adult mortality, respectively. The chemicals were also tested against the pre-imaginal stages of minute parasitoid in the host eggs of *S. cerealella* and *Cydia pomonella* by dipping the parasitized host eggs in the solutions of field doses/100 ml: 350 ppm and 1,000 ppm of imidacloprid and fenpyroximate, respectively, to investigate pesticides effects on larvae (3 days after parasitism), pre-pupae (6 days after parasitism) and pupae (9 days after parasitism) of minute parasitoid.

The results demonstrated 94.4, 96.24 and 69.8% mean adult emergence from eggs of *S. cerealella* treated with imidacloprid at larval, prepupal and pupal stages, respectively, while indicated 80.7, 89.18 and 37.1% emergence, when treated with fenpyroximate at aforementioned stages, respectively. *Cydia pomonella* showed 82.9, 89.6 and 83.4% emergence of tiny parasitoids in response to treatment with imidacloprid at larvae, prepupae and pupae, respectively, and resulted in 76.3, 64.2 and 78.2% emergence when treated with fenpyroximate at the same stages, respectively.

Both imidacloprid and fenpyroximate had no effect on longevity and progeny production of the parasitoid adults emerging from treated eggs in comparison to the control. Overall, results of this study demonstrated a relative safety of fenpyroximate

to *T. cacoeciae*, but imidacloprid showed deleterious effects on the adults of parasitoid.

Saljoqi et al., 2012 studied different concentrations (0.2, 0.15, 0.1, 0.05 and 0.01%; 0.2% stock solution was equal to 8 ml/970 ml) of spinosad against *T. chilonis* to assess the chemical's effects on emergence rates, emergence time, parasitism, residual toxicity and walking behavior of the parasitoid. The host eggs of *S. cerealella* were previously sprayed with the said concentrations of the insecticide and were exposed for parasitism. Only 0.01 concentration was statistically at par with control, while 0.2% concentration resulted in minimum parasitism by the fresh females exposed to the treated eggs. However, all the treated concentration demonstrated parasitism significantly different from control by the females emerged from the host eggs treated when the parasitoid was in egg and larvae stages. The emergence time (days) was not significantly different among 0.01, 0.05, 0.10, and control regarding egg, larval and pupal treatments. All concentrations caused 100% adult mortality within 15 minutes. Spinosad also decreased the total movement of the minute wasp.

Hussain et al., 2012 tested selected insecticides (at dose ml/ha) including spinosad 240SC (198), lufenuron 50EC (494), flubendiamide 480SC (432), chlorantraniliprole 20SC (72), emamectin benzoate 1.9EC (494) and imidacloprid 200SL (618) against immature and adult stages of *T. chilonis*, in order to assess adult survival and emergence under laboratory conditions. The host eggs of *S. cerealella* were dipped in the solution of pesticides on the 1st, 3rd, 4th, 5th, 7th and 8th day after parasitism. These days corresponded to the developmental stages of *T. chilonis*: eggs (1d), larvae (3d), pre-pupae (4d), early pupae (5d), pupae (7d), and late pupae or one day before adult emergence (8d).

Parasitized eggs treated with spinosad after different intervals post parasitism resulted in the lowest emergence among the tested chemicals. The application of emamectin benzoate and lufenuron to 1-day post-parasitism host eggs, imidacloprid, emamectin benzoate and lufenuron on the 3rd day after parasitism, imidacloprid, emamectin benzoate, flubendiamide and lufenuron 4, 5 and 7 days after parasitism, respectively, exhibited minimal adverse effect on the emergence of parasitoids and were found to be compatible with the parasitoid. Treating eggs 8 days after parasitism with chlorantraniliprole resulted in maximum emergence of parasitoid, although not significantly different from lufenuron and emamectin benzoate.

The assessment of adult toxicity 3 hours after application of chlorantraniliprole revealed maximum survival (42%) and did not differ significantly with lufenuron, with 36% survival. While emamectin benzoate was found to be a toxic insecticide, reducing survival of the *T. chilonis* to 18%, it was not significantly different from imidacloprid with 22% adult survival. All the chemicals were considered toxic and did not differ significantly regarding adult survival of *T. chilonis* at 24 h post application with survival range between 8.0 to 14% compared to control treatment with 92% survival.

Delpuech and Delahaye (2013) determined the effect of LD₂₀ of deltamethrin on the behavior of *T. brassicae* females foraging in a patch of host eggs. Female parasitoids exposed to insecticide parasitized fewer host eggs; spent more time on unsuitable previously parasitized host eggs than on untreated controls. An increase in antennal and ovipositor rejection of previously parasitized host eggs was also induced by the insecticide.

Khan et al., 2015 evaluated the effects of pesticides, including insecticides, m- iticides, fungicides and herbicide on the adult survival and foraging behavior of *T. pretiosum*. Fipronil, dinotefuran, spinetoram, tolfenpyrad and abamectin resulted in nearly 100% mortality of adults within 24 h of exposure to treated cotton leaves compared to controls at field doses. Acetamiprid was comparatively less toxic to adult survival of parasitic wasps at field dose, while glufosinate ammonium was the only chemical demonstrated increased toxicity among the non-toxic materials at both of two- or four fold of field dose. The remaining chemicals were all harmless to the adult survival, including buprofezin, pyraclostrobin, spiromesifen, a mixture of trifloxystrobin and tebuconazole, chlorantraniliprole, glyphosate, cyflumetofen, glufosinate ammonium, flonicamid, flubendiamide, novaluron, spirotetramat and myclobutanil: they induced < 10% adult mortality at recommended field dose, and these pesticides exhibited no significant change in toxicity from the RFC even at 4x dose.

Foraging behavior (number of drumming, stinging or feeding bouts) of parasitoids on the first encountered host egg (*Helicoverpa zea*) treated with acetamiprid, chlorantraniliprole, cyflumetofen, glufosinate ammonium, glyphosate, myclobutanil, the mixture of trifloxystrobin + tebuconazole, spiromesifen, spinetoram, tolfenpyrad or abamectin by comparison with the water-treated (control) eggs demonstrated that foraging behavior of parasitic wasps was affected only by tolfenpyrad among the materials tested: female parasitoids on tolfenpyrad-treated eggs drummed the host with greater frequency, stung the treated host more frequently and engaged in more feeding bouts than females on eggs of any other treatment. Although the average total duration of the respective drumming, stinging and feeding bouts was not affected by tolfenpyrad. The second treated egg also demonstrated results for foraging behavior similar to

those observed for the first egg, except the host feeding behavior was less common than on the first egg.

Chapter 3

MATERIALS AND METHODS

The assessment studies on the effect of 15 novel/selected pesticides against *Trichogramma chilonis* (Ishii) were carried out during 2010-2011. The pesticides tested against the minute wasp in the laboratory of Nuclear Institute for Food and Agricu-

lture (NIFA), Tarnab, Peshawar, KPK, Pakistan, included insecticides, herbicides, fungicides and miticides. The 15 pesticides studied belong to 15 different classes of pesticides, including HaNPV, spirotetramat, chlorantraniliprole, acetamiprid, spinetoram, fipronil, abamectin, spiromesifen, haloxyfop-p-methyl, bispyribac sodium, nicosulfuron, myclobutanil, the mixture of chlorothalonil + procymidone, the mixture of pyraclostrobin + metiram and the mixture of trifloxystrobin + tebuconazole (Table 1). All chemicals were evaluated at three concentrations: x (field dose used in Pakistan), 0.5x (half field dose), 2x (twice field dose) to discover their effects on different life stages (egg, larva, pupae, and adult) of *T. chilonis*. The brief descriptions of used pesticides (Table 1) in the current laboratory work of PhD research are given below.

3.1) Brief Description of Pesticides Used in Laboratory

3.1.1) HaNPV

The nuclear polyhedroviruses (NPV) are microbial insecticides belonging to the sub group Baculoviruses, and have been used to manage pest insects, predominantly moths and butterflies in cereal crops and vegetables. The polygonal structure of the virus known as the capsid protects and facilitates the virus's infestation of host cells and helps in virus reproduction. The virus strains are released and start reproduction in the host by rupturing the capsid. The NPV *Baculovirus heliothis* control *sheliothin*-es spp, an important pest group in at least 30 crops worldwide. The *Helicoverpa armigera* nuclear polyhedrovirus (HaNPV) is an important virus strain for controlling the important cotton pest *H. armigera*. In Pakistan, the field dose of HaNPV is 80 ml/acre, and it is traded with the name "Helicovex".

3.1.2) Spirotetramat

Spirotetramat is a novel insecticide and is the first member of a new chemical class the cyclic ketoenols. It is derived from tetramic acid and has a novel mode of action that inhibits acetyl CoA carboxylase, a key enzyme in fatty acid biosynthesis (lipid biosynthesis inhibitor), which leads to the death of immature stages of sucking pest insects within 2 to 10 days after application. It is used to manage sucking insect pests including aphids, scales (soft and armoured), mealybugs, whiteflies, psyllids and selected thrip species at a field concentration of 125 ml/acre (a.i) in crops and vegetables including cotton, chilli, mango, citrus, potato, tomato, cucurbits, brinjal, lettuce, cabbage, cauliflower, onion and spinach.

Spirotetramat reduces fecundity, fertility and survival of pest progeny, and its impact on development of pest juvenile stages provides long residual control. It has the trade name “Movento” 24% SC in Pakistan. Movento is a mixture of spirotetramat (125 ml/acre) and adjuvant (250 ml/acre), and is used to manage sucking pests particularly in wheat in Pakistan.

3.1.3) Chlorantraniliprole

The insecticide chlorantraniliprole belongs to a novel chemical class called anthranilic diamides and is selective to beneficial arthropods, manufactured by DuPont. It activates insect ryanodine receptors, which stimulates release of calcium from the internal store of smooth and striated muscle, and results in impaired muscle regulation, paralysis, and insect death, due to abnormalities in the normal contraction of muscles.

Chlorantraniliprole acts mainly by ingestion and has little contact activity. Foliar formulation of this new chemical has the trade name “Coragen” 20% SC, and has recommended field doses ranging from 50 ml-80 ml/acre in Pakistan for control of m-

ainly lepidopterous insects (moths and butterfly), such as American bollworms, bollworms and armyworms in sugarcane, corn, rice, cotton, tomato, lady finger, cabbage, mustard, spinach, apple and peach, etc.

3.1.4) Acetamiprid

It is a synthetic organic chemical belonging to the class known as neonicotinoid insecticides. Acetamiprid is manufactured by Nippon Soda Co. Ltd. Japan, and is a broad spectrum insecticide with contact, stomach, and translaminar activity. It acts on target organisms by antagonizing the nicotine acetylcholine receptor of neural pathways of target insects. It controls sucking pests through foliar spray and/or soil treatments, including whiteflies, jassid, thrips, mealybug, leaf minor and aphids in a variety of crops, such as cotton, tobacco, melon, apples, cherries, house plants, lettuce, ornamental garden plants, pears, peppers, plums, potatoes and tomatoes. An acetamiprid field rate ranges from 50-125 gm/acre for different crops and vegetables and is available in the market with brand name “Mospilan” 20SP in Pakistan.

3.1.5) Spinetoram

Spinetoram is a broad spectrum new insecticide of the novel class Spinosyn, produced by the soil actinomycete microbe *Saccharopolyspora spinosa*, and manufactured by Dow AgroSciences USA. It affects the nervous system of insects, targeting nicotinic acetylcholine receptors and γ -aminobutyric acid (GABA) receptors existing on postsynaptic membranes in insect nervous systems, thereby disrupting normal neural transmission. It can be used to manage pests in cotton, corn, rice, cabbage, brinjal, potato, leafy vegetable, onion, chilli, tomato, cauliflower, melon, apple, mango, peach and plum, such as bollworm, armyworm, thrips, looper, *Heliothis* sp, leafminer flies, fruit worm, shoot and fruit borer, leaf folder, leaf hopper and whiteflies. It is available

with brand name “Radiant” 120SC in Pakistan and is used at field concentrations ranging from 50 to 100 ml/acre.

3.1.6) Fipronil

Fipronil is a broad-use insecticide manufactured by Bayer CropScience that belongs to the family phenylpyrazole. Fipronil interferes with the insect central nervous system by preventing the passage of chloride ions through the GABA (gamma aminobutyric acid) receptor and glutamate-gated chloride (GluCl) channels, components of the central nervous system. Thus, hyperexcitation of nerves and muscles of insects occurs by contamination with fipronil. The better efficacy on GABA by fipronil leads to specificity of the same insecticide on insects. Fipronil is commonly used in rice and sugarcane to control stem borers at field recommended concentration of 480 ml/acre and is available with trade name “Regent” 5% SC in Pakistan.

3.1.7) Abamectin

Abamectin is a member of the chemical class of avermectins and is a mixture containing more than 80% avermectin B1a and less than 20% avermectin B1b. The avermectins act as insecticides, acaricides and helminthicides, and are a natural fermentation product of the soil bacterium *Streptomyces avermitilis*. The poison is absorbed by the leaves during foliage spray and is ingested by the target pests. It acts on insects by disrupting neural and neuromuscular transmission, and acts on a specific type of synapse located only within the brain and is protected by the blood-brain barrier. Thus, it activates the chloride channel, causing the affected insect to become paralyzed, stop feeding, and die after a few days. Abamectin is used to control insect and mite pests of a range of agronomic, fruits, vegetables and ornamental crops and it is used by homeowners for control of fire ants. In Pakistan, the field recommended concentra-

tion of abamectin is 480 ml/100 liters and it is available with the brand name “Abamectin” 1.8EC.

3.1.8) Spiromesifen

Spiromesifen is a novel acaricide, belonging to the insecticide class ketoenols, is manufactured by Bayer CropScience, and acts as a lipid biosynthesis inhibitor. Spiromesifen disrupts lipogenesis by preventing formation of fatty acid and their chemical derivatives. The acaricide is used at field dose of 100 ml/acre, and is marketed with the name “Oberon” 240SC in Pakistan for management of whiteflies and all major mites including red mites, two-spotted spider mites and bud mites at all stages in the cotton, maize, brinjal, cabbage, cauliflower, chilli, cucurbit, potato, lettuce, spinach, tomato and strawberry, etc.

3.1.9) Haloxyfop-p-methyl

Haloxyfop-p-methyl belongs to the chemical class aryloxyphenoxypropionate, and is used as pre-and post-emergence selective foliar herbicides. They control annual and perennial grasses in sugar beet, oilseed, potatoes, leaf vegetables, onions, sunflowers, strawberries and other crops by absorption into the plant and cause inhibition of growth. In Pakistan the field dose is 350 ml/acre and the chemical is available in the market with trade name “Percept” 10.8EC.

3.1.10) Bispyribac sodium

Bispyribac sodium is a new active ingredient of the chemical class, the pyrimidinylthiobenzoates, and is a selective, systemic post-emergence herbicide, absorbed by foliage and roots, that moves throughout the plant tissue, and works by disrupting the synthesis of the plant enzyme acetolactate synthase (ALS), required for growth.

Polyethoxylated fatty alcohol is used as an adjuvant to increase the efficacy of the active ingredient and is added to the bispyribac sodium, and the mixture is used in turf for the control of winter grass, sedges, broad-leaved weeds, barnyard grass and is also moderately active on rice field bulrush. The chemical is applied at a field dose 80 gm/acre, and is marketed with the trade name “Clover” 200WP in Pakistan.

3.1.11) Nicosulfuron

Nicosulfuron is a new broad spectrum systemic selective herbicide belonging to the class sulfonylurea, and has been used in maize since the early 1990s for management of wide range of maize weeds, both annual and perennial grass weeds, sedges and broad-leaved weeds such as *Sorghum halepense* and *Agropyron repens*. The chemical is selective to maize, as the maize plant metabolizes the nicosulfuron into harmless substances.

Nicosulfuron is applied as a foliage application and the chemical is absorbed through leaves and reaches the meristematic region via xylem. In the meristematic region, the herbicide stops cell division by inhibiting the activity of acetolactase synthase, a key enzyme required for cell division and plant growth. This product is easy to use and does not wash into the soil. It is considered safe to non-target organisms. The recommended field doses are 30 gm/acre in Pakistan and it has the product brand name “Sun” 750WDG.

3.1.12) Myclobutanil

Myclobutanil is an active ingredient belonging to the triazole fungicides, and is manufactured by Dow AgroSciences. It is a steroid demethylation inhibitor, specifically inhibiting ergosterol biosynthesis. Ergosterol is a critical component of fungal cell membranes. It inhibits the enzyme C14 demethylase, which is involved in the sy-

ntesis of ergosterol. It is used for control of blossom blight (*Monilinia spp*), shothole (*Stigmina spp*), rust (*Tranzschelia spp*) and anthracnose (*Colletotrichum spp*) in almond. “Systhane” 20EW is the trade product used in Pakistan containing myclobutanil and it is applied at field dose of 35 ml/acre.

3.1.13) Mixture of Chlorothalonil and Procymidone.

a) Chlorothalonil is a broad-spectrum contact organochlorine fungicide. It has non-systemic action and is used in a foliar application as either curative or preventative fungicide, and has a long residual activity. Chlorothalonil is a multi-site inhibitor of various enzymes and other metabolic functions, thereby aimed to stop spore germination and causing death of growing cells. It controls diseases including early blight, late blight, fruit rot, rust, downy mildew, anthracnose and leaf spot in vegetables, small fruit, stone fruit, turf, ornamental and other agricultural crops especially barley and wheat.

b) Procymidone is a dicarboximide fungicide, used as a seed dressing, pre-harvest spray or post-harvest dip of lupins, grapes, stone fruit and strawberries. It disrupts endocrine processes (androgen receptor antagonist), is absorbed through roots, and is transported to leaves and flowers. It controls fungi, e.g., *Sclerotinia*, *Monilia* and *Helminthosporium* spp. on fruit (including top fruit, strawberries and raspberries), vines, vegetables (including tomatoes, peas and beans), ornamentals, cereals, sunflowers, oilseed rape, soya beans, peanuts, and tobacco, etc. In Pakistan, chlorothalonil (33.3% w/w) is combined with procymidone (16.7% w/w) and is applied at a field dose of 500 gm/acre with the trade name “Protocol” 500WP to control powdery mildew, etc.

3.1.14) Mixture of Pyraclostrobin and Metiram

a) Pyraclostrobin is a preventive fungicide (prohibits germination of spore), belonging to the fungicide group methoxy-carbamate, an extraction of the fungus *Strobilurus tenacellus*. It is a quinone outside inhibitor. It actively inhibits respiration and subsequent energy production within mitochondria, effectively stopping any cellular activity within the fungal cells, and is used for controlling scab and powdery mildew in tomato and other vegetables.

b) Metiram belongs to a class of chemicals known as ethylene bisdithiocarbamates (EBDCs). It also contributes to control of scab and powdery mildew in tomato and other vegetables. In Pakistan, the product is marketed with “CabrioTop” 600-WDG, which contains both pyraclostrobin (5% w/w) + and metiram, (55% w/w), and is applied at field spray rate of 250 gm/acre.

3.1.15) Mixture of Trifloxystrobin and Tebuconazole

a) Trifloxystrobin is a broad-spectrum fungicide with preventative and specific curative activity, and displaying rain-fastness. It is applied as foliar spray on a wide range of agricultural and horticultural crops in temperate, sub-tropical and tropical climates in open fields, or protected culture under glass and plastic. The chemical is used to manage phytopathogenic fungi belonging to four classes: Ascomycetes, Deuteromycetes, Basidiomycetes and Oomycetes, and causing diseases such as grape and cucurbit powdery mildew, apple scab and powdery mildew, peanut leaf spot, and brown patch of turf grasses. The fungicide works by interfering with respiration in phytopathogenic fungi. Thus, trifloxystrobin inhibits fungal spore germination and mycelial growth.

b) Tebuconazole is a member of the triazole fungicides, used to treat plant pathogenic fungi causing diseases such as leaf spot, leaf speckle on banana, rust on wh-

eat, beans and oats, scald/powdery mildew on barley, leaf spot and net blotch of peanut, foliar diseases on cereal crops and other diseases such as powdery mildew on peas, white root rot on onion, black spot on papaw, *Sclerotinia sclerotiorum* on chrysanthemum, leaf rust and stem rust in ryegrass and fescue seed crops. In Pakistan, the mixture of trifloxystrobin and tebuconazole is marketed as “Nativo” 750WG and is applied at the field dose of 65 gm/acre.

3.2) Rearing of *Sitotroga cerealella* Olivier on Wheat Grain

Eggs of *Sitotroga cerealella* Olivier were sprinkled on sterilized grains in a plastic tray (30 cm x 18cm) in the laboratory of Entomology Division, NIFA, Peshawar, (Pakistan). The young larvae hatched and infested the grain within a week. The infested wheat was then shifted to plastic rearing jars (15cm x 20 cm), and their openings were subsequently covered with a piece of cotton cloth, and they were maintained in the laboratory at average conditions of $24 \pm 6^{\circ}\text{C}$, $65 \pm 10\%$ relative humidity (RH) and 16:8 (L:D) until adults emerged after 20-25 days.

Regular collections of emerged moths from the rearing jars every 24 hours were carried out by an electric suction apparatus in the oviposition jar (10 cm x 15 cm in dimension) covered at bottom by mesh (mesh no.30 to 40 pore size). After completion of moth collection, the jar was placed over the corn flour in a metal/plastic tray, and was given a single turn to adhere the flour to the jar mesh at the bottom for egg laying. The jar was then carefully placed on metal/plastic tray until next day (24 hours) allowing the moths to have sufficient time to lay eggs in the flour. Several oviposition jars were prepared in the same way. Next day, the host eggs were collected by sieving the flour and the eggs were used in the experimental work as well as for maintenance of *S. cerealella* culture in the laboratory.

3.3) Rearing of *Trichogramma chilonis* (Ishii) on *S. cerealella*

S. cerealella is one of the few hosts which can be easily reared under artificial conditions for use in mass production of *Trichogramma* (Bastos *et al.*, 2006). *S. cerealella* eggs were used to maintain culture of *T. chilonis* in the laboratory. Approximately 1000-1300 fresh host eggs were used to sprinkle on and glued to paper card (5 x 8 cm), and the card was allowed to dry for 1-2 hrs. The dried card was exposed to parasitism for 24 h in glass jars (5 cm x 12 cm) containing approximately 30 to 40 adults (mixed-gender) of *T. chilonis*, and the jar was placed in the lamp light in order to obtain good parasitism by the tiny wasp. Minute drops of honey diluted in water were stuck to the inner wall of jar as food for the adult parasitoids. The opening of the glass jar was tightly covered with muslin cloth to prevent escape of the adults. Subsequently, the parasitized card was removed and was transferred to another glass jar of the same size, and the jar was incubated at the $23 \pm 3^{\circ}\text{C}$, $70 \pm 10\%$ RH and 14:10 (L:D) conditions until adult emergence. Several parasitized cards were obtained in the same manner on a daily basis, and were incubated at the same conditions to produce stock culture of *T. chilonis* for maintenance of culture and to use in the experimental work.

3.4) Preparation of Different Concentrations of Pesticides Solution

Commercially available formulated pesticides (Table 1) were diluted with tap water to prepare their respective stock solutions: taking the required amount of the formulated pesticides, and were mixed with amount of water required in the laboratory under the stated conditions. The stock solution was diluted (serial dilutions) and three different concentrations (reagents of laboratory solutions) of pesticides were prepared for use in the experiments by the formula: $C_1V_1 = C_2V_2$, where C_1 and V_1 are the concentration and volume of commercial pesticides/stock solution, respectively, while

e C_2 and V_2 are the concentration and volume of the required pesticide solutions (diluted), respectively.

3.5) Preparation for Testing of Pesticides Against Pre-imaginal Stages of *T. chilonis*

Approximately 200-300 fresh *S. cerealella* eggs were glued to the hard paper card (approximately 5 x 8 cm), and allowed to dry 1-2 h before offering to parasitoids in glass jars (5 cm x 12 cm) containing approximately 14 to 20 mixed-gender adults of *T. chilonis* depending on the number of host eggs on the card in the laboratory at average conditions of 24 ± 6 °C, $65 \pm 10\%$ RH and 16:8 (L:D). After 24 h of exposure, the parasitized card was subsequently removed and was cut to small card strips (approximately 0.8 cm x 8 cm). The trial involved 10 replicated card strips, each containing 20-40 host eggs, prepared separately for each of three concentrations: field dose (x), twice field dose (2x) and half field dose (0.5x) of each pesticide. The above mentioned card strips were treated at appropriate time post-parasitization for the different immature stages that were: less than 1 day (< 24 h) for eggs stage, 3 days (72 h) for larvae and 6 days (144 h) for pupae.

3.6) Testing Against Egg Stage of *T. chilonis*

Fifteen pesticides were tested against the egg stage of *T. chilonis* by dipping paper card strips (0.8 cm x 8 cm) each containing approximately 10-15 parasitized host eggs (< 24 h) for 1-2 seconds in the pesticide solution or in water (untreated: control) of different treatments. The trial consisted of dipping ten strip cards in each of three doses of each treatment (stage), in order to assess pesticide-wise, dose-wise and stage-wise effects of pesticides on the most sensitive immature stage (egg) of *T. chilonis* regarding emergence and parasitism. The card strip was then removed; the excess

of pesticide solution or water was removed with filter paper, and card was subsequently air dried at room temperature for 1 h. Each dried, parasitized card strip was transferred into a vial (1cm x10 cm), and was incubated at controlled conditions: $23 \pm 3^{\circ}\text{C}$, $70 \pm 10\%$ (RH) and 14:10 (L: D) until adult emergence.

Card strips (approximately 0.8 x 8 cm each) of approximately 200 to 300 fresh *S. cerealella* eggs (< 24 h old) were prepared with host eggs sprinkled on the glued card, and were exposed to the newly emerged adult parasitoids (including 4-8 females) in each of the ten vials of each concentrations or in water (untreated: control) of different treatments, for 24 h in the laboratory under the conditions aforementioned, to determine parasitism efficiency of the female parasitoids emerged from host eggs treated with the pesticides or water when parasitoids were in egg stage by recording the number of the parasitizing females in each vial, as newly emerged from the treated or control eggs during the first 24 h (period of exposure of new card to parasitism), in order to assess the effect of the pesticides on parasitism capacity of *T. chilonis* regarding both dose and life stage. Minute drops of honey diluted in water were stuck to the inner wall of vial as food for the adult parasitoids. All the female parasitoids parasitizing the newly exposed card were removed to make sure that no parasitism could continue beyond 24 h and the exposed card was transferred to a separate vial, and was incubated at the same conditions as described earlier until complete parasitoid pupation.

Table 1. Label descriptions of the pesticides used in the experiments

Trade name and formulation	Active ingredient	Chemical class	Field rate
Coragen 200 SC	chlorantraniliprole	Anthranilicdiamide	80-ml
HaNPV	HaNPV (7.5×10^{12} OB/L)	Biopesticides	80-ml
Movento 240SC	spirotetramat	Tetramic acids	125ml+adj 250-ml
Radiant 120SC	spinetoram	Spinosyns	100-ml
Mospilon 20SP	acetamiprid	Neonicotinoid	125-gm
Regent 50SC	fipronil	Phenylpyrazol	480- ml
Oberon 240SC	spiromesifen	Tetronic acids	250-ml
Abamectin 1.8EC	abamectin	Avermectin or Glycoside	480-ml
Clover 200WP	bispyribac sodium (20%w/w)+ adjuvant (polyethoxylated fatty alcohol, 99.50 %w/w)	Pyrimidinylthiobenzoates	80-gm
Sun 750WDG	nicosulfuron	Sulfonylurea	30-gm
Percept 10.8EC	haloxyfop-p-methyl	Aryloxyphenoxypropionates	350- ml
Systhane 20EW	myclobutanil	Triazole	35-ml
Nativo 750WG	trifloxystrobin (25%w/w) +tebuconazole (50% w/w)	Mandelamide + Triazole	65-gm
CabrioTop 600 WDG	pyraclostrobin (5% w/w)+ metiram (55% w/w)	Methoxy-carbamates + Ethylene bisdithiocarbamates	250-gm
Protocol 500 WP	chlorothalonil (33.3%w/w) + procymidone (16.7% w/w)	Organochlorine + Dicarboximide	500-gm

WP (wetable powder); EC (emulsifiable concentrate); SP (soluble powder); WDG (water- dispersable granule); SC (soluble concentrate); WG (wetable granule); EW (emulsifiable oil in solution in water)

Twelve pesticides were tested to evaluate parasitism by *T. chilonis*.

The number of pupae of the pesticide dipped card, and also number of the specimens emerged from the pupae of the pesticides dipped card, number of females parasitizing the newly exposed card (determined after 24 h period by sex identification of the adults parasitoid emerged from the pupae of the pesticide dipped card under binocular microscope after removal of the parasitized card), and the number of pupae of the exposed card (after 7 days of parasitism) were determined by magnifying lens without dissecting them (generally one *T. chilonis* adult emerged from single parasitized *S. cerealella* eggs) and recorded separately for each concentration and stage treated with pesticide, in order to assess both emergence success as well as parasitism efficacy of the parasitoids emerging from eggs treated during parasitoids' egg stage. Parasitism was quantified by the number of darkened host eggs, the darkness (indication of parasitism) being due to the deposition of granules of urate inside the chorion of the host eggs by the *Trichogramma* larva (Pratissoli *et al.*, 2005).

3.7) Testing Against Larval and Pupal Stages of *T. chilonis*

The newly parasitized eggs strip cards, similar to those used in the egg treatment, were incubated for 3 days (72 h) and 6 days (144 h) for larval and pupal assays, respectively, under the aforementioned incubation conditions, and at those points were treated with 14 pesticides or tap water (control) in the same way as described in the assay of egg stage. However, 12 chemicals were evaluated for their effects on the parasitism by the females emerged from the host eggs treated when parasitoids were in egg and larval stages, while 13 chemicals were tested against the host eggs when parasitoids were in pupal stage in order to determine impacts on the parasitism capacity of the *T. chilonis* emerged from the treated eggs. The adult emergence and parasitism

efficiency of the *T. chilonis* females emerged from the pesticides or water treated eggs were determined and evaluated for both stages in the same way as aforementioned in the egg stage treatment.

3.8) Testing the Pesticides to Assess Parasitism (no-choice test) of Previously Treated Host Eggs by *T. chilonis*

Laboratory experiments were conducted to evaluate effects of 15 pesticides on parasitism of treated eggs by *T. chilonis* in a no-choice design. Approximately 100 to 175 fresh *S. cerealella* eggs were glued on hard paper card (5x8 cm). The card was dried for 1-2 hours and was subsequently cut into several strips (0.9 x 8cm each) in such a manner that each card strip contained approximately 20-35 host eggs.

Card strips were treated by dipping for 1-2 seconds for each pesticide solution and dose (x, 2x, and 0.5x doses), in addition to water. Each card was dried at the aforementioned laboratory conditions and was subsequently transferred to a glass vial (1 cm x 10 cm) containing one pair of *T. chilonis* (< 24 h old). Minute drops of honey diluted in water were stuck to the inner wall of vial as feeding. The vial was exposed to light for 24 h for completion of parasitization. The trial consisted of 10 replications for each concentration and treatment. The exposed parasitizing female was removed after 24 h from each vial and all the vials were incubated at aforementioned conditions until pupae formation. The data were recorded separately for all doses and all the pesticides by counting darkened eggs (pupae) 7 days after exposure to the parasitoids.

3.9) Testing the Pesticides Against Adult Stage of *T. chilonis*

The field doses of 11 pesticides were tested against adult *T. chilonis* to assess their effects on susceptibility of most susceptible life stage (residual toxicity/persiste-

nce), and 15 pesticides were tested to assess effect on parasitism. Adult *T. chilonis* (10-15 mixed gender) were exposed to vials treated with pesticides for 24 h, and simultaneously exposed/surviving females were provided with fresh *S. cerealella* eggs cards, containing approximately 200 to 300 eggs (< 24 h old) to assess parasitism during the exposure period in each of 10 glass vials of each treatment interval, i.e., 1, 5, 10 and 15 days, in addition to 10 vials of untreated controls. The number of females parasitizing card in each vial was recorded to determine the parasitism efficiency of minute parasitoid in response to treatment or untreated control. Minute drops of honey diluted in water were stuck to the inner wall of vial as feeding food resource for the parasitoids. The experiments for all the intervals were conducted on the same day for several chemicals, and a single water treated control was set up for the chemicals used in the experiment to compare the results.

The exposed card was removed after 24 h, transferred to a separate vial, and was subsequently incubated at conditions described earlier, until pupal formation. Residual toxicity was determined and recorded for 24 h by counting the dead adults. Parasitism data was recorded by counting pupae 7 days after parasitism. Pesticides were evaluated for their effects on adult survival, and parasitism by the survived females.

3.10) Data Calculations

The experiments in laboratory were conducted using a completely randomized design (CRD) with ten replications for each dose/treatment of pesticide and for control (water treated). The data obtained were used to assess the effects of pesticides on the emergence, residual toxicity and parasitism by *T. chilonis*. Means $\pm$ standard error (SE) of data were also calculated.

The mean parasitoid emergence for each treatment of pesticide was calculated:

as all emerged adults in each treatment divided by all parasitized eggs in the treatment, while the mean parasitism for each treatment was determined by the division of all the parasitized eggs by all the parasitizing females for each treatment of all the pesticides. While the percentage of adult mortality for 24 h were calculated by dividing the adults died during 24 hours by the total adult number used in the treatment, multiplied by 100. The total number of adults of *T. chilonis* emerged or the number of host eggs parasitized per treatment (dose) for the same stage of the parasitoid were converted to a percentage using the control treatment as reference (maximum possible emergence or parasitism): the determination of percent emergence of *T. chilonis* relative to control for each dose was determined by:

$$\text{Percent emergence} = \frac{\text{No. of emerged adults in treatment}}{\text{No. of emerged adults in control}} \times 100$$

Similarly, the percent parasitism relative to control (untreated) was determined by:

$$\text{Percent parasitism} = \frac{\text{No. of parasitizing eggs in treatment}}{\text{No. of parasitized eggs in control}} \times 100$$

The effect of the insecticides on percent reduction in emergence or parasitism compared to the untreated was calculated by the formula: $E (\%) = (1 - E_t/E_c) \times 100$, where “E” is the effect of the pesticide on the biological control agent being measured as either reduction in adult emergence or parasitism rate compared to the untreated, “E_t” is the emergence or parasitism rate observed on each pesticide treatment and “E_c” is the emergence or parasitism rate observed on the untreated: control (Manzoni *et al.*, 2007). The control values were used as the denominator in the percentage calculation for each of the variables.

If mortality exceeded 20% in the control batch, the entire test was rejected. If

mortality in the controls was between 5% and 20%, then the percentage mortality of adults *T. chilonis* for each pesticide was corrected using the Abbott's formula (Abbott, 1925; WHO, 2009); corrected (%) mortality with control (water treated) was determined by:

$$\text{Corrected (\% adult mortality)} = \frac{X - Y}{(100 - Y)} \times 100$$

Where X means percentage of mortality in pesticide treatment, while, Y is percentage of mortality in control treatment. Thus, adult mortality (due to residual toxicity) expressed as percentage mortality of 24 hours data was transformed (by the use of Abbott's formula) prior to analysis.

3.11) Statistical Analysis

The data were tested for normality (Shapiro and Wilk, 1965), and the data did not fit a normal distribution or homogeneity of variance. Therefore, data on emergence and parasitism were square-root transformed previously to analysis to normalize the data. The data were analyzed using a general linear model through factorial analysis of variance (two-way ANOVA) for each of the response variables, and the differences between treatments were analyzed statistically by Tukey's HSD all-pairwise multiple comparison tests ($p = 0.001$ or 99.9% CI) by using the computer-based statistical software package "Statistix" version 9.

The assessment of reduction in emergence (%) or reduction in parasitism (%) over controls was carried out by toxicity categories (laboratory and field scales) of International Organization for Biological Control (IOBC)/West Palaearctic Regional Section (WPRS) (Hassan 1994; Sterk *et al.*, 1999): 1= harmless ($E < 30\%$); 2 = slight harmful ($30 \leq E \leq 79\%$); 3 = moderately harmful ($79 < E \leq 99\%$); 4 = harmful (> 99

%), where “E” is the effect of the pesticide on the biological control agent being measured as the reduction in percentage of emergence or parasitism over control.

While assessment of reduction in adult mortality (corrected % mortality) of *T. chilonis* under laboratory conditions was carried out by toxicity ranking according to Brunner *et al.*, 2001: pesticides were classified low toxic if < 20% corrected mortality was observed, moderate toxic if > 20% and < 70% corrected mortality was observed and highly toxic if > 70% corrected mortality was observed.

The members of the IOBC/WPRS Working Group “Pesticides and Beneficial Organisms” evaluated harmful activity duration (persistence) of insecticides against predators and parasitoids under laboratory conditions that include: A, short-lived (duration of harmful activity of < 5 days); B, slightly persistent (5–15 days); C, moderately persistent (16–30 days) and D, persistent (>30 days) (Sterk *et al.*, 1999).

Chapter 4

RESULTS

The results of the current studies on the “assessment of lethal and sublethal effects of some novel pesticides on *Trichogramma chilonis* (Ishii)” are described here. A highly significant interaction ($P < 0.0001$) was found between the pesticides and doses for effect on emergence of adults, parasitism of untreated host eggs by females emerged from treated host eggs, parasitism of the previously treated host eggs, adult mortality based on residual toxicity, and parasitism of untreated host eggs by the females exposed to the treated surface on glass vial.

The results obtained were classified as: 1) section 1-A: immature mortality; section 1-B: parasitism by the females emerged from host eggs treated when parasitoids were in egg, larval and pupal stage; 2) section 2: parasitism by female of treated host eggs; 3) section 3-A: mortality of adults exposed to the dry residues on glass surface for 24 h; and section 3-B: effects of pesticides on parasitism efficiency of females exposed to the treated surface on glass vial for 24 h. The results are discussed below.

Section: 1-A

EFFECT OF PESTICIDES ON THE EMERGENCE OF *T. Chilonis*

4.1) Percent Emergence of *T. chilonis* When Treated at Egg Stage within Host Eggs

4.1.1) Anova on percent emergence after egg exposure

Pesticides were evaluated for their effects on percent emergence of *T. chilonis*

from host eggs (*S. cerealella*) treated at egg stage of minute parasitoids. The results of analysis of variance revealed (Table 2) that the interaction between pesticides and doses was significant regarding effect on the emergence of *T. chilonis* ($df = 42, 540$; $f = 806.12$; $p < 0.0001$).

4.1.2) Comparison of pesticide-dose means of percent emergence of *T. chilonis* regarding treatment of egg stage

a) x dose

Percentage emergence (mean) of *T. chilonis* from host eggs (*S. cerealella*) based on field recommended dose (x) revealed (Table 2) that among the pesticides tested, fipronil, followed by abamectin and spinetoram were the most toxic pesticides for emergence with $< 10\%$ emergence, while acetamiprid showed 25.04% emergence, followed by spiromesifen (65.22%), and were all found statistically significantly different not only from each other but also from the remaining pesticides used in the treatment ($p \leq 0.001$).

Maximum percentage of emergence was observed for myclobutanil (94.34), followed by mixture of trifloxystrobin + tebuconazole (93.91), bispyribac sodium (93.36) and haloxyfop-p-methyl (89.26), and these were not differed statistically from each other ($p > 0.001$). Emergence in the remaining pesticide treatments ranging from 83.21 to 86.17%, and did not differ significantly from each other ($p > 0.001$).

b) 2x dose

The twice field dose demonstrated (Table 2) that the most toxic pesticide for emergence of *T. chilonis* was fipronil, followed by spinetoram, abamectin and acetamiprid, which resulted in $< 6\%$ emergence, Fipronil, spinetoram, abamectin were found statistically at par with each other ($p > 0.001$). Spiromesifen treatment led to

60.90% emergence. Acetamiprid and spiromesifen were found significantly different from each other and also from the remaining pesticides ($p \leq 0.001$) tested at this dose against the egg stage of the beneficial parasitic wasp. Emergence from eggs treated with mixture of pyraclostrobin + metiram, chlorantraniliprole and nicosulfuron were 79.18%, 80.13% and 83.33%, respectively, and were all statistically at par with each other ($p > 0.001$). While emergence from the remaining treatments ranged from 85.43 to 96.48%.

c) 0.5x dose

The 0.5x dose results regarding percentage of emergence (mean) of minute parasitoids demonstrated (Table 2) that the most toxic chemical was fipronil, followed by abamectin, which led to 0.68 and 7.60% emergence, respectively, followed by spinetoram (28.58%), acetamiprid (33.94%), and spiromesifen (72.85%), and were all found significantly different not only from each other but also from the remaining chemicals tested with the egg stage ($p \leq 0.001$). The remaining pesticides resulted in emergence ranging from 82.83 to 96.24%.

d) Comparison of pesticide dose treatments with control

Comparison of means of doses with their respective control treatments (mean) based on percent emergence (Table 2) showed that all three doses of spinetoram, abamectin, fipronil, acetamiprid and spiromesifen were significantly different from their respective control treatments ($p \leq 0.001$). Similarly, 2x dose of mixture of pyraclostrobin + metiram also differed significantly its control treatment ($p \leq 0.001$). However, all the doses treated for the remaining pesticides were found statistically at par with their respective control treatments ($p > 0.001$).

Table 2. Percentage emergence (mean $\pm$ SE) of *T. chilonis* from host eggs (*S. cerealella*) treated with different pesticides when parasitoids were in egg stage, and means comparison based on square root transformed data (Tukey's HSD, $P = 0.001$ or 0.1%)

S.no	Pesticides	Doses (mean $\pm$ S.E)			
		2x	x	0.5x	Control
1	HaNPV	87.36 $\pm$ 9.57 defghijkl	83.21 $\pm$ 10.42 jklm	83.71 $\pm$ 8.74 ijklm	88.00 $\pm$ 8.50 cdefghijk
2	Spirotetramat	85.43 $\pm$ 2.99 ghijklm	85.48 $\pm$ 5.79 ghijklm	85.16 $\pm$ 4.38 ghijklm	85.62 $\pm$ 7.78 ghijklm
3	Chlorantraniliprole	80.13 $\pm$ 7.28 lm	86.17 $\pm$ 3.87 fghijklm	86.46 $\pm$ 3.47 efghijklm	86.55 $\pm$ 4.69 efghijkl
4	Acetamiprid	5.41 $\pm$ 1.28 s	25.04 $\pm$ 3.31q	33.94 $\pm$ 5.13 p	80.73 $\pm$ 8.50 klm
5	Spinetoram	0.66 $\pm$ 0.46 uv	9.17 $\pm$ 3.01r	28.58 $\pm$ 3.93 q	90.85 $\pm$ 3.31 bcdefghi
6	Fipronil	0.37 $\pm$ 0.37 uv	0.00 $\pm$ 0.00 v	0.68 $\pm$ 0.52 uv	92.20 $\pm$ 3.35 abcdefg
7	Abamectin	1.06 $\pm$ 0.52 u	3.42 $\pm$ 0.91 t	7.60 $\pm$ 1.26 r	91.24 $\pm$ 3.31 bcdefgh
8	Spiromesifen	60.90 $\pm$ 7.19 o	65.22 $\pm$ 7.42 o	72.85 $\pm$ 5.71n	89.40 $\pm$ 8.06 bcdefghij
9	Haloxypop-p-methyl	88.43 $\pm$ 3.50 cdefghij	89.26 $\pm$ 2.53 bcdefghij	86.88 $\pm$ 3.32 defghijkl	89.57 $\pm$ 3.55 bcdefghij
10	Bispyribac sodium	90.82 $\pm$ 2.78 bcdefghi	93.36 $\pm$ 2.93 abcdef	95.05 $\pm$ 2.75 abc	96.67 $\pm$ 3.33 ab

S.no	Pesticides	2x	x	0.5x	Control
11	Nicosulfuron	83.33 ± 4.82 ijklm	83.50 ± 2.86 jklm	83.27 ± 4.08 jklm	83.57 ± 5.54 ijklm
12	Myclobutanil	95.20 ± 1.51 abc	94.34 ± 2.89 abcd	95.32 ± 2.06 abc	95.63 ± 2.30 abc
13	Chlorothalonil + Procymidone	89.15 ± 1.87 bcdefghij	83.95 ± 4.07 hijklm	89.68 ± 2.46 bcdefghij	89.95 ± 2.12 bcdefghij
14	Pyraclostrobin + Metiram	79.18 ± 6.36 mn	86.14 ± 7.91fghijklm	82.83 ± 4.18 jklm	89.51 ± 3.55 bcdefghij
15	Trifloxystrobin + Tebuconazole	96.48 ± 1.60 ab	93.91 ± 3.23 abcde	96.24 ± 2.45 ab	100.00 ± 0.00 a
ANOVA results based on sqrt transformed data (emergence based on egg treatment)					
—		df	f	P	Remarks
Pesticides		14	6489.64	< 0.0001	sig
Doses		3	4667.82	< 0.0001	sig
Pesticides * Doses		42	806.12	< 0.0001	sig

Means followed by the same letter within a column/among columns are not significantly different (Turkey's HSD, $p > 0.001$).

4.1.3) Percentage reduction in emergence by pesticides and dose over control of *T. chilonis* when treated at egg stage, and IOBC/WPRS toxicity ranking

a) x dose

At field dose (Table 3), HaNPV, spirotetramat, chlorantraniliprole, mixture of pyraclostrobin + metiram, mixture of trifloxystrobin + tebuconazole, mixture of chlorothalonil + procymidone, myclobutanil, nicosulfuron, bispyribac sodium and haloxyfop-p-methyl yielded < 7% reduction in emergence, while spiromesifen demonstrated 27.04% reduction, and all the aforementioned chemicals were ranked as harmless for emergence of the tiny wasp. While the most toxic chemical, ranked as harmful for emergence, was fipronil, followed by abamectin and spinetoram (both moderately harmful). Acetamiprid was slightly harmful for emergence of parasitoid.

b) 2x dose

At this dose (Table 3) HaNPV, spirotetramat, chlorantraniliprole, haloxyfop-p-methyl, bispyribac sodium, nicosulfuron, myclobutanil, chlorothalonil+ procymidone, pyraclostrobin + metiram and trifloxystrobin + tebuconazole were all harmless for emergence of *T. chilonis*: all resulting in < 12% reduction in emergence relative to controls. Fipronil and spinetoram were harmful, followed by both abamectin and acetamiprid (moderately harmful), and spiromesifen was slightly harmful for emergence.

c) 0.5x dose

Half field dose demonstrated (Table 3) that fipronil was the most toxic chemical: ranked as harmful for emergence of *T. chilonis*, followed by abamectin (moderately harmful), both spinetoram and acetamiprid (slightly harmful). The remaining chemicals were all harmless for emergence of tiny parasitoids with $\leq 18.51\%$ reduction in parasitism over control.

Table 3. Percentage reduction (mean) in emergence over control of *T. chilonis* from the host eggs (*S. cerealella*) treated with different pesticides when parasitoids were in egg stage, and ranking of toxicity (IOBC/WPRS ranking)

S.no	Pesticides	Doses-wise percent reduction in emergence, and IOBC ranking							Control (Emer %)
		2x	Class	x	Class	0.5x	Class	Mean reduction	
1	HaNPV	0.73	1	5.44	1	4.88	1	3.68	88.00
2	Spirotetramat	0.22	1	0.16	1	0.54	1	0.31	85.62
3	Chlorantraniliprole	7.42	1	0.44	1	0.11	1	2.65	86.55
4	Acetamiprid	93.30	3	68.98	2	57.95	2	73.41	80.73
5	Spinetoram	99.27	4	89.91	3	68.54	2	85.91	90.85
6	Fipronil	99.60	4	100.00	4	99.26	4	99.62	92.20
7	Abamectin	98.84	3	96.25	3	91.67	3	95.59	91.24
8	Spiromesifen	31.87	2	27.04	1	18.51	1	25.81	89.40
9	Haloxypop-p-methyl	1.27	1	0.34	1	3.00	1	1.54	89.57
10	Bispyribac sodium	6.05	1	3.42	1	1.68	1	3.72	96.67
11	Nicosulfuron	0.29	1	0.08	1	0.36	1	0.25	83.58
12	Myclobutanil	0.45	1	1.35	1	0.33	1	0.71	95.63
13	Chlorothalonil +Procymidone	0.89	1	6.67	1	0.30	1	2.62	89.95
14	Pyraclostrobin + Metiram	11.54	1	3.76	1	7.46	1	7.59	89.51
15	Trifloxystrob + Tebuconazole	3.52	1	6.09	1	3.76	1	4.46	100

Ranking: class 1= harmless ($E < 30\%$); 2 = slight harmful ($30 \leq E \leq 79\%$); 3 = moderately harmful ($79 < E \leq 99\%$); 4 = harmful ($> 99\%$)

4.1.4) Percentage emergence relative to control of *T. chilonis* regarding treatment of egg stage

a) x dose

The relative emergence (means) of dose treatments with respective controls (untreated) of *T. chilonis* at field dose (x) demonstrated (Fig 1) that no emergence occurred with treatment of fipronil (0.00%), followed by abamectin (3.75%), spinetoram (10.09%) and acetamiprid (31.02%). Spiromesifen resulted in emergence (%): 72.96, followed by chlorantraniliprole (93.33), trifloxystrobin + tebuconazole (93.91), HaN-PV (94.56), pyraclostrobin + metiram (96.24), bispyribac sodium (96.58), myclobutanil (98.65), chlorothalonil + procymidone (99.56), haloxyfop-p-mrthyl (99.66), spirotetramat (99.84) and nicosulfuron (99.92%) relative to controls.

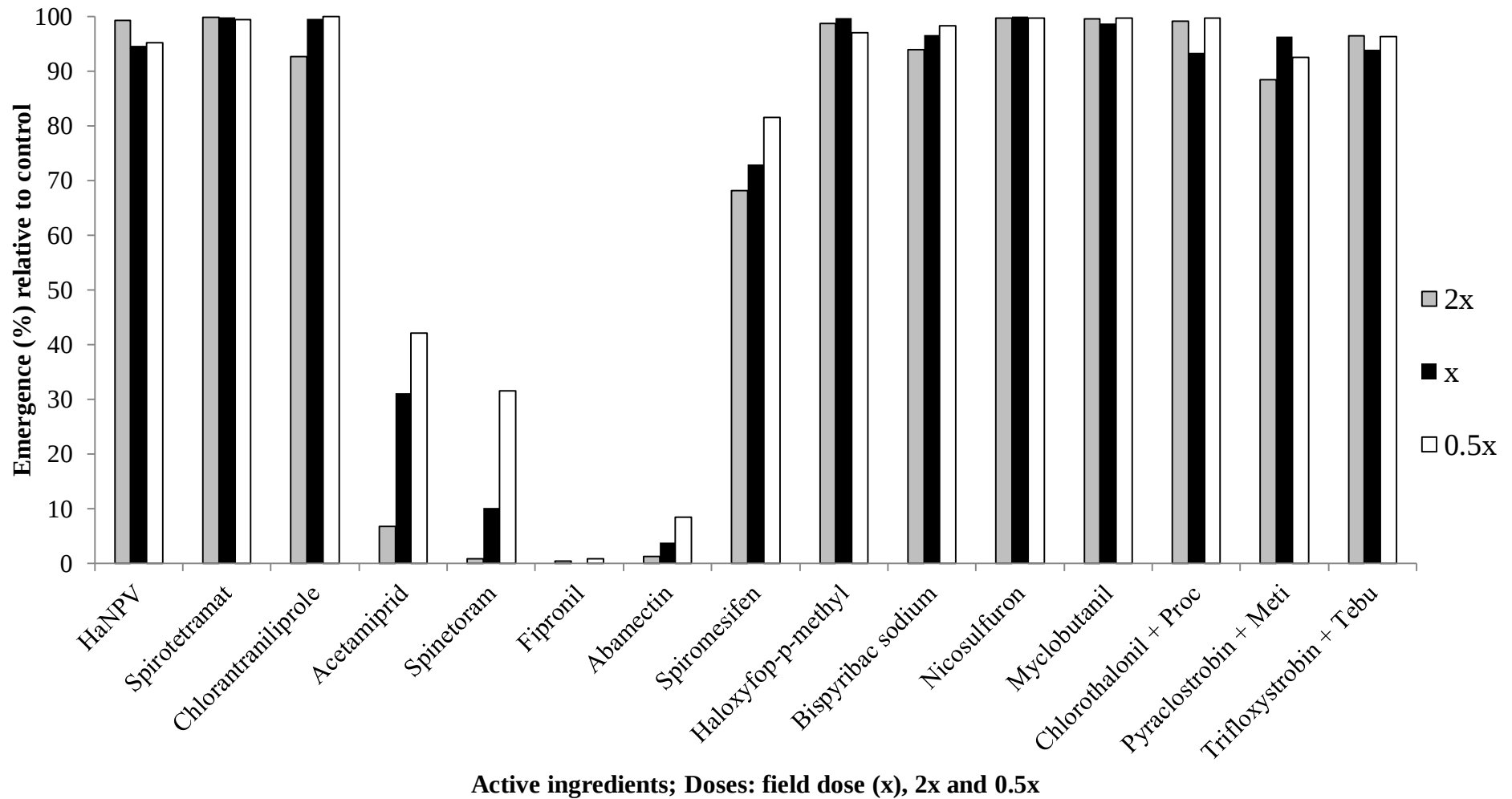
b) 2x dose

This dose (Fig 1) showed that treatment with fipronil led to 0.40% emergence of tiny wasp relative to controls, followed by spinetoram (0.73%), abamectin (1.16%), and acetamiprid (6.70%). Spiromesifen led to 68.13% emergence. The remaining pesticides resulted in emergence of *T. chilonis* ranging from 88.46 to 99.78% relative to controls.

c) 0.5x dose

Fipronil was the most toxic among the used chemicals even at this lower than field dose (Fig 1): causing lowest emergence of 0.74% of tiny parasitoid, followed by abamectin (8.33%), spinetoram (31.46%) and acetamiprid (42.05%). Spiromesifen led to 81.49% emergence. The remaining chemicals demonstrated emergence by tiny parasitoids ranging from 92.54 to 99.89% relative to controls.

Fig. 1 Percent emergence (mean) relative to control of *T. chilonis* from host eggs (*S. cerealella*) treated with different pesticides when parasitoids were in egg stage



4.2) Percent Emergence of *T. chilonis* When Treated at Larval Stage within Host Eggs

4.2.1) Anova on percent emergence after larval exposure

Pesticides were assessed for their effects on percent emergence of *T. chilonis* from host eggs treated at larval stage of parasitoids. The anova (Table 4) for the interaction of pesticides and doses demonstrated significant effects on emergence of *T. chilonis* ($df = 39, 504$; $f = 850.65$; $p < 0.0001$).

4.2.2) Comparison of pesticide-dose means of percent emergence of *T. chilonis* regarding treatment of larval stage

a) x dose

Percentage emergence (mean) of *T. chilonis* from host eggs (*S. cerealella*) based on field recommended dose (x) revealed (Table 4) that among all the pesticides, abamectin, followed by spinetoram and fipronil were the most toxic pesticides with < 12% emergence of parasitic wasps. While emergence from eggs treated with acetamiprid was 59.68%, followed by haloxyfop-p-methyl and spiromesifen were 69.23%, 71.43%, respectively. The remaining pesticides demonstrated emergence of tiny wasp ranging from 82.78 to 94.01%.

Acetamiprid and fipronil were significantly different from each other and also from the remaining used chemicals ($p \leq 0.001$). While spinetoram was significantly different from all used chemicals except abamectin ($p \leq 0.001$). Spiromesifen and haloxyfop-p-methyl were also statistically at par with each other among the tested products ($p > 0.001$). HaNPV, spirotetramat, bispyribac sodium, pyraclostrobin + metiram and trifloxystrobin + tebuconazole were statistically at par with each other ($p > 0.001$). Although, both of chlorantraniliprole and HaNPV were statistically at par with nicos-

ulfuron, trifloxystrobin + tebuconazole and myclobutanil ($p > 0.001$), yet they were significantly different from each other ($p \leq 0.001$).

b) 2x dose

Based on this dose the means results of percentage emergence of *T. chilonis* (Table 4) revealed that fipronil was the most toxic insecticide among the tested pesticides, followed by abamectin and spinetoram, with $< 6\%$ emergence, followed by acetamiprid (47.16%), spirotetramat (63.00%), spiromesifen (64.95%), and haloxyfop-p-methyl (69.67%). The remaining pesticides exhibited emergence ranging from 85.49 to 95.27%. Fipronil, abamectin, spinetoram and acetamiprid were significantly different from each other, and also from the remaining tested pesticides ($p \leq 0.001$). Spiromesifen was significantly not different from each spirotetramat and haloxyfop-p-methyl ($p > 0.001$). HaNPV, chlorantraniliprole, myclobutanil, bispyribac sodium and nicosulfuron were statistically at par with each other ($p > 0.001$). Similarly, HaNPV, pyraclostrobin + metiram, bispyribac sodium, nicosulfuron and trifloxystrobin + tebuconazole were also found statistically at par with each other ($p > 0.001$).

c) 0.5x dose

At this dose, percentage emergence (mean) of parasitoid demonstrated that the most toxic chemical (Table 4) was spinetoram, followed by abamectin i.e. with $< 18\%$ emergence, and fipronil (42.33%), which were significantly different from each other and also from the remaining tested pesticides ($p \leq 0.001$). While haloxyfop-p-methyl and spiromesifen showed 75.09 and 80.42% emergence, respectively, and were statistically at par with each other ($p > 0.001$). Spiromesifen was also statistically similar with each acetamiprid and spirotetramat ($p > 0.001$). While myclobutanil and pyraclostrobin + metiram were significantly different from each other ($p \leq 0.001$). The remaining pesticides resulted in emergence of *T. chilonis* ranging from 83.04 to 95.15%.

d) Comparison of pesticide dose treatments with control

Comparison of means of doses with their respective control treatments (mean) based on percent emergence (Table 4) showed that all three doses of each spinetoram, fipronil, abamectin, spiromesifen and haloxyfop-p-methyl were significantly different from their respective control treatments ($p \leq 0.001$). Similarly, 2x dose of spirotetramat, and both 2x and x doses of acetamiprid also indicated significant differences from their respective control treatments ($p \leq 0.001$). However, the three doses of the remaining pesticides were statistically at par with each other and with their respective control treatments ($p > 0.001$).

Table 4. Percentage emergence (mean $\pm$ SE) of *T. chilonis* from the host eggs (*S. cerealella*) treated with different pesticides when parasitoids were in larval stage, and means comparison based on square root transformed data (Tukey's HSD, P = 0.001 or 0.1%)

S.no	Pesticides	Doses (mean $\pm$ SE)			
		2x	x	0.5x	Control
1	HaNPV	91.64 $\pm$ 3.21 abcdefgh	87.23 $\pm$ 4.70 fghijk	89.70 $\pm$ 2.97 abcdefghi	92.09 $\pm$ 4.09 abcdefg
2	Spirotetramat	63.00 $\pm$ 1.82 p	82.78 $\pm$ 5.54 kl	83.04 $\pm$ 4.54jkl	86.02 $\pm$ 6.28 ghijkl
3	Chlorantraniliprole	95.27 $\pm$ 3.48 ab	94.01 $\pm$ 0.83 abcde	95.15 $\pm$ 1.92 abc	95.38 $\pm$ 1.84 a
4	Acetamiprid	47.16 $\pm$ 5.01 q	59.68 $\pm$ 2.90 p	83.06 $\pm$ 4.00 jkl	86.93 $\pm$ 3.90 fghijk
5	Spinetoram	5.44 $\pm$ 3.27 u	5.03 $\pm$ 1.05 u	11.59 $\pm$ 2.95 t	90.89 $\pm$ 6.73 abcdefgh
6	Fipronil	0.00 $\pm$ 1.39 w	11.39 $\pm$ 2.81 t	42.33 $\pm$ 4.88 r	90.24 $\pm$ 6.73 abcdefghi
7	Abamectin	2.94 $\pm$ 0.89 v	4.34 $\pm$ 2.36 u	17.20 $\pm$ 3.10 s	90.89 $\pm$ 3.29 abcdefgh
8	Spiromesifen	64.95 $\pm$ 7.48 op	71.43 $\pm$ 4.06 n	80.42 $\pm$ 3.31 lm	88.59 $\pm$ 3.08 cdefghijk
9	Haloxypop-p-methyl	69.67 $\pm$ 6.61 no	69.23 $\pm$ 8.07 no	75.09 $\pm$ 7.21 mn	89.51 $\pm$ 7.88 abcdefghi
10	Bispyribac sodium	88.75 $\pm$ 5.11 abcdefghijk	84.00 $\pm$ 6.06 ijkl	89.05 $\pm$ 3.82 abcdefghijk	89.66 $\pm$ 6.28 abcdefghi

S.no	Pesticides	2x	x	0.5x	Control
11	Nicosulfuron	89.23 ± 1.44 abcdefghi	89.20 ± 2.33 abcdefghi	89.29 ± 3.09 abcdefghi	89.71± 6.28 abcdefghi
12	Myclobutanil	94.70 ± 2.92 abcde	93.25 ± 2.84 abcdef	94.82 ± 2.27 abcde	95.08 ± 1.99 abcd
13	Pyraclostrobin + Metiram	85.49 ± 10.00 hijkl	84.30 ± 6.18 ijkl	86.82 ± 8.83 fghijk	89.51 ± 7.88 abcdefghi
14	Trifloxystrobin + Tebuconazole	88.27 ± 3.06 efghijk	88.21 ± 2.77 efghijk	88.48 ± 1.57 defghijk	88.64 ± 3.35 bcdefghijk
ANOVA Results based on sqrt transformed data (emergence based on larval treatment)					
—		df	f	p	Remark
Pesticides		13	6082.78	< 0.0001	sig
Doses		3	4886.32	< 0.0001	sig
Pesticides * Doses		39	850.65	< 0.0001	sig

Means followed by the same letter within a column/among columns are not significantly different (Tukey's HSD, $p > 0.001$)

4.2.3) Percentage reduction in emergence by pesticides and dose over control of *T. chilonis* when treated at larval stage, and IOBC/WPRS toxicity ranking

a) x dose

The most toxic chemical for emergence at field dose (Table 5) was abamectin, followed by spinetoram and fipronil, and they were ranked as moderately harmful for emergence. Acetamiprid was categorized as slightly harmful for emergence of tiny wasp. The remaining chemicals were ranked as harmless for emergence of tiny parasitoid: they demonstrated < 7% reduction in emergence over control (Table 5), except haloxyfop-p-methyl and spiromesifen that caused 22.66 and 19.37% reduction in emergence over control of *T. chilonis*, respectively.

b) 2x dose

The pesticides tested and found harmless for emergence at the aforementioned field dose were also found harmless for emergence even at this dose: causing < 5% reduction over control, except for haloxyfop-p-methyl, spiromesifen and spirotetramat, which reduced emergence over control ranging from 22.17 to 26.76%. Nevertheless, fipronil was the most toxic chemical and was ranked as harmful, followed by abamectin and spinetoram (moderately harmful) and acetamiprid was slightly harmful for emergence of *T. chilonis*.

c) 0.5x dose

Half-x dose revealed (Table 5) that spinetoram and abamectin were categorized as moderately harmful for emergence, followed by fipronil (slightly harmful). The remaining pesticides were found harmless for emergence of tiny wasp: causing < 5% reduction in emergence except spiromesifen and haloxyfop-p-methyl, which resulted in 9.22 and 16.10% reduction in emergence over controls, respectively.

Table 5. Percentage reduction (mean) in emergence over control of *T. chilonis* from the host eggs (*S. cerealella*) treated with different pesticides at larval stage of parasitoids, and ranking of toxicity (IOBC/WPRS ranking)

S.no	Pesticides	Doses-wise percent reduction in emergence, and IOBC ranking							Control (Emer %)
		2x	Class	x	Class	0.5x	Class	Mean reduction	
1	HaNPV	0.49	1	5.28	1	2.6	1	2.79	92.09
2	Spirotetramat	26.76	1	3.77	1	3.47	1	11.34	86.03
3	Chlorantraniliprole	0.11	1	1.44	1	0.24	1	0.6	95.38
4	Acetamiprid	45.75	2	31.35	2	4.45	1	27.18	86.93
5	Spinetoram	94.02	3	94.47	3	87.25	3	91.91	90.89
6	Fipronil	100	4	87.37	3	53.09	2	80.15	90.24
7	Abamectin	96.77	3	95.22	3	81.08	3	91.02	90.89
8	Spiromesifen	26.68	1	19.37	1	9.22	1	18.42	88.59
9	Haloxypop-p-methyl	22.17	1	22.66	1	16.1	1	20.31	89.51
10	Bispyribac sodium	1.01	1	6.32	1	0.68	1	2.67	89.66
11	Nicosulfuron	0.54	1	0.58	1	0.48	1	0.53	89.72
12	Myclobutanil	0.4	1	1.92	1	0.27	1	0.86	95.08
13	Pyraclostrobin + Metiram	4.49	1	5.81	1	3	1	4.43	89.51
14	Trifloxystrobin + Tebuconazole	0.42	1	0.49	1	0.19	1	0.36	88.64

Ranking: class 1= harmless ($E < 30\%$); 2 = slight harmful ($30 \leq E \leq 79\%$); 3 = moderately harmful ($79 < E \leq 99\%$); 4 = harmful ($> 99\%$)

4.2.4) Percentage emergence relative to control of *T. chilonis* regarding treatment of larval stage

a) x dose

The relative emergence (means) of dose treatments with respective controls (untreated) of *T. chilonis* at field dose (x) demonstrated (Fig 2) that least emergence was caused by the most toxic chemicals for emergence, including abamectin (4.78%), followed by spinetoram (5.53%) and fipronil (12.63%). Acetamiprid and haloxyfop-p-methyl led to 68.65 and 77.34% emergence, respectively, followed by spiromesifen (80.63%), bispyribac sodium (93.68%), pyraclostrobin + metiram (94.19%), HaNPV (94.72%), spirotetramat (96.23%), myclobutanil (98.08%), chlorantraniliprole (98.56%), nicosulfuron (99.42%) and trifloxystrobin + tebuconazole (99.51%) relative to controls.

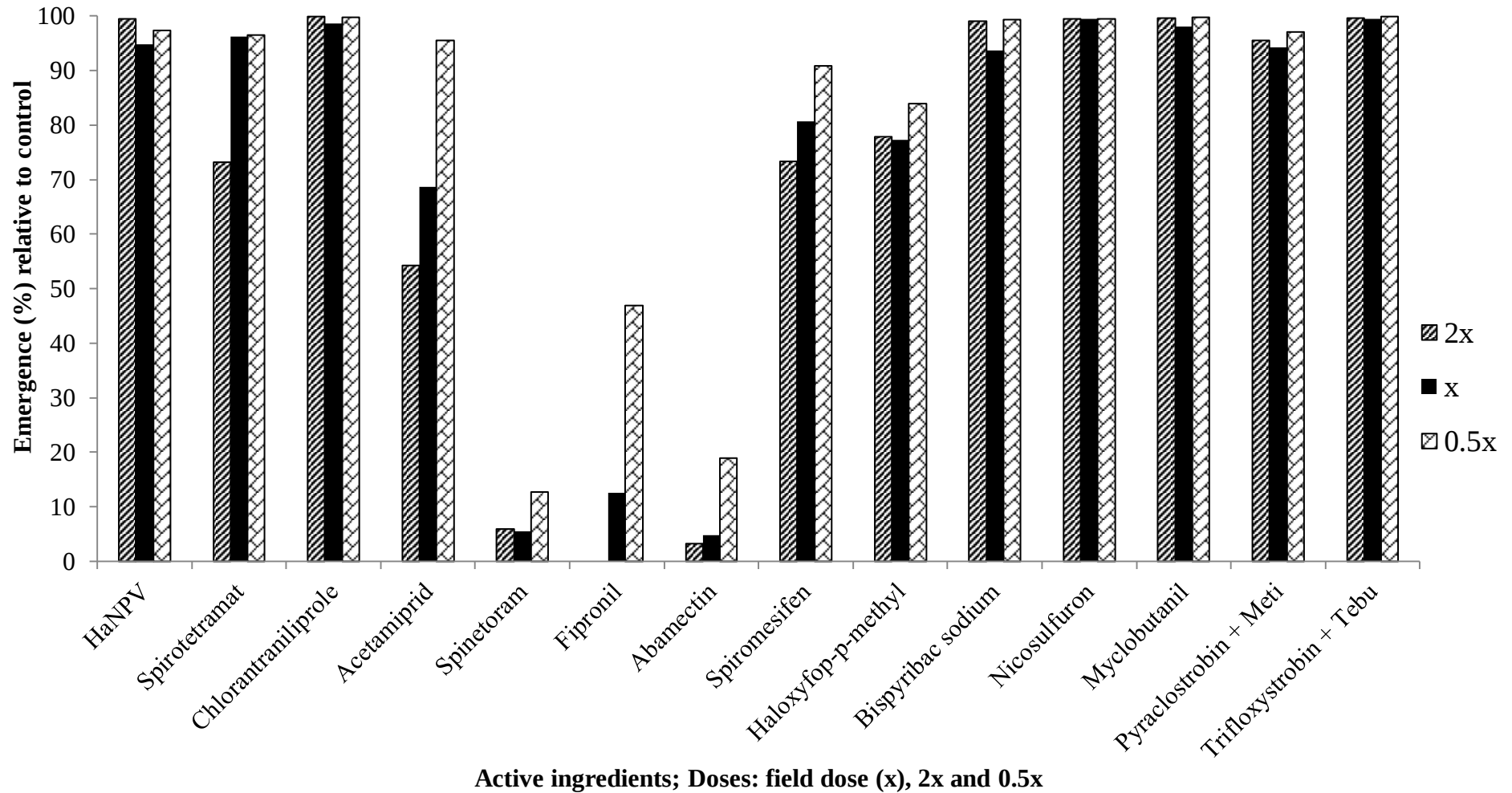
b) 2x dose

Fipronil at twice field dose (Fig 2) resulted in no emergence of *T. chilonis* i.e., 0.00%, followed by abamectin (3.23%), and spinetoram (5.98%). Acetamiprid, spirotetramat, spiromesifen and haloxyfop-p-methyl, led to 54.25, 73.24, 73.32, and 77.83%, emergence, respectively. The remaining chemicals led to emergence ranging from 95.51 to 99.60% relative to controls.

c) 0.5x dose

Spinetoram demonstrated highest toxicity to emergence even at this dose (Fig 2): yielding 12.75% emergence, followed by abamectin and fipronil with emergence of 18.92 and 46.91%, respectively. The remaining pesticides led to emergence of *T. chilonis* ranging from 83.90-99.81% relative to controls.

Fig. 2 Percent emergence (mean) relative to control of *T. chilonis* from host eggs (*S. cerealella*) treated with different pesticides at larval stage of parasitoids



4.3) Percent Emergence of *T. chilonis* When Treated at Pupal Stage within Host Eggs

4.3.1) Anova on percent emergence after pupal exposure

Pesticides were evaluated for their effect on percent emergence of *T. chilonis* from host eggs treated at pupal stage of parasitoids. The anova (Table 6) demonstrated that emergence of *T. chilonis* wasps was significantly affected as indicated by the interaction between pesticides and doses ($df = 39, 504$; $f = 623.25$; $p < 0.0001$).

4.3.2) Comparison of pesticide-dose means of percent emergence of *T. chilonis* regarding treatment of pupal stage

a) x dose

Percentage emergence (mean) of *T. chilonis* from host eggs (*S. cerealella*) based on field dose (x) revealed (Table 6) that spinetoram was the most toxic pesticide for emergence, exhibiting 2.09% emergence, followed by abamectin (19.12%), acetamiprid (28.99%) and fipronil (69.43%), and were all found significantly different not only from each other but also from the remaining pesticides tested against the pupal stage ($p \leq 0.001$). The remaining chemicals demonstrated emergence of parasitoids ranging from 81.83 to 93.32%.

HaNPV, chlorantraniliprole, spirotetramat, nicosulfuron, spiromesifen, pyraclostrobin + metiram, trifloxystrobin + tebuconazole and haloxyfop-p-methyl were statistically similar to each other ($p > 0.001$), while bispyribac sodium were statistically at par with the said chemicals except each nicosulfuron and pyraclostrobin + metiram ($p \leq 0.001$). Similarly, myclobutanil did not differ significantly from each HaNPV, pyraclostrobin + metiram, chlorantraniliprole, trifloxystrobin + tebuconazole and nicosulfuron ($p > 0.001$).

b) 2x dose

Accordingly 2x dose (Table 6), spinetoram was the most toxic pesticide, which resulted in 1.82% emergence, followed by acetamiprid (6.35%), abamectin (19.65%) and fipronil (28.43%). All these chemicals were significantly different from each other or from the remaining tested pesticides ($p \leq 0.001$). Parasitoids exposed to the remaining pesticides demonstrated emergence success ranging from 79.76 to 93.11%.

HaNPV, spirotetramat, chlorantraniliprole, spiromesifen, trifloxystrobin + tebuconazole, bispyribac sodium and nicosulfuron were statistically at par with each other ($p > 0.001$), except the last two, were significantly different from each other ($p \leq 0.001$). Similarly, pyraclostrobin + metiram, nicosulfuron, haloxyfop-p-methyl and myclobutanil were statistically similar ($p > 0.001$). While haloxyfop-p-methyl was significantly different from each spirotetramat and bispyribac sodium ($p \leq 0.001$).

c) 0.5x dose

Spinetoram was the most toxic chemical for emergence (Table. 6) even at half field dose: causing 6.36% emergence, followed by abamectin (52.56%), acetamiprid (60.86%) and fipronil (72.72%), and all were significantly different from each other and from the remaining chemicals ($p \leq 0.001$). The remaining chemicals resulted in emergence ranging from 83.84 to 93.17%, and were all statistically similar with each other ($p > 0.001$), except bispyribac sodium, which showed significant difference with each myclobutanil and pyraclostrobin + metiram ($p \leq 0.001$).

d) Comparison of pesticide dose treatments with control

Comparison of means of doses with their respective control treatments (mean) based on percent emergence (Table 6) revealed that all the treated doses of each acetamiprid, spinetoram, fipronil and abamectin were significantly different from their res-

pective control treatments ($p \leq 0.001$). While 2x dose of pyraclostrobin + metiram and haloxyfop-p-methyl also yielded significant differences from their respective control treatments ($p \leq 0.001$). The treated doses of the remaining pesticides were all statistically similar to each other and to their respective control treatments ($p > 0.001$).

Table 6. Percentage emergence (mean $\pm$ SE) of *T. chilonis* adults from the host eggs (*S. cerealella*) treated with different pesticides at pupal stage of parasitoid, and means comparison based on square root transformed data (Tukey's HSD, P = 0.001 or 0.1%)

S.no	Pesticides	Doses (mean $\pm$ SE)			
		2x	x	0.5x	Control
1	HaNPV	87.06 $\pm$ 1.94 abcdefghi	87.28 $\pm$ 5.65 abcdefghi	87.38 $\pm$ 2.74 abcdefghi	87.56 $\pm$ 5.85 abcdefghi
2	Spirotetramat	89.31 $\pm$ 2.11 abcdefg	88.99 $\pm$ 2.47 abcdefg	89.38 $\pm$ 2.47 abcdefg	89.40 $\pm$ 3.32 abcdefg
3	Chlorantraniliprole	86.37 $\pm$ 5.20 cdefghi	86.43 $\pm$ 7.91 bcdefghi	86.60 $\pm$ 5.18 abcdefghi	86.70 $\pm$ 4.46 abcdefghi
4	Acetamiprid	6.35 $\pm$ 2.18 p	28.99 $\pm$ 3.43 n	60.86 $\pm$ 5.82 l	85.54 $\pm$ 5.36 efghij
5	Spinetoram	1.82 $\pm$ 0.83 q	2.09 $\pm$ 0.77 q	6.36 $\pm$ 10.00 p	86.22 $\pm$ 8.39 defghij
6	Fipronil	28.43 $\pm$ 5.27 n	69.43 $\pm$ 4.21 k	72.72 $\pm$ 5.10 k	87.85 $\pm$ 5.85 abcdefghi
7	Abamectin	19.65 $\pm$ 2.43 o	19.12 $\pm$ 3.26 o	52.56 $\pm$ 6.56 m	86.11 $\pm$ 8.39 efghij
8	Spiromesifen	87.61 $\pm$ 3.94 abcdefghi	89.77 $\pm$ 2.33 abcdefg	90.12 $\pm$ 1.87 abcdefg	90.47 $\pm$ 3.92 abcdef
9	Haloxypop-p-methyl	81.96 $\pm$ 5.38 hij	88.69 $\pm$ 5.37 abcdefgh	88.33 $\pm$ 4.17 abcdefghi	89.40 $\pm$ 3.32 abcdefg
10	Bispyribac sodium	93.11 $\pm$ 2.73 abcd	93.32 $\pm$ 6.18 ab	93.17 $\pm$ 2.24 abc	93.37 $\pm$ 2.04 a
11	Nicosulfuron	85.11 $\pm$ 3.25 efghij	85.20 $\pm$ 5.08 efghij	90.55 $\pm$ 2.64 abcdef	90.69 $\pm$ 4.36 abcde

S.no	Pesticides	2x	x	0.5x	Control
12	Myclobutanil	82.03 ± 7.19 hij	81.83 ± 5.52 ij	83.84 ± 4.65 fghij	85.19 ± 3.86 efghij
13	Pyraclostrobin + Metiram	79.76 ± 3.03 j	83.46 ± 4.02 ghij	85.80 ± 3.45 efghij	87.23 ± 5.18 abcdefghi
14	Trifloxystrobin + Tebuconazole	87.30 ± 4.06 abcdefghi	87.35 ± 3.68 abcdefghi	86.99 ± 9.68 abcdefghi	87.44 ± 3.47 abcdefghi
ANOVA results based on sqrt transformed data (emergence based on pupal treatment)					
—		df	f	p	Remark
Pesticides		13	4319.20	< 0.0001	sig
Doses		3	2806.18	< 0.0001	sig
Pesticides * Doses		39	623.25	< 0.0001	sig

Means followed by the same letter within a column/among columns are not significantly different (Tukey's HSD, $p > 0.001$)

4.3.3) Percentage reduction in emergence by pesticides and dose over control of *T. chilonis* when treated at pupal stage, and IOBC/WPRS toxicity ranking

a) x dose

The highest percentage reduction (means) in emergence of *T. chilonis* over controls was observed (Table 7) with spinetoram, i.e., 97.58% (moderately harmful), followed by abamectin (77.79%) and acetamiprid (66.11%) as both of slightly harmful for the emergence by tiny parasitoid. The remaining pesticides resulted in < 6.5% (except fipronil, which led to 20.97%) reduction in emergence over control and were all harmless for emergence of tiny parasitoid.

b) 2x dose

Spinetoram and acetamiprid were both moderately harmful for emergence of parasitoids (Table 7). While abamectin and fipronil were ranked as slightly harmful for emergence. The remaining pesticides resulted in reduction in emergence ranging from 0.10 to 8.56% over control, and they were all rated harmless for the emergence of *T. chilonis* even at this dose.

c) 0.5x dose

0.5x dose showed (Table 7) that spinetoram was the only chemical as moderately harmful for emergence of parasitic wasps, followed by abamectin (slightly harmful). The remaining pesticides were harmless for emergence of *T. chilonis*.

Table 7. Percentage reduction (mean) in emergence over control of *T. chilonis* from the host eggs (*S. cerealella*) treated with different doses of pesticides at pupal stage of parasitoids, and ranking of pesticides according to IOBC/WPRS

S.no	Pesticides	Doses-wise percent reduction in emergence, and IOBC ranking							Control (Eme %)
		2x	Class	x	Class	0.5x	Class	Mean reduction	
1	HaNPV	0.58	1	0.32	1	0.2	1	0.37	87.56
2	Spirotetramat	0.1	1	0.47	1	0.03	1	0.2	89.4
3	Chlorantraniliprole	0.39	1	0.32	1	0.11	1	0.27	86.7
4	Acetamiprid	92.57	3	66.11	2	28.85	1	62.51	85.55
5	Spinetoram	97.88	3	97.58	3	92.63	3	96.03	86.23
6	Fipronil	67.64	2	20.97	1	17.23	1	35.28	87.86
7	Abamectin	77.18	2	77.79	2	38.96	2	64.65	86.11
8	Spiromesifen	3.16	1	0.76	1	0.38	1	1.44	90.47
9	Haloxypop-p-methyl	8.32	1	0.8	1	1.2	1	3.44	89.4
10	Bispyribac sodium	0.28	1	0.05	1	0.21	1	0.18	93.37
11	Nicosulfuron	6.14	1	6.05	1	0.15	1	4.12	90.69
12	Myclobutanil	3.72	1	3.95	1	1.59	1	3.08	85.2
13	Pyraclostrobin + Metiram	8.56	1	4.32	1	1.64	1	4.84	87.23
14	Trifloxystrobin+ Tebuconazole	0.16	1	0.1	1	0.51	1	0.26	87.44

Ranking: class 1= harmless ($E < 30\%$); 2 = slight harmful ($30 \leq E \leq 79\%$); 3 = moderately harmful ($79 < E \leq 99\%$); 4 = harmful ($> 99\%$).

4.3.4) Percentage emergence relative to control of *T. chilonis* regarding treatment of pupal stage

a) x dose

The relative emergence (means) of dose treatments with respective controls (untreated) of *T. chilonis* at field dose (x) demonstrated (Fig 3) that the least emergence (%) occurred with spinetoram (2.42), followed by abamectin (22.21), acetamiprid (33.89), fipronil (79.03), nicosulfuron (93.95), pyraclostrobin+ metiram (95.68), myclobutanil (96.05), haloxyfop-p-methyl (99.20), spiromesifen (99.24), spirotetramat (99.53), both of chlorantraniliprole and HaNPV (99.68), trifloxystrobin + tebuconazole (99.90) and bispyribac sodium (99.95) relative to controls.

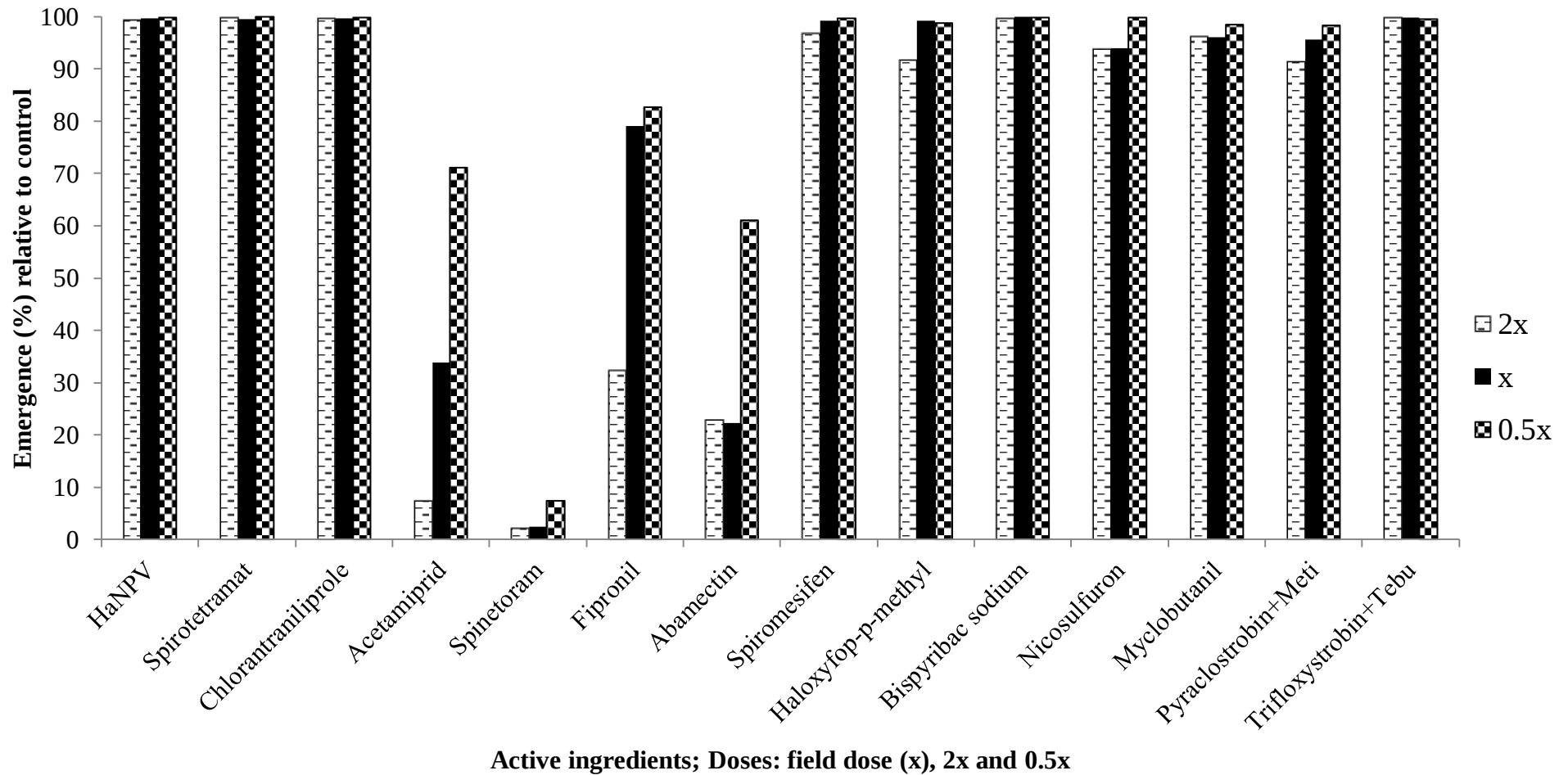
b) 2x dose

Spinetoram was the most toxic for emergence at this dose among the test products (Fig. 3), causing 2.12% emergence, followed by acetamiprid (7.43%), abamectin (22.82%) and fipronil (32.36%). The remaining pesticides resulted in emergence ranging from 91.44 to 99.90% relative to controls.

c) 0.5x dose

Spinetoram was the most toxic to emergence even at this lower than field dose (Fig 3) among the tested chemicals, causing 7.37% emergence of tiny wasp, followed by abamectin (61.04%), acetamiprid (71.15%) and fipronil (82.77%), while the remaining pesticides resulted > 98% of emergence relative to controls.

Fig. 3 Percent emergence (mean) relative to control of *T. chilonis* from host eggs (*S. cerealella*) treated with different pesticides when parasitoids were in pupal stage



4.3.5) Dose-as well as stage-wise effects of pesticide on percent reduction in emergence over control of *T. chilonis*, and IOBC toxicity ranking

The dose-and stage-wise effects of chemicals on emergence of *T. chilonis* from the treated host eggs of *S. cerealella*, when parasitoids were in each egg, larval and pupal stage, demonstrated that fipronil was harmful to parasitoid at each of three doses (x, 2x and 0.5x doses) treated in the egg treatment (Table 3), but demonstrated reduced toxicity in the larval treatment from harmful at 2x, moderately harmful at x, and slightly harmful for emergence at 0.5 x dose (Table 5). Fipronil also showed reduced toxicity to wasps as slightly harmful at 2x, while harmless for emergence at both of x and 0.5x doses regarding treatment of pupae (Table 7).

Abamectin demonstrated decreased toxicity from moderately harmful for emergence in egg and larval treatments (Table 3, 5, respectively) to slightly harmful for emergence of parasitoids in pupal treatment regarding all treated doses (Table 7).

Spinetoram was harmful for emergence at 2x, moderately harmful at x, and was slightly harmful at 0.5x dose regarding egg treatment (Table 3), but was consistently moderately harmful for emergence of tiny parasitoids at all three doses used in the treatment of larvae and pupae (Table 5, 7).

Acetamiprid was moderately harmful for emergence at 2x dose, and slightly harmful at x and 0.5x doses in egg treatment (Table 3). In the larval treatment (Table 5), acetamiprid showed reduced toxicity as slightly harmful at 2x and x doses, and harmless for emergence at 0.5x dose. However, in the pupal treatment (Table 7), acetamiprid was moderately harmful at 2x dose, slightly harmful at x dose, while harmless for emergence of *T. chilonis* at 0.5x dose.

The remaining pesticides were harmless for emergence of *T. chilonis* at the all doses used against egg, larvae and pupae (Tables 3, 5, 7, respectively) except spiromesifen, which was slightly harmful for emergence (i.e., 31.87% reduction in emergence), when treated at 2x dose at the egg stage.

Section: 1-B

EFFECT OF PESTICIDES ON THE PARASITISM BY *T. chilonis* EMERGED FROM TREATED HOST EGGS

4.4) Parasitism (mean) by *T. chilonis* Emerged from the Host Eggs Treated at Egg Stage of Parasitoid

4.4.1) Anova on parasitism after egg exposure

Pesticides were evaluated for their effect on parasitism by *T. chilonis*, exposed to pesticides at egg stage within host eggs (*S. cerealella*). The analysis of variance (Table 8) for interaction between pesticides and doses revealed that parasitism by female *T. chilonis* was significantly affected by treatments with different pesticides and doses ($df = 33, 432$; $f = 228.24$; $p < 0.0001$).

4.4.2) Comparison of pesticide-dose means of parasitism by *T. chilonis* regarding egg treatment

a) x dose

Parasitism of host eggs (*S. cerealella*) by *T. chilonis* based on FRC (x) showed (Table 8) that maximum mean parasitism (27.17) was by the females emerged from the host eggs (*S. cerealella*) treated at egg stage of *T. chilonis* with pyraclostrobin and metiram, followed by chlorantraniliprole (24.40), myclobutanil (24.28), spirotetramat (23.71), spiromesifen (22.32), haloxyfop-p-methyl (22.22), trifloxystrobin+tebuconazole (21.62), HaNPV (21.60), chlorothalonil + procymidone (21.03), bispyribac sodium (21.03) and nicosulfuron (17.47), while acetamiprid severely affected parasitism efficacy of the emerged females, and resulted in complete failure of parasitism (0.00).

Chlorantraniliprole, spirotetramat, spiromesifen, haloxyfop-p-methyl and myc-

lobutanil were found statistically at par with each other ($p > 0.001$). Similarly, HaNPV, spiromesifen, bispyribac sodium, chlorothalonil + procymidone, trifloxystrobin + tebuconazole and haloxyfop-p-methyl also demonstrated no significant difference with each other ($p > 0.001$). Spirotetramat was statistically similar with each HaNPV, chlorantraniliprole, spiromesifen, haloxyfop-p-methyl, trifloxystrobin + tebuconazole and myclobutanil ($p > 0.001$). Acetamiprid, nicosulfuron and pyraclostrobin + metiram were significantly different from each other and were also significantly different from the aforementioned pesticides tested against egg stage ($p \leq 0.001$).

b) 2x dose

Females emerged from eggs treated at this dose (Table 8) with the most toxic pesticide, acetamiprid, showed no successful parasitism (0.00), followed by mean parasitism by chlorothalonil + procymidone (13.42), nicosulfuron (16.26), trifloxystrobin + tebuconazole (18.48), bispyribac sodium (21.06), haloxyfop-p-methyl (22.04), spiromesifen (22.34), HaNPV (23.05). While myclobutanil, pyraclostrobin + metiram, spirotetramat and chlorantraniliprole demonstrated comparable mean parasitism by *T. chilonis* ($\geq 24.29 \leq 24.69$) when treated at egg stage.

HaNPV, spirotetramat, pyraclostrobin + metiram, myclobutanil, spiromesifen, haloxyfop-p-methyl and chlorantraniliprole were statistically at par with each other ($p > 0.001$), except the last two chemicals were significantly different from each other ($p \leq 0.001$). Similarly, bispyribac sodium was statistically similar with each HaNPV, haloxyfop-p-methyl and spiromesifen ($p > 0.001$). However, acetamiprid, nicosulfuron, chlorothalonil + procymidone and trifloxystrobin + tebuconazole were significantly different from each other and were also significantly different from the remaining pesticides tested at this dose against egg stage ($p \leq 0.001$).

c) 0.5x dose

This dose revealed (Table 8) that pyraclostrobin + metiram showed highest parasitism (mean) by parasitoids, i.e. 27.77, followed by spirotetramat (25.35), myclobutanil (24.27), trifloxystrobin + tebuconazole (23.99), chlorantraniliprole (23.95), and bispyribac sodium (23.15). While acetamiprid was the most toxic pesticide for parasitism: resulted in mean parasitism 2.00. Females exposed to the remaining chemicals parasitized from 21 to 22.37 hosts/female.

HaNPV, spiromesifen, haloxyfop-p-methyl, bispyribac sodium and chlorothalonil + procymidone were statistically at par with each other ($p > 0.001$). Similarly, spirotetramat, chlorantraniliprole, nicosulfuron, myclobutanil, bispyribac sodium and trifloxystrobin + tebuconazole were also significantly not different from each other ($p > 0.001$). Pyraclostrobin + metiram were statistically similar only with spirotetramat ($p > 0.001$). However, acetamiprid was significantly different from the remaining pesticides tested against egg stage of *T. chilonis* ($p \leq 0.001$).

d) Comparison of pesticide dose treatments with control

Comparison of means of doses with their respective controls (mean) based on parasitism (Table 8) by *T. chilonis* showed that all doses of each acetamiprid and haloxyfop-p-methyl were significantly different from their respective controls. Similarly, 2x dose of pyraclostrobin + metiram, and both 2x and x doses of each chlorothalonil + procymidone, nicosulfuron, bispyribac sodium and trifloxystrobin + tebuconazole were also found significantly different from their respective controls. All treated doses of remaining chemicals were statistically at par with their respective controls.

Table 8. Parasitism (mean $\pm$ S.E) by *T. chilonis* emerged from the host eggs (*S. cerealella*) treated at egg stage of parasitoid with different pesticides, and means comparison based on square root transformed data (Tukey's HSD, P = 0.001 or 0.1%)

S.no	Pesticides	Doses (means $\pm$ S.E)			
		2x	x	0.5x	Control
1	HaNPV	23.05 $\pm$ 2.28 efghi	21.60 $\pm$ 2.27 hi	21.00 $\pm$ 1.26 i	23.17 $\pm$ 1.86 efghi
2	Spirotetramat	24.35 $\pm$ 3.34 defg	23.71 $\pm$ 2.26 defgh	25.35 $\pm$ 3.16 bcde	25.52 $\pm$ 8.77 bcde
3	Chlorantraniliprole	24.69 $\pm$ 2.51cdef	24.40 $\pm$ 1.98 defg	23.95 $\pm$ 2.16 defgh	24.93 $\pm$ 2.88 cde
4	Acetamiprid	0.00 $\pm$ 0.00 n	0.00 $\pm$ 0.00 n	2.00 $\pm$ 0.21 m	23.07 $\pm$ 4.44 efghi
5	Spiromesifen	22.34 $\pm$ 9.76 fghi	22.32 $\pm$ 6.61 fghi	21.67 $\pm$ 7.25 hi	22.43 $\pm$ 4.96 fghi
6	Haloxypop-p-methyl	22.04 $\pm$ 2.93 ghi	22.22 $\pm$ 1.86 ghi	22.37 $\pm$ 2.78 fghi	28.66 $\pm$ 4.23 a
7	Bispyribac sodium	21.06 $\pm$ 1.28 i	21.03 $\pm$ 1.08 i	23.15 $\pm$ 2.03 efghi	23.56 $\pm$ 0.86 defgh
8	Nicosulfuron	16.26 $\pm$ 0.91 k	17.47 $\pm$ 2.21 jk	21.84 $\pm$ 2.42 defgh	24.31 $\pm$ 7.71 defg
9	Myclobutanil	24.29 $\pm$ 1.83 defg	24.28 $\pm$ 0.83 defg	24.27 $\pm$ 1.63 defg	24.35 $\pm$ 0.99 defg
10	Chlorothalonil + Procymidone	13.42 $\pm$ 1.36 l	21.03 $\pm$ 1.70 i	22.11 $\pm$ 2.38 ghi	23.55 $\pm$ 1.70 defgh
11	Pyraclostrobin + Metiram	24.31 $\pm$ 4.10 defg	27.17 $\pm$ 2.53 abc	27.77 $\pm$ 4.48 ab	28.79 $\pm$ 4.23 a
12	Trifloxystrobin +Tebuconazole	18.48 $\pm$ 1.46 j	21.62 $\pm$ 2.15 hi	23.99 $\pm$ 3.28 defgh	25.68 $\pm$ 4.37 bcd

ANOVA results based on sqrt transformed data (parasitism based on egg treatment)				
–	df	f	P	Remark
Pesticides	11	2100.62	< 0.0001	sig
Doses	3	817.92	< 0.0001	sig
Pesticides * Doses	33	228.24	< 0.0001	sig

Means followed by the same letter within a column/among columns are not significantly different (Tukey's HSD, $p > 0.001$)

4.4.3) Percentage reduction in parasitism over control by *T. chilonis* against pesticides and dose when treated at egg stage, and IOBC/WPRS toxicity ranking

a) x dose

The field dose percentage reduction (mean) in parasitism over control (Table 9) demonstrated that acetamiprid resulted in the highest reduction in parasitism over control, i.e., 100% (harmful for parasitism). The remaining pesticides were all harmless for parasitism by the female wasps emerged from the treated eggs.

b) 2x dose

Acetamiprid led to highest reduction (100%) in parasitism over control (Table 9), followed by chlorothalonil + procymidone and nicosulfuron, and both were slightly harmful for parasitism. The remaining chemicals were harmless for parasitism by *T. chilonis* even at this dose.

c) 0.5x dose

Acetamiprid is the only toxic chemical for parasitism by the emerged female parasitoids (Table 9), i.e. moderately harmful for parasitism. The remaining pesticides were considered harmless for parasitism by *T. chilonis* emerged from treated host eggs.

Table 9. Percentage reduction in parasitism over control by *T. chilonis* emerged from host eggs (*S. cerealella*) treated with different pesticides at egg stage of parasitoid, and ranking of pesticides according to IOBC/WPRS

S.no	Pesticides	Dose-wise percent reduction in parasitism, and IOBC ranking							Control (mean parasitism)
		2x	Class	x	Class	0.5x	Class	Mean reduction	
1	HaNPV	0.50	1	6.76	1	9.35	1	5.54	23.17
2	Spirotetramat	4.56	1	7.06	1	0.67	1	4.10	25.52
3	Chlorantraniliprole	0.96	1	2.14	1	3.93	1	2.34	24.93
4	Acetamiprid	100.00	4	100.00	4	91.33	3	97.11	23.07
5	Spiromesifen	0.41	1	0.47	1	3.41	1	1.43	22.43
6	Haloxypop-p-methyl	23.11	1	22.47	1	21.94	1	22.51	28.66
7	Bispyribac sodium	10.61	1	10.73	1	1.74	1	7.70	23.56
8	Nicosulfuron	33.10	2	28.13	1	10.15	1	23.79	24.31
9	Myclobutanil	0.24	1	0.29	1	0.31	1	0.28	24.35
10	Chlorothalonil + Procymidone	43.00	2	10.69	1	6.12	1	19.94	23.55
11	Pyraclostrobin + Metiram	15.54	1	5.62	1	3.52	1	8.22	28.79
12	Trifloxystrobin +Tebuconazole	28.03	1	15.79	1	6.57	1	16.80	25.68

Ranking: class 1= harmless ($E < 30\%$); 2 = slight harmful ($30 \leq E \leq 79\%$); 3 = moderately harmful ($79 < E \leq 99\%$); 4 = harmful ($> 99\%$).

4.4.4) Percentage parasitism relative to control by *T. chilonis* regarding treatment of egg stage

a) x dose

The relative parasitism (means) of field-dose treatments (x) with respective controls of host eggs (*S. cerealella*) by *T. chilonis* (Fig 4), emerged from *S. cerealella* eggs treated at egg stage of parasitoids, showed acetamiprid as the most toxic pesticide for parasitism, with no parasitism (0.00%). Maximum parasitism was observed in myclobutanil (99.71%), followed by spiromesifen (99.53%), chlorantraniliprole (97.86%), pyraclostrobin + metiram (94.38%), HaNPV (93.24%), spirotetramat (92.94%), chlorothalonil + procymidone (89.31%), bispyribac sodium (89.27%), trifloxystrobin + tebuconazole (84.21%) and nicosulfuron (71.87%).

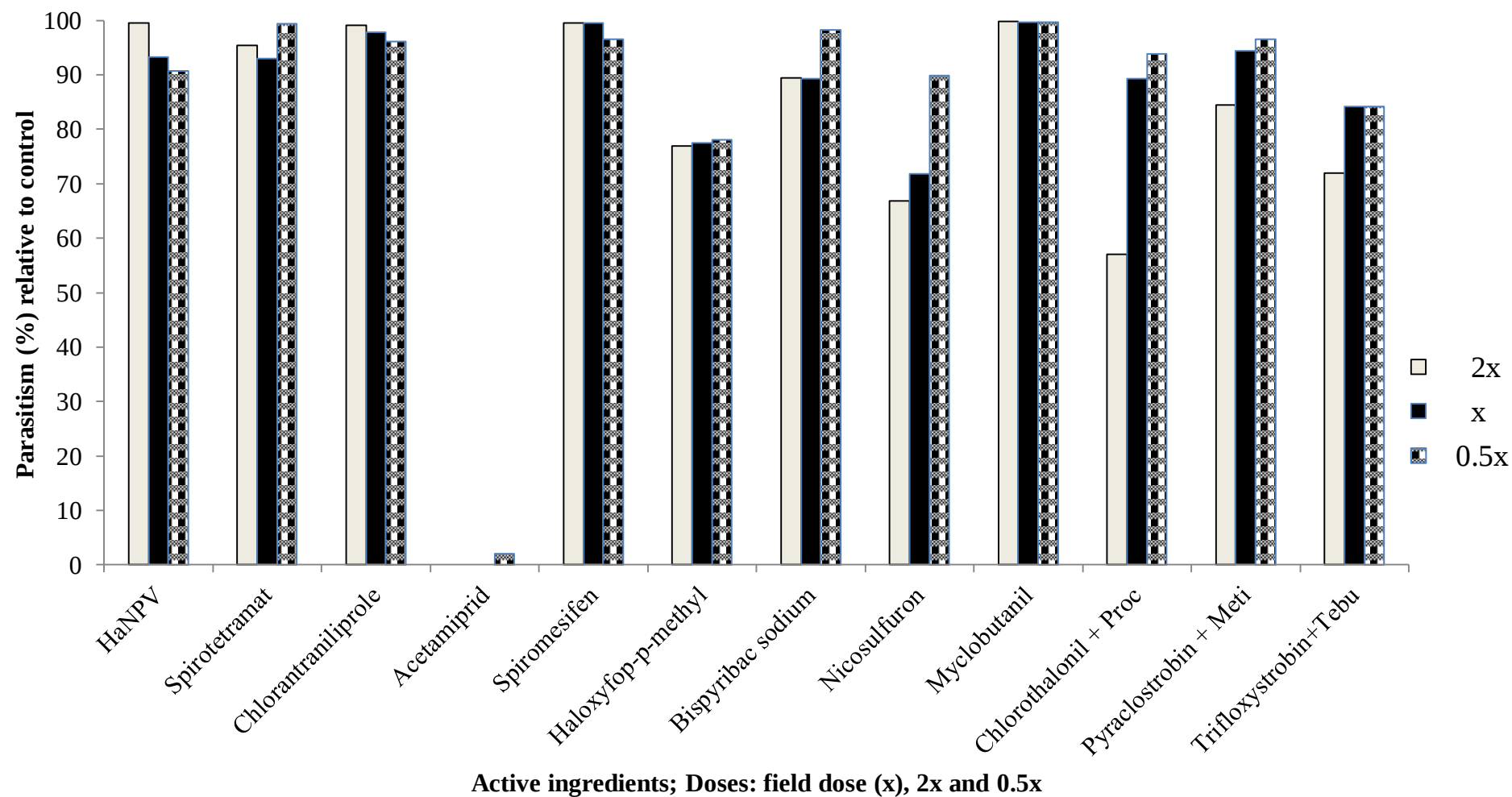
b) 2x dose

Highest sensitivity for parasitism by *T. chilonis* was observed for acetamiprid (Fig 4): parasitism (0.00%) relative to control, followed by chlorothalonil + procymidone, which led to 57.01% parasitism, nicosulfuron (66.90%), trifloxystrobin + tebuconazole (71.97%), haloxyfop-p-methyl (76.89%), pyraclostrobin + metiram (84.46%), bispyribac sodium (89.39%), spirotetramat (95.44%), chloraniliprole (99.04%), HaNPV (99.50%), spiromesifen (99.59%) and myclobutanil (99.76%).

c) 0.5x dose

As with the other two doses, parasitoids exposed to acetamiprid produced the lowest rates of parasitism: 0.00% (Fig 4), followed by haloxyfop-p-methyl (78.06%). Parasitoids exposed during the egg stage to the remaining pesticides produced parasitism ranging from 84.21 to 99.69%.

Fig. 4 Percent parasitism (mean) relative to control of *S. cerealella* eggs by *T. chilonis* emerged from host eggs (*S. cerealella*) treated at egg stage of parasitoids



4.5) Parasitism by *T. chilonis* Emerged from Host Eggs When Treated at Larval Stage

4.5.1) Anova on parasitism after larval exposure

Pesticides as well as their used doses affected parasitism by *T. chilonis* emerged from host eggs when treated at larval stage. The anova interaction between pesticides and doses (Table 10) revealed significant effects on the parasitism by females *T. chilonis* (df = 33, 432; $f = 99.80$; $p < 0.0001$).

4.5.2) Comparison of pesticide-dose means of parasitism by *T. chilonis* regarding larval treatment

a) x dose

Number of hosts (*S. cerealella*) parasitized by female *T. chilonis* emerged from the host eggs (*S. cerealella*) treated with different pesticides at parasitoid larval stage at field dose (x) demonstrated (Table 10) that among all the pesticides, bispyribac sodium showed maximum mean parasitism (26.92) by wasps, followed by HaNPV (26.52), nicosulfuron (26.33), pyraclostrobin + metiram (26.32), trifloxystrobin + tebuconazole (23.94), haloxyfop-p-methyl (23.88), spirotetramat (23.10), myclobutanil (22.02), chlorantraniliprole (21.97), acetamiprid (20.58), spiromesifen (19.48) and fipronil (7.78).

HaNPV, nicosulfuron, pyraclostrobin + metiram, trifloxystrobin + tebuconazole, haloxyfop-p-methyl and bispyribac sodium were statistically at par with each other ($p > 0.001$). Similarly, spirotetramat, myclobutanil, chlorantraniliprole and acetamiprid were significantly not different from each other ($p > 0.001$). Spiromesifen was statistically similar to with each chlorantraniliprole, myclobutanil and acetamiprid, while haloxyfop-p-methyl was statistically at par with each myclobutanil and trifloxys-

trobin + tebuconazole ($p > 0.001$). However, females in the fipronil treatment parasitized significantly fewer hosts than females in all other pesticides tested against larvae ($p \leq 0.001$).

b) 2x dose

Twice-field dose showed that maximum mean parasitism (27.51) by *T. chilonis* was observed (Table 10) for HaNPV, followed by bispyribac sodium (26.82), pyraclostrobin + metiram (26.37), nicosulfuron (26.07), haloxyfop-p-methyl (23.14), trifloxystrobin + tebuconazole (22.51), chlorantraniliprole (21.68), spirotetramat (20.75), spiromesifen (19.68) and acetamiprid (10.15).

HaNPV, nicosulfuron and pyraclostrobin + metiram were statistically at par with each other ($p > 0.001$). Similarly, spirotetramat, chlorantraniliprole, haloxyfop-p-methyl, bispyribac sodium and trifloxystrobin + tebuconazole were statistically similar with each other ($p > 0.001$). However, acetamiprid and fipronil were found significantly different from each other and also from the remaining tested pesticides ($p \leq 0.001$). Both of pyraclostrobin + metiram and haloxyfop-p-methyl were significantly not different from each nicosulfuron and bispyribac sodium ($p > 0.001$). No significant difference was existed among myclobutanil, spirotetramat, chlorantraniliprole and spiromesifen ($p > 0.001$). Furthermore, spiromesifen was not differed significantly from trifloxystrobin + tebuconazole ($p > 0.001$).

c) 0.5x dose

Similarly to the field dose, fipronil was the single most toxic among the used chemicals (Table 10) which led to mean parasitism of 10.02 by *T. chilonis*, followed by spiromesifen with mean parasitism of 19.85, chlorantraniliprole (22.53), spirotetramat (23.07), acetamiprid (23.11), pyraclostrobin + metiram (24.08), trifloxystrobin + tebuconazole (24.10), haloxyfop-p-methyl (24.29), HaNPV (25.92), bispyribac sodi-

um (26.16), nicosulfuron (26.77) and myclobutanil (27.97).

HaNPV, spirotetramat, acetamiprid, haloxyfop-p-methyl, bispyribac sodium pyraclostrobin + metiram and trifloxystrobin + tebuconazole were statistically at par with each other ($p > 0.001$). Chlorantraniliprole was also statistically similar with each spirotetramat, acetamiprid, haloxyfop-p-methyl, pyraclostrobin + metiram, spiromesifen and trifloxystrobin + tebuconazole ($p > 0.001$). Similarly, both HaNPV and bispyribac sodium demonstrated no significance difference with each nicosulfuron and myclobutanil ($p > 0.001$). Nicosulfuron did not differ significantly from each HaNPV, haloxyfop-p-methyl, bispyribac sodium, pyraclostrobin + metiram and trifloxystrobin + tebuconazole ($p > 0.001$). However, females in the fipronil treatment parasitised significantly fewer hosts than females in the remaining pesticides tested against larval stage ($p \leq 0.001$).

d) Comparison of pesticide dose treatments with control

The comparison of means of doses with respective control treatments based on parasitism regarding treatment of larvae of minute parasitoid (Table10) revealed that the three treated doses of acetamiprid and fipronil were significantly different from their respective control treatments ($p \leq 0.001$). Similarly, 2x dose of bispyribac sodium and spirotetramat, and both 2x and x doses of myclobutanil were also significantly different from their respective control treatments ($p \leq 0.001$). However, all doses of the remaining pesticides, treated against the larval stage of *T. chilonis*, were statistically at par with their respective control treatments ($p > 0.001$).

Table 10. Parasitism (mean $\pm$ S.E) by *T. chilonis* emerged from eggs of *S. cerealella* treated with different pesticides at larval stage of parasitoid, and means comparison based on square root transformed data (Tukey's HSD, P = 0.001 or 0.1%)

S.no	Pesticides	Doses (mean $\pm$ S.E)			
		2x	x	0.5x	Control
1	HaNPV	27.51 $\pm$ 6.03 abc	26.52 $\pm$ 6.31 abcde	25.92 $\pm$ 6.13 abcdefg	27.74 $\pm$ 3.57 ab
2	Spirotetramat	20.75 $\pm$ 1.94 jklmn	23.10 $\pm$ 3.99 fghijk	23.07 $\pm$ 3.97 fghijk	24.24 $\pm$ 1.07 cdefghi
3	Chlorantiprole	21.68 $\pm$ 2.02 ijklmn	21.97 $\pm$ 2.59 ijklmn	22.53 $\pm$ 4.45 hijklm	23.28 $\pm$ 0.96 efghijk
4	Acetamiprid	10.15 $\pm$ 5.96 o	20.58 $\pm$ 2.36 klmn	23.11 $\pm$ 3.23 fghijk	29.46 $\pm$ 3.92 a
5	Fipronil	0* $\pm$ 0.00 q	7.78 $\pm$ 1.31 p	10.02 $\pm$ 1.99 o	21.50 $\pm$ 5.29 ijklm
6	Spiromesifen	19.68 $\pm$ 2.31 mn	19.48 $\pm$ 2.23 mn	19.85 $\pm$ 3.25 lmn	22.24 $\pm$ 1.74 hijklm
7	Haloxypop-p-methyl	23.14 $\pm$ 3.70 ghijkl	23.88 $\pm$ 9.02 defghij	24.29 $\pm$ 8.60 cdefghi	25.53 $\pm$ 5.19 bcdefgh
8	Bispyribac sodium	23.32 $\pm$ 2.07 efghijk	26.92 $\pm$ 1.55 abcd	26.16 $\pm$ 1.66 abcdefg	27.03 $\pm$ 1.07 abcd
9	Nicosulfuron	26.07 $\pm$ 2.00 abcdefg	26.33 $\pm$ 2.99 abcdefg	26.77 $\pm$ 4.01 abcd	26.94 $\pm$ 1.07 abcd
10	Myclobutanil	18.31 $\pm$ 2.35 n	22.02 $\pm$ 1.47 ijklm	27.97 $\pm$ 2.30 ab	28.15 $\pm$ 1.65 ab
11	Pyraclostrobin + Metiram	26.37 $\pm$ 3.56 abcdef	26.32 $\pm$ 5.07 abcdefg	24.08 $\pm$ 6.28 defghi	26.38 $\pm$ 5.18 abcdef
12	Trifloxystrobin + Tebuconazole	22.51 $\pm$ 2.15 hijklm	23.94 $\pm$ 1.70 defghij	24.10 $\pm$ 1.63 cdefghi	24.23 $\pm$ 0.94 cdefghi

ANOVA results based on sqrt transformed data (parasitism based on larval treatment)				
–	df	f	p	Remark
Pesticides	11	628.38	< 0.0001	sig
Doses	3	445.43	< 0.0001	sig
Pesticides * Doses	33	99.80	< 0.0001	sig

Means followed by the same letter within a column/among columns are not significantly different (Tukey's HSD, $p > 0.001$).

Asterisks (*) indicate no parasitism due to no emergence

4.5.3) Percentage reduction in parasitism over control by *T. chilonis* against pesticides and dose when treated at larval stage, and IOBC/WPRS toxicity ranking

a) x dose

The percentage reduction (mean) in parasitism over control by pesticide-dose based on field dose demonstrated (Table 11) that fipronil caused highest reduction in parasitism over control by *T. chilonis*, i.e. 63.81%, followed by acetamiprid (30.13%), and both were slightly harmful for parasitism. The remaining pesticides were all harmless for parasitism by parasitoid, with < 7% reduction over control, except spiromesifen and myclobutanil, which demonstrated, 12.79 and 21.79%, reduction in parasitism over control, respectively.

a) 2x dose

Acetamiprid caused highest reduction in parasitism (65.55%) over control (Table 11) by the females emerged from host eggs treated at this dose, followed by myclobutanil (34.95%), and both were rated slightly harmful. While the remaining pesticides were ranked harmless for parasitism except fipronil, and showed < 15% reduction in parasitism over control. The data on the effect of fipronil on parasitism is not available due to complete failure of emergence from the treated larvae.

b) 0.5x dose

The highest degree of disruption for parasitism by parasitic wasps among the tested chemicals (Table 11) was by fipronil: by reducing the parasitism 53.41% (slightly harmful) over control. The remaining pesticides were all harmless for parasitism, and led to reductions in parasitism over control ranging from 0.54 to 10.74%, except for acetamiprid (21.54%).

Table 11. Percentage reduction (mean) in parasitism over control of host eggs (*S. cerealella*) by *T. chilonis* emerged from host eggs treated at larval stage of parasitoid, and ranking of pesticides according to IOBC/WPRS

S.no	Pesticides	Doses-wise percent reduction in parasitism, and IOBC ranking							Control (mean parasitism)
		2x	Class	x	Class	0.5x	Class	Mean reduction	
1	HaNPV	0.81	1	4.38	1	6.57	1	3.92	27.74
2	Spirotetramat	14.40	1	4.71	1	4.84	1	7.98	24.25
3	Chlorantraniliprole	4.50	1	3.25	1	0.75	1	2.83	22.71
4	Acetamiprid	65.55	2	30.13	2	21.54	1	39.07	29.46
5	Fipronil	-	-	63.81	2	53.41	2	58.61	21.50
6	Spiromesifen	11.53	1	12.42	1	10.74	1	11.56	22.24
7	Haloxypop-p-methyl	9.34	1	6.44	1	4.85	1	6.88	25.53
8	Bispyribac sodium	0.78	1	0.38	1	3.20	1	1.45	27.03
9	Nicosulfuron	3.22	1	2.24	1	0.61	1	2.02	26.94
10	Myclobutanil	34.95	2	21.79	1	0.63	1	19.12	28.15
11	Pyraclostrobin + Metiram	0.02	1	0.22	1	8.71	1	2.98	26.38
12	Trifloxystrobin + Tebuconazole	7.11	1	1.19	1	0.54	1	2.95	24.23

Ranking: class 1= harmless ($E < 30\%$); 2 = slight harmful ($30 \leq E \leq 79\%$); 3 = moderately harmful ($79 < E \leq 99\%$); 4 = harmful ($> 99\%$).

4.5.4) Percentage parasitism relative to control by *T. chilonis* regarding treatment of larval stage

a) x dose

The relative parasitism (means) of field-dose treatments (x) with respective controls of host eggs (*S. cerealella*) by *T. chilonis* (Fig 5), emerged from *S. cerealella* eggs treated at larval stage of parasitoids, demonstrated that among all the pesticides tested, fipronil was the most toxic pesticide for parasitism, led to percentage parasitism (36.19) relative to control, followed by acetamiprid (69.87), myclobutanil (78.21), spiromesifen (87.58), haloxyfop-p-methyl (93.56), spirotetramat (95.29), HaNPV (95.62), chlorantraniliprole (96.75), nicosulfuron (97.76), trifloxystrobin + tebuconazole (98.81), bispyribac sodium (99.62) and pyraclostrobin + metiram (99.78%).

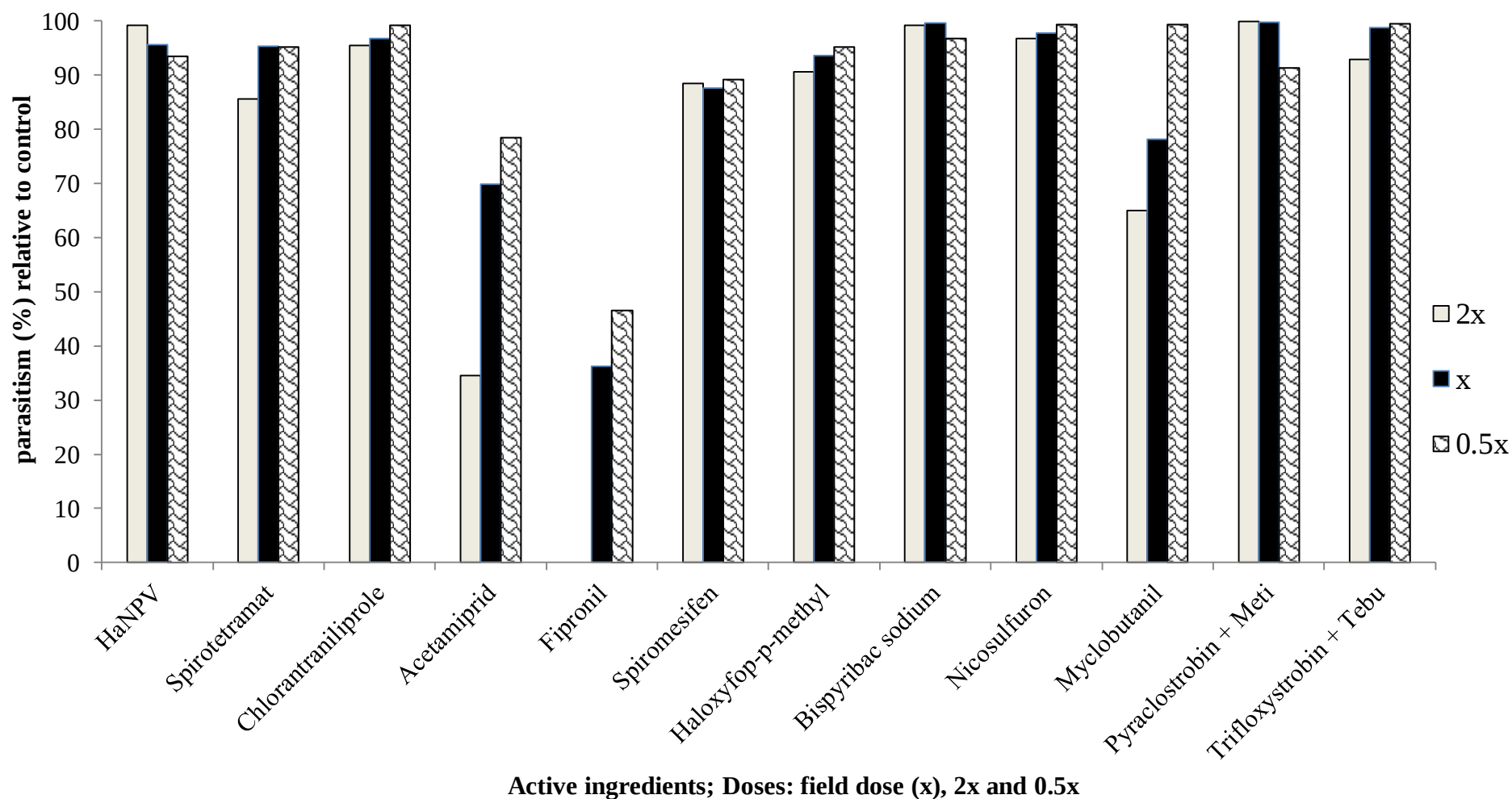
b) 2x dose

Females treated with fipronil while in the larval stages at 2x dose (Fig. 5) completely failed to parasitize the host eggs, While acetamiprid led to 34.45% parasitism, followed by myclobutanil (65.05%), spirotetramat (85.60%) and spiromesifen (88.47%). Parasitoids in the remaining pesticides treatments showed parasitism ranging from 90.66 to 99.98% relative to control

c) 0.5x dose

Fipronil was the most toxic chemical to parasitism (Fig. 5): resulted in 46.59% parasitism, followed by acetamiprid (78.46%) and spiromesifen (89.26%). While trifloxystrobin + tebuconazole showed maximum percentage parasitism (99.46). The remaining pesticides resulted in parasitism ranging from 91.29 to 99.37% relative to control.

Fig. 5 Percent parasitism (mean) relative to control of *S. cerealella* eggs by *T. chilonis* emerged from host eggs (*S. cerealella*) treated with different pesticides when parasitoids were in larval stage



4.6) Parasitism by *T. chilonis* Emerged from Host Eggs When Treated at Pupal stage

4.6.1) Anova on parasitism after pupal exposure

Pesticides were evaluated for their effects on parasitism (mean) by *T. chilonis* females emerged from host eggs when treated at parasitoid pupal stage. The anova (Table 12) for interaction between pesticides and doses revealed significant effects on parasitism by *T. chilonis* wasps ($df = 36, 468$; $f = 209.65$; $p < 0.0001$).

4.6.2) Comparison of pesticide-dose means of parasitism by *T. chilonis* regarding larval treatment

a) x dose

The mean parasitism of host eggs (*S. cerealella*) by female *T. chilonis* treated at field dose (x) with different pesticides while in the pupal stage within the host eggs (*S. cerealella*) demonstrated (Table 12) that among the tested pesticides, pyraclostrobin + metiram demonstrated maximum mean parasitism of 28.30 by the wasp, followed by chlorantraniliprole (26.81), spiromesifen (25.64), trifloxystrobin + tebuconazole (25.42), HaNPV (25.14), haloxyfop-p-methyl (24.88), myclobutanil (24.53), spirotetramat (24.32), bispyribac sodium (24.02) and nicosulfuron (20.91). The most toxic chemical was abamectin for parasitism: resulting in mean parasitism (0.8), followed by acetamiprid (11.34) and fipronil (13.71).

HaNPV, spirotetramat, chlorantraniliprole, spiromesifen, bispyribac sodium, haloxyfop-p-methyl, myclobutanil and trifloxystrobin + tebuconazole were statistically at par with each other ($p > 0.001$). Similarly, pyraclostrobin + metiram were statistically similar with each HaNPV, chlorantraniliprole, spiromesifen and trifloxystrobin + tebuconazole ($p > 0.001$). However, acetamiprid, fipronil, abamectin and nicosulfuron

ron were significantly different from each other, and also from the remaining pesticides tested against the pupae ($p \leq 0.001$).

b) 2x dose

Treated at twice-field dose, abamectin was the most toxic chemical for parasitism (Table 12) resulting in complete failure of parasitoids to emerge from the host eggs to parasitize the host eggs. Acetamiprid led to mean parasitism of 0.50, followed by fipronil (5.36), myclobutanil (19.94), nicosulfuron (21.02), trifloxystrobin + tebuconazole (23.69), spirotetramat (23.91) and haloxyfop-p-methyl (24.46). The remaining pesticides resulted in mean parasitism ranging from 25.33 to 27.45.

HaNPV, spirotetramat, chlorantraniliprole, spiromesifen, haloxyfop-p-methyl, bispyribac sodium and trifloxystrobin + tebuconazole were statistically at par with each other ($p > 0.001$), except chlorantraniliprole and trifloxystrobin + tebuconazole were significantly different from each other ($p \leq 0.001$). Similarly, pyraclostrobin + metiram were significantly not different from each HaNPV, spiromesifen, haloxyfop-p-methyl, bispyribac sodium ($p > 0.001$). Nicosulfuron was statistically similar with myclobutanil and trifloxystrobin + tebuconazole ($p > 0.001$). Acetamiprid and abamectin were also statistically similar with each other ($p > 0.001$), but they differed significantly from the remaining pesticides tested against the parasitoids ($p \leq 0.001$). Fipronil demonstrated significantly different from the remaining pesticides tested against pupae of *T. chilonis* ($p \leq 0.001$).

c) 0.5x dose

Abamectin was the most toxic chemical for parasitism at this dose (Table 12): led to mean parasitism of 2.41, followed by acetamiprid (12.50), fipronil (14.95), nicosulfuron (21.54), spirotetramat (24.33), myclobutanil (24.57) and haloxyfop-p-meth-

yl (24.95). The remaining chemicals showed mean parasitism ranging from 25.08 to 27.70.

HaNPV, chlorantraniliprole, spiromesifen, bispyribac sodium, haloxyfop-p-methyl, pyraclostrobin + metiram, trifloxystrobin + tebuconazole and myclobutanil were statistically at par with each other ($p > 0.001$). Similarly, nicosulfuron was statistically similar to spirotetramat ($p > 0.001$). However, acetamiprid, fipronil and abamectin were significantly different from each other, and also from the remaining chemicals tested against pupal stage of *T. chilonis* ($p \leq 0.001$).

d) Comparison of pesticide dose treatments with control

The comparison of means of doses with respective control treatments based on parasitism regarding treatment of pupae of minute parasitoid (Table12) demonstrated that all used doses of acetamiprid, fipronil and abamectin, and only 2x dose of myclobutanil were significantly different from their respective control treatments ($p \leq 0.001$). The three treated doses of each remaining pesticides were statistically at par with each other and with their respective controls ($p > 0.001$).

Table 12. Parasitism (mean $\pm$ S.E) of *S. cerealella* eggs by *T. chilonis* emerged from host eggs (*S. cerealella*) treated with different pesticides at pupal stage of parasitoid, and means comparison based on square root transformed data (Tukey's HSD, P= 0.001 or 0.1%)

S.no	Pesticides	Doses: (Mean $\pm$ S.E)			
		2x	x	0.5x	Control $\pm$ S.E
1	HaNPV	26.12 $\pm$ 2.06 abcdefghi	25.14 $\pm$ 3.76 bcdefghijkl	27.70 $\pm$ 1.75 abcd	27.83 $\pm$ 2.51 abc
2	Spirotetramat	23.91 $\pm$ 4.81 hijklmn	24.32 $\pm$ 2.76 fghijklm	24.33 $\pm$ 3.26 fghijklm	24.55 $\pm$ 2.08 defghijklm
3	Chlorantraniliprole	27.16 $\pm$ 2.83 abcdefgh	26.81 $\pm$ 2.75 abcdefghi	26.78 $\pm$ 1.95 abcdefghi	27.23 $\pm$ 4.83 abcdefg
4	Acetamiprid	0.50 $\pm$ 0.18 vw	11.34 $\pm$ 2.88 s	12.50 $\pm$ 2.17 rs	22.67 $\pm$ 2.10 jklmnop
5	Fipronil	5.36 $\pm$ 1.33 t	13.72 $\pm$ 2.69 qr	14.95 $\pm$ 1.97 q	26.32 $\pm$ 2.51 abcdefghi
6	Abamectin	0.00 $\pm$ 0.00 w	0.80 $\pm$ 0.15 v	2.41 $\pm$ 1.15 u	22.36 $\pm$ 0.95 klmnop
7	Spiromesifen	25.33 $\pm$ 2.89 abcdefghijk	25.64 $\pm$ 1.92 abcdefghij	25.08 $\pm$ 4.33 bcdefghijkl	25.93 $\pm$ 2.23 abcdefghi
8	Haloxypop-p-methyl	24.46 $\pm$ 6.08 efghijklm	24.88 $\pm$ 3.76 cdefghijkl	24.96 $\pm$ 3.97 cdefghijkl	25.03 $\pm$ 3.73 cdefghijkl
9	Bispyribac sodium	25.91 $\pm$ 2.18 abcdefghi	24.02 $\pm$ 2.21 ghijklm	25.79 $\pm$ 0.46 abcdefghi	26.56 $\pm$ 2.43 abcdefghi
10	Nicosulfuron	21.02 $\pm$ 1.25 nop	20.91 $\pm$ 1.97 op	21.54 $\pm$ 2.01 mnop	22.18 $\pm$ 2.04 lmnop
11	Myclobutanil	19.94 $\pm$ 5.31 p	24.53 $\pm$ 2.58 defghijklm	24.57 $\pm$ 0.27 defghijkl	24.59 $\pm$ 2.56 defghijkl

S.no	Pesticides	2x	x	0.5x	Control ± S.E
12	Pyraclostrobin + Metiram	27.45 ± 1.78 abcdef	28.30 ± 1.05 ab	27.58 ± 0.92 abcde	28.59 ± 1.10 a
13	Trifloxystrobin + Tebuconazole	23.69 ± 2.18 ijklmno	25.42 ± 5.03 abcdefghijk	25.17 ± 2.56 bcdefghijkl	25.65 ± 2.69 abcdefghij
ANOVA results based on sqrt transformed data (parasitism based on pupal treatment)					
—		df	f	p	Remark
Pesticides		12	1727.80	< 0.0001	sig
Doses		3	856.88	< 0.0001	sig
Pesticide * Doses		36	209.65	< 0.0001	sig

Means followed by the same letter within a column/among columns are not significantly different (Tukey's HSD, $p > 0.001$)

4.6.3) Percentage reduction in parasitism over control by *T. chilonis* against pesticides and dose when treated at pupal stage, and IOBC/WPRS toxicity ranking

a) x dose

Pesticide-dose based percentage reduction (mean) in parasitism over control by female *T. chilonis* while treated in the pupal stage within host eggs at field dose demonstrated (Table 13) that abamectin was moderately harmful for parasitism with 96.42% reduction in parasitism, followed by slightly harmful acetamiprid (49.97%) and fipronil (47.88%). The remaining chemicals were harmless for parasitism by *T. chilonis*: resulting in < 10% reduction in parasitism over control.

a) 2x dose

The twice field dose demonstrated (Table 13) that abamectin resulted in 100% reduction in parasitism by *T. chilonis* emerged from treated eggs, and was classified as harmful for parasitism, followed by moderately harmful chemicals with reduction in parasitism, including acetamiprid (97.79%) and fipronil (79.64%). The remaining pesticides were categorized as harmless for parasitism: resulting reduction in parasitism over controls ranged from 0.24 to 18.92% over control.

b) 0.5x dose

The most toxic chemical for the parasitism even at this lower than field dose (Table 13) among the tested pesticides was abamectin, resulting in 89.24% reduction in parasitism over controls, ranked as moderately harmful, followed by each slightly harmful acetamiprid (44.84%) and fipronil (43.18%). The remaining chemicals were harmless for parasitism: caused < 4% reduction in parasitism over control.

Table 13. Percentage reduction (mean) in parasitism over control of *S. cerealella* eggs by *T. chilonis* emerged from the host eggs (*S. cerealella*) treated at pupal stage of parasitoid, and IOBC ranking of toxicity

S.no	Pesticides	Doses-wise percent reduction in parasitism, and IOBC ranking							Control (mean parasitism)
		2x	Class	x	Class	0.5x	Class	Mean reduction	
1	HaNPV	6.15	1	9.68	1	0.48	1	5.44	27.83
2	Spirotetramat	2.62	1	0.96	1	0.91	1	1.50	24.55
3	Chlorantraniliprole	0.24	1	1.53	1	1.65	1	1.14	27.23
4	Acetamiprid	97.79	3	49.97	2	44.84	2	64.20	22.67
5	Fipronil	79.64	3	47.88	2	43.18	2	56.90	26.32
6	Abamectin	100.00	4	96.42	3	89.24	3	95.22	22.36
7	Spiromesifen	2.34	1	1.14	1	3.29	1	2.26	25.93
8	Haloxypop-p-methyl	2.31	1	0.60	1	0.31	1	1.07	25.03
9	Bispyribac sodium	2.42	1	9.53	1	2.88	1	4.94	26.56
10	Nicosulfuron	5.23	1	5.72	1	2.91	1	4.62	22.18
11	Myclobutanil	18.92	1	0.23	1	0.07	1	6.40	24.59
12	Pyraclostrobin + Metiram	4.00	1	1.02	1	3.54	1	2.85	28.59
13	Trifloxystrobin + Tebuconazole	7.64	1	0.90	1	1.89	1	3.48	25.65

Ranking: class 1= harmless ($E < 30\%$); 2 = slight harmful ($30 \leq E \leq 79\%$); 3 = moderately harmful ($79 < E \leq 99\%$); 4 = harmful ($> 99\%$)

4.6.4) Percentage parasitism relative to control by *T. chilonis* emerged from host eggs treated at pupal stage of parasitoids

a) x dose

The relative parasitism (means) of field-dose treatments (x) with respective controls of host eggs (*S. cerealella*) by female *T. chilonis* (Fig. 6), treated while in pupal stage within eggs of same grain moth, demonstrated that among all the pesticides used, abamectin was most toxic pesticide for parasitism, i.e., causing 3.58% parasitism, followed by acetamiprid (50.32%), fipronil (52.12%). While myclobutanil showed maximum percent parasitism, i.e. 99.77, followed by haloxyfop-p-methyl (99.40), trifloxystrobin + tebuconazole (99.10), spirotetramat (99.04), pyraclostrobin + metiram (98.98), spiromesifen (98.86), chlorantraniliprole (98.47), nicosulfuron (94.28), bispiribac sodium (90.47) and HaNPV (90.32) relative to control.

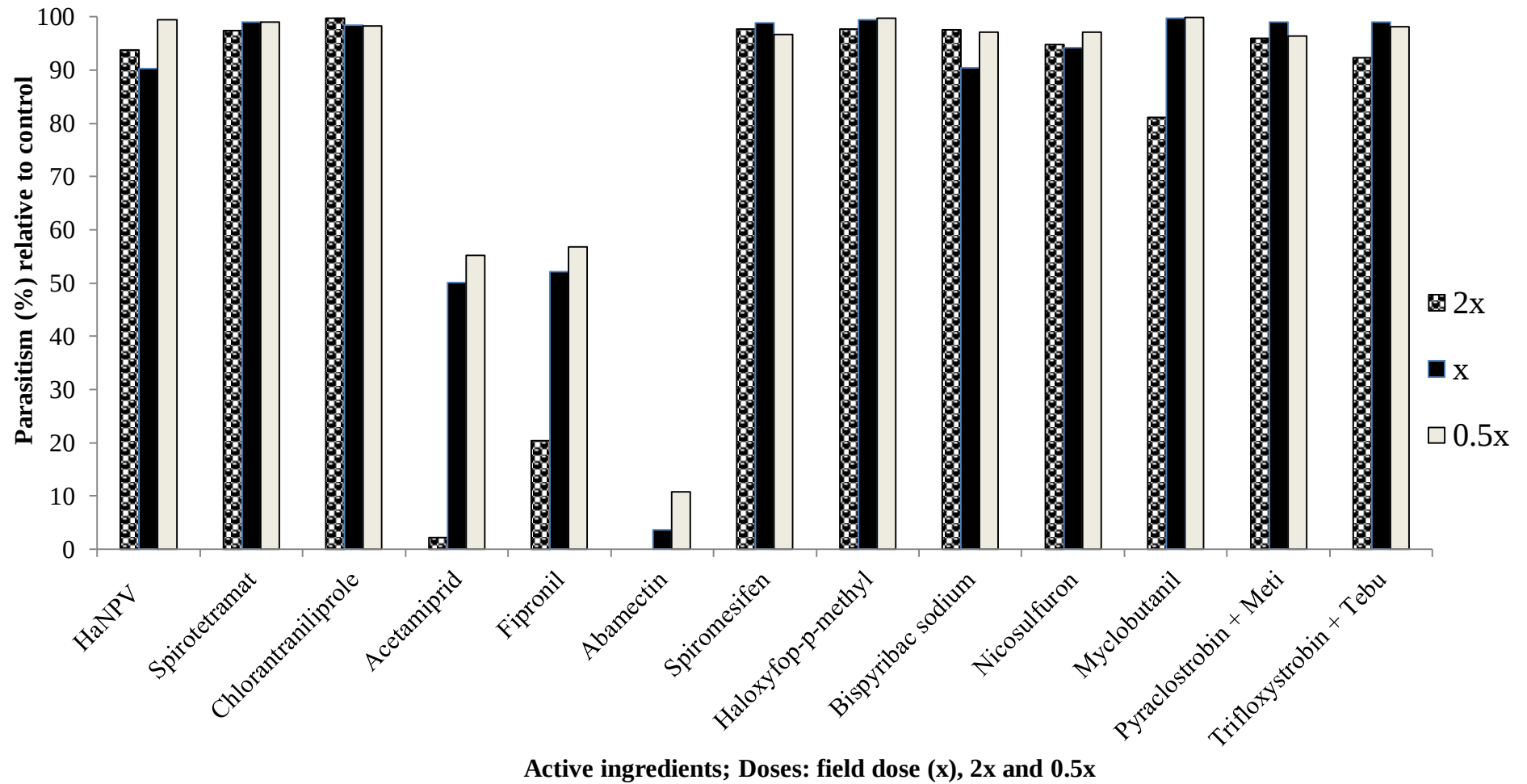
b) 2x dose

Due to 100% mortality of wasp pupae (Fig. 6), abamectin was the most toxic chemical, resulted in 0.00% successful parasitism relative to controls, followed by acetamiprid (2.21%) and fipronil (20.36%). While highest percent parasitism was observed for chlorantraniliprole (99.76), followed by haloxyfop-p-methyl, spiromesifen, bispiribac sodium, spirotetramat, pyraclostrobin, nicosulfuron, HaNPV, trifloxystrobin + tebuconazole and myclobutanil (97.69, 97.66, 97.58, 97.38, 96.10, 94.77, 93.85, 92.36 and 81.08, respectively).

c) 0.5x dose

Abamectin was the most toxic (Fig. 6): 10.76% parasitism (lowest parasitism) relative to controls, followed by acetamiprid (55.16%) and fipronil (56.82%). The remaining chemical treatments exhibited > 96% parasitism relative to controls.

Fig. 6 Percent parasitism (mean) relative to control of *S. cerealella* eggs by *T. chilonis* emerged from the host eggs (*S. cerealella*) treated with different pesticides when parasitoids were in pupal stage



4.6.5) Dose-as well as stage-wise effects of pesticides on percent reduction in parasitism by female *T. chilonis* emerged from treated host eggs

In the current studies, pesticide effects on parasitism of *S. cerealella* by female *T. chilonis* emerged from the treated host eggs (*S. cerealella*) showed variability regarding dose and life stage treated. Acetamiprid is the only chemical which induced reduced parasitism (%) by the wasps, and was rated as harmful and slightly harmful at x dose through moderately harmful and harmless for parasitism at 0.5x dose regarding egg and larval treatment, respectively (Table 9, 11, respectively). Acetamiprid also rated as moderately harmful at 2x through slightly harmful for parasitism at both x and 0.5x doses when parasitoids were treated at pupal stage (Table 13).

Nicosulfuron and chlorothalonil + procymidone showed rise in toxicity for parasitism i.e., from harmless and slightly harmful at x through 2x dose regarding egg treatment (Table 9). However, they were found harmless for parasitism at all doses treated against larvae and pupae (Table 11, 13, respectively). Myclobutanil demonstrated reduction in parasitism as slightly harmful and harmless for parasitism at 2x and x dose, respectively, in the larval treatment (Table 11), while the same chemical was rated as harmless for parasitism at all doses by the female wasps emerged from the treated egg and pupal stages (Table 9, 13, respectively).

Abamectin and fipronil demonstrated as harmful and moderately harmful for parasitism by wasps at 2x, respectively, but both showed reduced toxicity as moderately and slightly harmful for parasitism at x and 0.5x doses, respectively, regarding treatment of pupal stage (Table 13). Data regarding effects of both of chemicals on parasitism for egg and larval treatments is not available because there was no emergence of *T. chilonis* from the egg and larval stages treated with abamectin and fipronil.

The remaining pesticides were found harmless for parasitism by parasitoids at all three doses in treatment of all stages of *T. chilonis*, including HaNPV, spirotetramat, chlorantraniliprole, spiromesifen, haloxyfop-p-methyl, bispyribac sodium, pyraclostrobin + metiram, and trifloxystrobin + tebuconazole.

Section: 2

EFFECT OF PESTICIDES ON THE PARASITISM (NO-CHOICE TEST)

4.7) Effect of Experimental Pesticides on the Parasitism of Previously Treated Host Eggs by *T. chilonis* (No-Choice Test)

4.7.1) Anova on parasitism after exposure to treated host eggs

The interaction results of analysis of variance (Table 14) for pesticides and doses showed that parasitism of previously treated host eggs (*S. cerealella*) by *T. chilonis* was significantly affected with treatments of different pesticides and doses ($df = 42$, 540 ; $f = 618.35$; $p < 0.0001$)

4.7.2) Comparison of pesticide-dose means of parasitism by *T. chilonis* (no-choice test)

a) x dose

Parasitism (mean) by single female *T. chilonis* exposed to each replicated card of previously treated host eggs (*S. cerealella*) regarding no-choice test (Table 14) based on field dose (x) yielded maximum mean parasitism of 31.60 of the host eggs observed for eggs treated with pyraclostrobin + metiram, followed by bispyribac sodium and HaNPV (31.10 and 30.90, respectively), and these three chemicals were statistically at par with each other ($p > 0.001$), but demonstrated significant difference with the remaining tested chemicals ($p \leq 0.001$).

Mean parasitism in chlorantraniliprole was 24.80, followed by trifloxystrobin + tebuconazole (23.20), and both were statistically at par with each other ($p > 0.001$), but were significantly different from the remaining pesticides ($p \leq 0.001$). Myclobutanil observed mean parasitism of 19.70, followed by haloxyfop-p-methyl (17.20), acetamiprid (12.10), fipronil (9.60), spinetoram (8.10), abamectin (6.30) and spiromesif-

en (5.30): these were all significantly different from each other and also from the remaining chemicals tested at field dose ($p \leq 0.001$). Similarly, mean parasitism in the spirotetramat (28.50), chlorothalonil + procymidone (28.40) and nicosulfuron (27.90) treatments were all statistically similar with each other ($p > 0.001$), but were significantly different from the remaining tested chemicals ($p \leq 0.001$).

b) 2x dose

Maximum parasitism was observed on host eggs treated at this dose with HaNPV (Table 14) i.e., 30.70, followed by bispyribac sodium (28.50), chlorothalonil + procymidone (28.20), pyraclostrobin + metiram (26.20), nicosulfuron (25.50), chlorantraniliprole (24.70), spirotetramat (18.00), trifloxystrobin + tebuconazole (16.10), myclobutanil (16.00), haloxyfop-p-methyl (15.30), acetamiprid (8.10), spinetoram (4.70), abamectin (4.40) and both fipronil and spiromesifen (1.00).

HaNPV, spirotetramat and acetamiprid were significantly different from each other and from the remaining tested chemicals ($p \leq 0.001$). Chlorantraniliprole, pyraclostrobin + metiram and nicosulfuron were statistically at par with each other ($p > 0.001$). Similarly, spinetoram and fipronil were statistically similar only with abamectin and spiromesifen, respectively ($p > 0.001$). Furthermore, trifloxystrobin + tebuconazole, myclobutanil and haloxyfop-p-methyl were also found significantly not different from each other ($p > 0.001$), but were significantly different from the remaining tested chemicals ($p \leq 0.001$). Chlorothalonil + procymidone were statistically similar with each bispyribac sodium and pyraclostrobin + metiram tested against host eggs ($p > 0.001$).

c) 0.5x dose

The chemicals most toxic for parasitism were (Table 14) abamectin, spiromes-

ifen and spinetoram, which led to mean parasitism of 6.70, 7.70 and 9.50, respectively. Fipronil resulted in mean parasitism of 20.00, followed by myclobutanil (20.10), acetamiprid (23.50), chlorantraniliprole (27.90), chlorothalonil + procymidone (28.40), nicosulfuron (28.50), spirotetramat (30.70), HaNPV (31.00), trifloxystrobin + tebuconazole (31.20), bispyribac sodium (31.20) and pyraclostrobin + metiram (31.30).

HaNPV, spirotetramat, haloxyfop-p-methyl, bispyribac sodium, pyraclostrobin + metiram and trifloxystrobin + tebuconazole were not significantly different from each other ($p > 0.001$) but demonstrated significance difference with the remaining treated pesticides ($p \leq 0.001$). Similarly, chlorantraniliprole, nicosulfuron and chlorothalonil + procymidone were also statistically similar with each other ($p > 0.001$). The chemicals tested and found statistically at par with each other ($p > 0.001$), but significantly different from the remaining chemicals ($p \leq 0.001$) were 1) Fipronil and myclobutanil, 2) acetamiprid and spinetoram, and 3) abamectin and spiromesifen.

c) Comparison of pesticide dose treatments with control

The comparison of means of doses with respective control treatments (Table 14) based on parasitism (no-choice test) demonstrated that all used doses of each HaNPV and chlorothalonil + procymidone were statistically at par with, while each chlorantraniliprole, acetamiprid, spinetoram, fipronil, abamectin, spiromesifen and myclobutanil were significantly different from their respective controls. Both x and 0.5x doses of bispyribac sodium, nicosulfuron and pyraclostrobin + metiram were statistically similar with their respective controls. Spirotetramat, haloxyfop-p-methyl and trifloxystrobin + tebuconazole have showed significance difference from their respective controls regarding both 2x and x doses.

Table 14. Parasitism (mean $\pm$ S.E) of previously treated host eggs (*S. cerealella*) by single female *T. chilonis* exposed to eggs treated with different pesticides, and means comparison based on square root transformation (Tukey's HSD, $P = 0.001$ or 0.1%)

S.no	Pesticides	Doses (means $\pm$ S.E)			
		2x	x	0.5x	Control
1	HaNPV	30.70 $\pm$ 2.39 ab	30.90 $\pm$ 4.01 ab	31.00 $\pm$ 1.87 a	31.50 $\pm$ 3.93 a
2	Spirotetramat	18.00 $\pm$ 2.75 k	28.50 $\pm$ 2.41 cd	30.70 $\pm$ 3.20 ab	31.50 $\pm$ 1.70 a
3	Chlorantraniliprole	24.70 $\pm$ 1.84 hi	24.80 $\pm$ 3.17 hi	27.900 $\pm$ 1.03 def	30.30 $\pm$ 3.23 abc
4	Acetamiprid	8.10 $\pm$ 2.02 p	12.10 $\pm$ 2.07 n	23.50 $\pm$ 1.96 i	26.40 $\pm$ 2.22 efgh
5	Spinetoram	4.70 $\pm$ 1.24 st	8.10 $\pm$ 1.69 p	9.50 $\pm$ 0.91 o	26.90 $\pm$ 2.22 defg
6	Fipronil	1.00 $\pm$ 0.38 u	9.60 $\pm$ 1.55 o	20.00 $\pm$ 1.60 j	31.00 $\pm$ 2.05 a
7	Abamectin	4.40 $\pm$ 1.16 t	6.30 $\pm$ 1.18 r	6.70 $\pm$ 0.99 qr	30.30 $\pm$ 3.23 abc
8	Spiromesifen	1.00 $\pm$ 0.35 u	5.30 $\pm$ 2.08 s	7.70 $\pm$ 2.72 pq	27.60 $\pm$ 2.04 def
9	Haloxypop-p-methyl	15.30 $\pm$ 2.82 m	17.20 $\pm$ 2.02 kl	31.30 $\pm$ 4.27 a	31.40 $\pm$ 1.70 a
10	Bispyribac sodium	28.50 $\pm$ 2.56 cd	31.10 $\pm$ 2.01 a	31.20 $\pm$ 1.89 a	31.40 $\pm$ 3.93 a
11	Nicosulfuron	25.50 $\pm$ 4.65 gh	27.90 $\pm$ 2.77 def	28.50 $\pm$ 3.05 cd	28.90 $\pm$ 3.64 bcd

S.no	Pesticides	2x	x	0.5x	Control
12	Myclobutanil	16.00 ± 2.78 lm	19.70 ± 4.70 j	20.10 ± 4.27 j	27.00 ± 4.24 defg
13	Chlorothalonil + Procymidone	28.20 ± 3.38 def	28.40 ± 3.35 cde	28.40 ± 4.62 cde	28.40 ± 3.64 cde
14	Pyraclostrobin + Metiram	26.20 ± 6.04 fgh	31.60 ± 2.27 a	31.30 ± 3.96 a	31.60 ± 1.70 a
15	Trifloxystrobin + Tebuconazole	16.10 ± 3.75 lm	23.20 ± 4.73 i	31.20 ± 3.34 a	31.40 ± 1.70 a
ANOVA results based on sqrt transformed data (parasitism of previously treated eggs: no-choice test)					
—		df	f	p	Remark
Pesticides		14	4431.44	< 0.0001	sig
Doses		3	9150.69	< 0.0001	sig
Pesticide * Doses		42	618.35	< 0.0001	sig

Means sharing same letter within a column/among columns are not significantly different (Tukey's HSD, $p > 0.001$)

4.7.3) Percentage reduction in parasitism over control by *T. chilonis* exposed to the previously treated host eggs, and IOBC/WPRS toxicity ranking

a) x dose

The percentage of reduction in parasitism (mean) over control by *T. chilonis* exposed to previously treated host eggs (*S.cerealella*) at field dose in the no-choice test (Table 15) revealed that maximum reduction in parasitism was by spiromesifen (80.80%) and was ranked as moderately harmful for parasitism, followed by the slightly harmful chemicals: abamectin, spinetoram, fipronil and acetamiprid. The remaining chemicals were all harmless for parasitism including, chlorothalonil+ procymidone and pyraclostrobin + metiram, both of which exhibited minimum reduction in parasitism (0.00%) over control, followed by bispyribac sodium (0.96) , HaNPV (1.90) and nicosulfuron (3.46). The remaining chemicals resulted in reduction in parasitism from 9.52 to 27.04% over control.

b) 2x dose

When the host eggs were previously treated with 2x concentration of pesticides (Table 15), the percentage of reduction (mean) in parasitism by the female *T. chilonis* showed that fipronil and spiromesifen were the most disruptive pesticides for parasitism, followed by abamectin, spinetoram, and these were all ranked moderately harmful for parasitism. Acetamiprid, followed by haloxyfop-p-methyl, trifloxystrobin + tebuconazole, spirotetramat and myclobutanil were all categorized slightly harmful for parasitism by the wasps. While minimum percentage reduction was observed by the female parasitoid on the host eggs previously treated with chlorothalonil + procymidone, followed by HaNPV, bispyribac sodium, nicosulfuron, pyraclostrobin + metiram and chlorantraniliprole as they demonstrated $\leq 18.48\%$ reduction in parasitism and were ranked as harmless for parasitism by *T. chilonis*.

d) 0.5x dose

Abamectin, spiromesifen, spinetoram and fipronil were found most toxic chemicals for parasitism when host eggs were treated with 0.5x dose of these pesticides previously to exposure to female *T. chilonis* (Table 15): they were all ranked as slightly harmful for parasitism by tiny wasps. While the remaining pesticides were harmless for parasitism: causing reduction in parasitism $\leq 10.98\%$, except myclobutanil which led to 25.56% reduction in parasitism by *T. chilonis*.

4.7.4) Dose-wise effects on parasitism of previously treated host eggs by *T. chilonis* (no-choice test)

The effect of pesticides through doses on parasitism (disruption of parasitism) of previously treated host eggs by *T. chilonis* showed that parasitism was affected by 9 chemicals (Table 15). Spirotetramat, trifloxystrobin + tebuconazole and myclobutanil demonstrated reduced toxicity for parasitism as slightly harmful at 2x dose, but were harmless at both x and 0.5x doses, while both of acetamiprid and haloxyfop-p-methyl also experienced reduced toxicity for parasitism from slightly harmful at both of 2x and x doses through harmless at 0.5x dose.

Spinetoram and fipronil decreased parasitism, and were rated as moderately harmful at 2x dose, but slightly harmful at both x and 0.5x doses. Abamectin was moderately harmful at 2x dose, but was slightly harmful for parasitism at both x and 0.5x doses. Spiromesifen was moderately harmful at both 2x and x doses, but was slightly harmful at 0.5x dose. The remaining chemicals were harmless for parasitism of the host eggs previously treated with pesticides at all treated doses.

Table 15. Percentage reduction (mean) in parasitism over control of previously treated host eggs (*S. cerealella*) by *T. chilonis*, and IOBC ranking of toxicity

S.no	Pesticides	Doses-wise percent reduction in parasitism, and IOBC ranking							Control (mean parasitis)
		2x	Class	x	Class	0.5x	Class	Mean reduction	
1	HaNPV	2.54	1	1.90	1	1.59	1	2.01	31.5
2	Spirotetramat	42.86	2	9.52	1	2.54	1	18.29	31.5
3	Chlorantraniliprole	18.48	1	18.15	1	7.92	1	14.82	30.3
4	Acetamiprid	69.32	2	54.17	2	10.98	1	44.82	26.4
5	Spinetoram	82.53	3	69.89	2	64.68	2	72.33	26.9
6	Fipronil	96.77	3	69.03	2	35.48	2	68.47	31.0
7	Abamectin	85.48	3	79.21	2	77.89	2	72.36	30.3
8	Spiromesifen	96.38	3	80.80	3	72.10	2	82.92	27.6
9	Haloxyp-p-methyl	51.27	2	45.22	2	0.32	1	30.29	31.4
10	Bispyribac sodium	9.24	1	0.96	1	0.64	1	8.26	31.4
11	Nicosulfuron	11.76	1	3.46	1	1.38	1	5.36	28.9
12	Myclobutanil	40.74	2	27.04	1	25.56	1	28.33	27.0
13	Chlorothalonil + Procymidone	0.70	1	0.00	1	0.00	1	3.02	28.4
14	Pyraclostrobin + Metiram	17.09	1	0.00	1	0.95	1	6.01	31.6
15	Trifloxystrobin + Tebuconazole	48.73	2	26.11	1	0.64	1	22.50	31.4

Ranking: class 1= harmless ($E < 30\%$); 2 = slightly harmful ($30 \leq E \leq 79\%$); 3 = moderately harmful ($79 < E \leq 99\%$); 4 = harmful ($> 99\%$)

4.7.5) Percent parasitism relative to control of previously treated host eggs by *T. chilonis* regarding no-choice test

a) x dose

The relative parasitism (means) of dose treatments with respective controls of host eggs (*S. cerealella*) by *T. chilonis*, previously treated with pesticides at field dose (x), demonstrated (Fig 7) that maximum parasitism (100%) relative to control were observed for host eggs treated with each chlorothalonil + procymidone and pyraclostrobin + metiram, followed by bispyribac sodium (99.04%), HaNPV (98.10%), nicosulfuron (96.54%), spirotetramat (90.48%), and chlorantraniliprole (81.85%). Minimum parasitism was observed when host eggs were treated with spiromesifen (19.20%), followed by abamectin (20.79%), spinetoram (30.11%), fipronil (30.97%), acetamiprid (45.83%), haloxyfop-p-methyl (54.78%) and myclobutanil (72.96%).

b) 2x dose

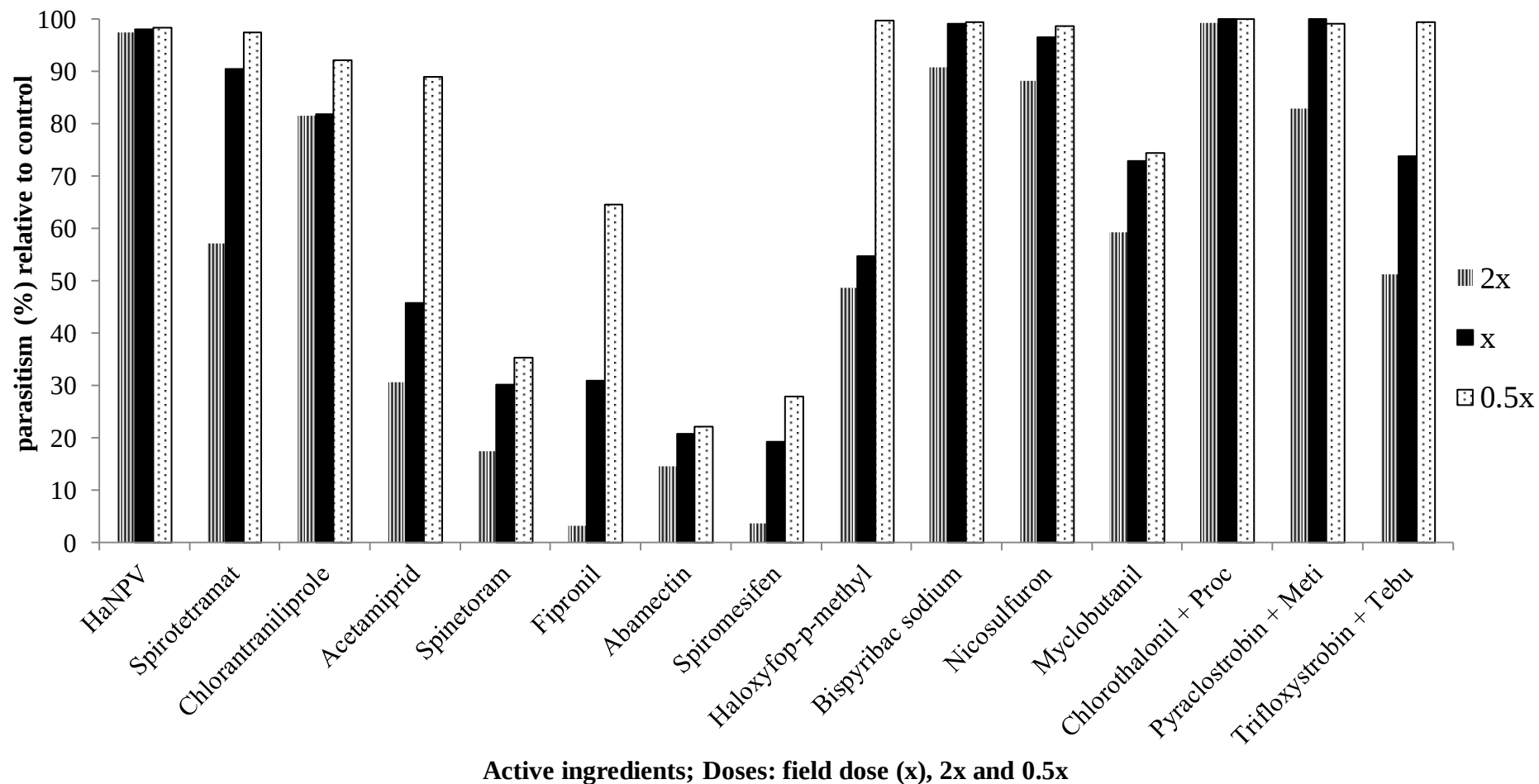
When the host eggs were treated at 2x dose (Fig 7) prior to exposure, the percentage parasitism (mean) relative to control by the wasps showed that fipronil and spiromesifen were the most toxic pesticides: resulting in 3.23% and 3.62% mean parasitism relative to controls, respectively, followed by abamectin (14.52%), spinetoram (17.47%), acetamiprid (30.68%), haloxyfop-p-methyl (48.73%), trifloxystrobin + tebuconazole (51.27%), spirotetramat (57.14%) and myclobutanil (59.26%). Maximum mean percent parasitism of 99.30% was observed where the host eggs were previously treated with chlorothalonil + procymidone, followed by HaNPV, bispyribac sodium, nicosulfuron, pyraclostrobin + metiram and chlorantraniliprole (97.46, 90.76, 88.24, 82.91 and 81.52%, respectively).

c) 0.5x dose

The host eggs previously treated at this dose (Fig 7) with abamectin, spirome-

sifen and spinetoram caused mean parasitism of 22.11, 27.90 and 35.32%, respectively, relative to control, followed by fipronil (64.52%) and myclobutanil (74.44%). Maximum percent parasitism (mean) by *T. chilonis* was recorded when host eggs were previously treated with chlorothalonil + procymidone (100%), followed by haloxyfop-p-methyl (99.68%), trifloxystrobin + tebuconazole (99.36%) and bispyribac sodium (99.36%), pyraclostrobin+ metiram (99.05%), nicosulfuron (98.62%), HaNPV (98.41%), spirotetramat (97.46%) and chlorantraniliprole (92.08%).

Fig. 7 Percent parasitism (mean) relative to control by *T. chilonis* of host eggs (*S. cerealella*), previously treated with different pesticides (no-choice test)



Section: 3-A

EFFECT OF PESTICIDES ON SURVIVAL OF ADULT *T. chilonis*

4.8) Testing the Persistence (Duration of Harmful Activity) of Pesticides Against Adult Stage of the *T. chilonis*

4.8.1) Anova on mortality of adult *T. chilonis*

Pesticides were evaluated for their effect on mortality (mean) of adults *T. chilonis* exposed to the treated surface of glass vial for 24 h. The analysis of variance based on interaction of pesticides and doses (Table 16) revealed that pesticides, as well as their doses significantly affected mortality of adult wasps ($df = 30, 396$; $f = 15.26$; $p < 0.0001$).

4.8.2) Comparison of pesticide-dose means of percent corrected mortality (corrected for control) of adult *T. chilonis* in response to exposure in different residual treatments of pesticides, and toxicity ranking (Brunner *et al.*, 2001)

Adult *T. chilonis* were exposed to 1-, 5-, 10- and 15-days-old dried pesticide residues for 24 h at field dose.

a) Adult mortality: 1-day-old residue

Exposure of randomly selected adults *T. chilonis* (Table 16) to the treated surface of the glass vial (1 x 10 cm) at field dose (Table 1) to 1-day (24 h) old pesticides residue resulted in 100% adult mortality by the fipronil and spinetoram during 24 h (in cases of 100% mortality in treatment, Abbott's formula has no effect), followed by acetamiprid (96.36) and abamectin (83.49%). The aforementioned four chemicals were ranked as highly toxic for the survival of adult *T. chilonis* (the ranking of adult toxicity in the laboratory trial based on corrected (%) mortality was according to Brunner

et al., 2001).

Bispyribac sodium with 54.86% mortality, followed by haloxyfop-p-methyl (36.45%), pyraclostrobin+metiram (34.53%), trifloxystrobin +tebuconazole (34.49%) and spiromesifen (29.46%) exhibited moderate toxicity for survival of adult parasitoids. Chlorantraniliprole and HaNPV had low toxicity to adult parasitoids among the tested chemicals, inducing 3.59 and 11.21% mortality, respectively, during 24 h of exposure to the treated surface.

The statistical analysis demonstrated that the chemicals statistically at par with each other ($p > 0.001$) were: 1) HaNPV and chlorantraniliprole, 2) acetamiprid, fipronil and spinetoram, 3) acetamiprid and abamectin, and 4) spiromesifen, haloxyfop-p-methyl, pyraclostrobin + metiram and trifloxystrobin + tebuconazole. However, bispyribac sodium was significantly different from the remaining pesticides tested against adult *T. chilonis* ($p \leq 0.001$).

b) Adult mortality: 5-day-old residue

Exposure for 24 h of randomly selected wasps to 5-day-old pesticide residue (Table 16) showed that fipronil inflicted 100% mortality on adult *T. chilonis*, followed by spinetoram, acetamiprid and abamectin, resulting in 98.99%, 95.56% and 81.90%, mortality, respectively. These four chemicals were ranked as highly toxic for adult survival of *T. chilonis*.

The chemicals moderately toxic for the adult parasitoid survival were bispyribac sodium with 51.15% corrected mortality, followed by pyraclostrobin + metiram and haloxyfop-p-methyl (22.56 and 21.02%, corrected mortality, respectively). The remaining pesticides were all categorized as low toxicity for the adult survival of tiny wasps with corrected mortality ranging from 4.79 to 17.23%.

The pesticides showed no significance difference from each other ($p > 0.001$) were 1) HaNPV, chlorantraniliprole, spiromesifen and trifloxystrobin + tebuconazole, 2) HaNPV, haloxyfop-p-methyl, trifloxystrobin + tebuconazole and pyraclostrobin + m- etiram, 3) acetamiprid, spinetoram and fipronil, and 4) acetamiprid and abamectin. However, bispyribac sodium was found significantly different from the remaining pesticides tested against the adults of *T. chilonis* ($p \leq 0.001$).

c) Adult mortality: 10-day-old residue

On exposure of randomly selected *T. chilonis* adults to 10-day-old pesticide residues demonstrated that both fipronil and spinetoram (Table 16) caused 100% adult mortality, followed by acetamiprid (95.37%) and abamectin (76.42%), and these were all ranked as highly toxic to adult wasps. The remaining pesticides were all of low toxicity for adult survival with corrected percent mortality ranging from 1.45 to 18.86%, except chlorantraniliprole which resulted in - 0.02 corrected mortality for control.

The pesticides found statistically similar with each other ($p > 0.001$) were 1) fipronil, spinetoram and acetamiprid, 2) spiromesifen, haloxyfop-p-methyl, bispyribac sodium, trifloxystrobin + tebuconazole and pyraclostrobin + metiram, 3) HaNPV, chlorantraniliprole, haloxyfop-p-methyl, bispyribac sodium and pyraclostrobin + metiram, and 4) HaNPV and spiromesifen. However, abamectin was found significantly different from the remaining pesticides tested against the wasp adult stage ($p \leq 0.001$).

d) Adult mortality: 15-day-old residue

Exposure of adult *T. chilonis* to 15-day-old residues (Table 16) resulted in fipronil, spinetoram, acetamiprid and abamectin being rated as highly toxic to adult *T. chilonis*, as they led to 98.13, 97.43, 95.27 and 71.30% mortality, respectively. Triflo-

xystrobin + tebuconazole resulted in 20.73% corrected mortality, and were ranked as moderately toxic for adults' survival. However, the remaining pesticides have < 8% corrected mortality for control, and were all ranked as low toxic for survival of adults of *T. chilonis*.

The pesticides found significantly not different from each other ($p > 0.001$) were 1) HaNPV, chlorantraniliprole, spiromesifen, haloxyfop-p-methyl, bispyribac sodium and pyraclostrobin + metiram, and 2) fipronil, spinetoram and acetamiprid. However, abamectin and trifloxystrobin + tebuconazole were significantly different from each other and from the remaining pesticides tested against the adult stage of parasitoids ($p \leq 0.001$).

e) Comparison among the residual treatments

The comparison of means of four residual treatments with each other (Table 16) based on adult mortality showed that 5-, 10-, 15- day treatments of haloxyfop-p-methyl were statistically at par with each other. Similarly, both bispyribac sodium and pyraclostrobin + metiram demonstrated 1- and 5- day treatments as statistically similar with each other, while 5- and 10- day treatments also showed similar trend. Spiromesifen and trifloxystrobin and tebuconazole revealed no significance difference among 5-, 10-, and 15- day treatments, however, the former chemical also showed no significance difference between 1- and 5- day treatment, while the later chemical also demonstrated both 1- and 15- day treatments as statistically at par with each other. All the remaining chemicals demonstrated all residual treatment as statistically at par with each other.

Table 16. Corrected (%) mortality corrected for control of adult *T. chilonis* after exposure to 1-, 5-, 10- and 15- days old pesticide residues at respective field dose for 24 h, and means comparison (Tukey's HSD, P = 0.001 or 0.1%)

S.no	Pesticides	(Age of pesticide residues and toxicity ranking); Corrected (%) mortality (mean $\pm$ S.E)								Mean $\pm$ S.E
		1 day	Class	5 days	Class	10 days	Class	15 days	Class	
1	HaNPV	11.21 $\pm$ 2.97 ijklmn	1	9.79 $\pm$ 2.78 ijklmn	1	1.45 $\pm$ 2.59 mn	1	3.10 $\pm$ 3.60 lmn	1	6.39 $\pm$ 2.42 f
2	Chlorantraniliprole	3.59 $\pm$ 2.32 lmn	1	4.79 $\pm$ 1.85 klmn	1	- 0.02 $\pm$ 1.66 n	1	0.94 $\pm$ 2.01 mn	1	2.32 $\pm$ 1.12 f
3	Acetamiprid	96.36 $\pm$ 1.86 ab	3	95.56 $\pm$ 1.82 abc	3	95.37 $\pm$ 1.91 abc	3	95.27 $\pm$ 2.55 abc	3	95.64 $\pm$ 0.25 a
4	Spinetoram	100* $\pm$ 0.00 a	3	98.99 $\pm$ 1.01 a	3	100* $\pm$ 0.00 a	3	97.43 $\pm$ 1.71 ab	3	99.10 $\pm$ 0.61 a
5	Fipronil	100* $\pm$ 0.00 a	3	100* $\pm$ 0.00 a	3	100* $\pm$ 0.00 a	3	98.13 $\pm$ 1.25 a	3	99.53 $\pm$ 0.47 a
6	Abamectin	83.49 $\pm$ 1.61 bcd	3	81.90 $\pm$ 2.36 cd	3	76.42 $\pm$ 1.16 d	3	71.30 $\pm$ 2.33 d	3	78.28 $\pm$ 2.78 b
7	Spiromesifen	29.46 $\pm$ 1.30 fgh	2	16.56 $\pm$ 1.59 hijkl	1	14.36 $\pm$ 2.21 ijklm	1	6.00 $\pm$ 1.27 klmn	1	16.59 $\pm$ 4.85 e
8	Haloxypop-p-methyl	36.45 $\pm$ 3.04 f	2	21.02 $\pm$ 2.62 ghij	2	8.55 $\pm$ 2.10 ijklmn	1	7.85 $\pm$ 2.84 jklmn	1	18.47 $\pm$ 6.71 de
9	Bispyribac sodium	54.86 $\pm$ 2.90 e	2	51.15 $\pm$ 4.09 e	2	10.74 $\pm$ 1.87 ijklmn	1	3.75 $\pm$ 2.75 lmn	1	30.13 $\pm$ 13.31 c
10	Pyraclostrobin + Metiram	34.53 $\pm$ 2.79 fg	2	22.56 $\pm$ 3.14 fghi	2	7.26 $\pm$ 2.32 jklmn	1	3.23 $\pm$ 1.81 lmn	1	16.89 $\pm$ 7.20 e

S.no	Pesticides	1 day	Class	5 days	Class	10 days	Class	15 days	Class	Mean ± S.E
11	Trifloxystrobin + Tebuconazole	34.49 ± 2.47 fg	2	17.23 ± 1.75 hijkl	1	18.86 ± 2.04 hijk	1	20.73 ± 1.49 ghij	2	22.83 ± 3.95 d
ANOVA results based on corrected (%) mortality (adult mortality based on residual toxicity)										
—		df	f		p		Remark			
Pesticides		10	1353.88		< 0.0001		sig			
Doses		3	127.50		< 0.0001		sig			
Pesticide * Doses		30	15.26		< 0.0001		sig			

Means followed by the same letter within a column/among columns are not significantly different (Tukey's HSD, $p > 0.001$).

The asterisk (*) means that in cases of 100% mortality in treatment, Abbott's formula has no effect.

Brunner *et al* (2001) adults toxicity ranking: 1 for low toxic ($E < 20\%$ corrected mortality); 2 for moderate toxic ($20 < E < 70\%$ corrected mortality); 3 for highly toxic ($E > 70\%$ corrected mortality), where E in this case stand for adult mortality.

Section: 3-B

EFFECT OF PESTICIDE DRIED RESIDUES ON PARASITISM BY *T. chilonis*

4.9) Testing the Persistence (Duration of Harmful Activity) of Pesticides Against Parasitism by the Females *T. chilonis* Exposed to Dried Pesticide Residues

4.9.1) Anova on parasitism by *T. chilonis*

Pesticides were evaluated for their effect on parasitism efficiency by female *T. chilonis* exposed to the treated surface of glass vial for 24 h. The analysis of variance (Table 17) for interaction of pesticides and doses revealed significant effects on parasitism by *T. chilonis* wasp (df = 56, 675; $f = 342.66$; $p < 0.0001$).

4.9.2) Comparison of pesticide-dose means of parasitism by *T. chilonis* in response to exposure in different residual treatments of pesticides

a) Parasitism: 1-day-old residue

Parasitism (mean) of fresh host eggs by female *T. chilonis* (Table 17) exposed for 24 h to 1-day-old pesticide residue at respective field dose in the glass vial demonstrated that the most toxic chemical for parasitism was acetamiprid, which resulted in mean parasitism of 0.69, followed by spinetoram (0.73), abamectin (2.31) and fipronil (4.84) during the 24-h exposure. While the host eggs treated with HaNPV resulted in highest mean parasitism (26.64) by wasps, followed by chlorantraniliprole (24.03), spiromesifen (23.37), chlorothalonil + procymidone (22.48), myclobutanil (20.44), haloxyfop-p-methyl (19.84) and trifloxystrobin + tebuconazole (19.46). The remaining pesticides exhibited mean parasitism ranging from 12.13 to 13.89.

HaNPV, fipronil and abamectin were significantly different from each other and from the remaining pesticides in the treated glass vials ($p \leq 0.001$). The pesticides

found statistically at par with each other ($p > 0.001$) were 1) chlorantraniliprole, spiromesifen and chlorothalonil + procymidone, 2) spirotetramat, pyraclostrobin + metiram and bispyribac sodium, 3) nicosulfuron, pyraclostrobin + metiram and bispyribac sodium, 4) myclobutanil, haloxyfop-p-methyl and trifloxystrobin + tebuconazole, 5) myclobutanil and chlorothalonil + procymidone, and 6) acetamiprid and spinetoram.

b) Parasitism: 5-day-old residue

The exposure of females of *T. chilonis* to 5-day-old residue of different pesticides showed (Table 17) that the most toxic chemical for parasitism was spinetoram, with 0.95 mean parasitism, followed by acetamiprid (1.66), abamectin (4.26) and fipronil (7.20). HaNPV led to highest mean parasitism i.e., 27.18, followed by chlorantraniliprole (26.82), haloxyfop-p-methyl (23.97), spiromesifen (23.75) and chlorothalonil + procymidone (23.04). The remaining pesticides demonstrated mean parasitism ranging from 13.39 to 20.74.

The pesticides found statistically similar with each other ($p > 0.001$) were 1) HaNPV and chlorantraniliprole, 2) bispyribac sodium and nicosulfuron, 3) spiromesifen, haloxyfop-p-methyl and chlorothalonil + procymidone, 4) myclobutanil and trifloxystrobin + tebuconazole, 5) spirotetramat and bispyribac sodium, and 6) spirotetramat, myclobutanil and trifloxystrobin + tebuconazole. However, chlorantraniliprole, pyraclostrobin + metiram, acetamiprid, spinetoram, fipronil and abamectin were significantly different from each other and from the remaining pesticides tested against the adults ($p \leq 0.001$).

c) Parasitism: 10-days-old residue

Female wasps exposed to 10-day-old pesticide residues demonstrated (Table 17) that the most toxic chemical for parasitism by the exposed female among the test-

ed chemicals was acetamiprid, which showed mean parasitism of 1.45, followed by spinetoram (2.31), abamectin (4.23) and fipronil (7.77). Both HaNPV and chlorantraniliprole exhibited highest parasitism (mean) among the tested pesticides, i.e., 27.55 and 27.05, respectively, followed by chlorothalonil + procymidone (25.34), spiromesifen (24.65) and haloxyfop-p-methyl (24.39). The remaining chemicals caused mean parasitism by *T. chilonis* from 17.23 to 21.09.

The pesticides found statistically at par with each other ($p > 0.001$) were 1) HaNPV, chlorantraniliprole and chlorothalonil + procymidone, 2) spirotetramat, nicosulfuron and pyraclostrobin + metiram, 3) bispyribac sodium and myclobutanil, and 4) spiromesifen, haloxyfop-p-methyl, chlorothalonil + procymidone and trifloxystrobin + tebuconazole. However, acetamiprid, spinetoram, fipronil and abamectin were significantly different from each other and also from the remaining pesticides tested against adult *T. chilonis* ($p \leq 0.001$).

d) Parasitism: 15-days-old residue

Parasitism by female *T. chilonis* exposed to treated surface 15 days after pesticide treatments (Table 17) was lowest with acetamiprid, with mean parasitism of 1.39, followed by spinetoram (3.36), abamectin (4.26) and fipronil (9.04). While chlorantraniliprole exhibited highest mean parasitism (27.47) among the tested chemicals, followed by HaNPV (26.82), chlorothalonil + procymidone (25.16), spiromesifen (24.84), trifloxystrobin + tebuconazole (24.57), haloxyfop-p-methyl (24.31) and bispyribac sodium (23.12). The remaining chemicals showed mean parasitism from 18.50 to 21.35.

The pesticides found significantly not different from each other ($p > 0.001$) were 1) HaNPV, chlorantraniliprole and chlorothalonil + procymidone, 2) HaNPV,

spiromesifen, chlorothalonil + procymidone and trifloxystrobin + tebuconazole, 3) spirotetramat, myclobutanil and pyraclostrobin + metiram, 4) spiromesifen, haloxyfop-p-methyl, bispyribac sodium, chlorothalonil + procymidone and trifloxystrobin + tebuconazole, 5) bispyribac sodium and myclobutanil, 6) pyraclostrobin + metiram and myclobutanil, 7) spinetoram and abamectin, and 8) spirotetramat, nicosulfuron and pyraclostrobin + metiram. However, acetamiprid and fipronil were significantly different from each other and from remaining pesticides tested against adult *T. chilonis* ($p \leq 0.001$).

e) Comparison of residual treatments with control

The comparison of means of four residual treatments with their respective control treatments (Table 17) based on parasitism demonstrated that all four treatments of HaNPV, spiromesifen and myclobutanil were statistically at par with their respective controls. While spirotetramat, acetamiprid, spinetoram, fipronil, abamectin, nicosulfuron, pyraclostrobin and trifloxystrobin + tebuconazole were significantly different from their respective controls. Similarly, 1-day treatments of chlorantraniliprole and haloxyfop-p-methyl have also showed significance difference with respective controls. The 15-day treatment of bispyribac sodium, while both 10-day and 15-day treatments of chlorothalonil + procymidone were statistically similar with their respective controls.

Table 17. Parasitism (mean) of *S. cerealella* eggs by *T. chilonis* exposed to 1-, 5-, 10- and 15-day-old pesticide residues at field dose for 24 h, and means comparison (Tukey's HSD, P = 0.001 or 0.1%)

S.no	Pesticides	Parasitism (mean $\pm$ S.E); (Age of pesticide residues)				
		1 day	5 days	10 days	15 days	Control
1	HaNPV	26.64 $\pm$ 6.52 abcdefgh	27.18 $\pm$ 3.23 abcde	27.55 $\pm$ 2.26 ab	26.82 $\pm$ 2.29 abcdefg	27.60 $\pm$ 2.89 ab
2	Spirotetramat	12.13 $\pm$ 1.13 A	18.97 $\pm$ 1.59 tuvw	18.23 $\pm$ 1.72 vwxy	20.37 $\pm$ 2.29 qrstu	24.66 $\pm$ 1.73 fghijk
3	Chlorantraniliprole	24.03 $\pm$ 2.75 ijklmn	26.82 $\pm$ 7.06 abcdefg	27.05 $\pm$ 3.60 abcde	27.47 $\pm$ 3.16 abc	28.03 $\pm$ 5.30 a
4	Acetamiprid	0.69 $\pm$ 0.45 I	1.66 $\pm$ 0.13 FG	1.45 $\pm$ 0.09 GH	1.39 $\pm$ 0.59GH	24.85 $\pm$ 1.73 efghijk
5	Spinetoram	0.73 $\pm$ 0.27 I	0.95 $\pm$ 0.13 HI	2.31 $\pm$ 0.67 F	3.36 $\pm$ 0.66 E	25.87 $\pm$ 2.25 abcdefghi
6	Fipronil	4.84 $\pm$ 0.43 D	7.20 $\pm$ 1.55 C	7.77 $\pm$ 2.57 BC	9.04 $\pm$ 2.35 B	25.04 $\pm$ 3.49 defghijk
7	Abamectin	2.31 $\pm$ 0.50 F	4.26 $\pm$ 0.90 DE	4.23 $\pm$ 0.82 DE	4.26 $\pm$ 1.06 DE	24.68 $\pm$ 1.37 fghijkl
8	Spiromesifen	23.37 $\pm$ 2.15 jklmno	23.75 $\pm$ 1.75 ijklmn	24.65 $\pm$ 1.50 fghijkl	24.84 $\pm$ 1.77 efghijk	25.05 $\pm$ 3.58 cdefghijk
9	Haloxypop-p-methyl	19.84 $\pm$ 1.82 rstuv	23.97 $\pm$ 2.91 ijklmn	24.39 $\pm$ 1.70 hijklm	24.31 $\pm$ 1.93 ijklm	24.60 $\pm$ 1.73 ghijkl
10	Bispyribac sodium	13.65 $\pm$ 2.65 zA	17.58 $\pm$ 1.65 wxy	21.01 $\pm$ 3.97 pqrst	23.12 $\pm$ 2.90 jklmnop	24.65 $\pm$ 4.42 fghijkl
11	Nicosulfuron	13.89 $\pm$ 1.26 z	17.02 $\pm$ 2.57 y	17.38 $\pm$ 1.70 xy	18.50 $\pm$ 2.98 uvwxy	21.93 $\pm$ 2.54 nopqr

S.no	Pesticides	1 day	5 days	10 days	15 days	Control
12	Myclobutanil	20.44 ± 1.94 qrstu	20.74 ± 1.98 qrst	21.09 ± 1.19 pqrs	21.35 ± 1.18 opqrs	22.41 ± 1.52 mnopq
13	Chlorothalonil + Procymidone	22.48 ± 3.21 lmnopq	23.04 ± 2.09 klmnop	25.34 ± 2.70 bcdefghij	25.16 ± 1.58 cdefghijk	26.95 ± 2.40 abcdef
14	Pyraclostrobin + Metiram	12.72 ± 2.46 zA	13.39 ± 1.60 zA	17.23 ± 1.91 xy	20.42 ± 3.15 qrstu	25.05 ± 1.53 cdefghijk
15	Trifloxystrobin + Tebuconazole	19.46 ± 3.51 stuvw	20.01 ± 0.84 rstuv	24.34 ± 1.93 hijklm	24.57 ± 0.76 ghijklm	27.32 ± 1.08 abcd
ANOVA results based on sqrt transformed data (parasitism based on residual toxicity)						
—		df	F	p	Remark	
Pesticides		14	5392.14	< 0.0001	sig	
Doses		4	4123.45	< 0.0001	sig	
Pesticide * Doses		56	342.66	< 0.0001	sig	

Means followed by the same small or capital letter within a column/among columns are not significantly different (Tukey's HSD, $p > 0.001$).

4.9.3) Percentage parasitism relative to controls by *T. chilonis* exposed in different residual treatments

Percentage parasitism (mean) of fresh eggs of *S. cerealella* relative to controls by female *T. chilonis* exposed to 1-, 5-, 10- and 15 day-old dried residues of pesticide at respective field recommended dose for 24 h in the glass vial were evaluated

a) Parasitism: 1-day-old residue

Females exposed for 24 h to 1-day-old residue (Fig 8) showed that maximum percentage parasitism (96.52) relative to control was observed for surface treated with HaNPV, followed by spiromesifen (93.29), myclobutanil (91.21), chlorantraniliprole (85.72), chlorothalonil + procymidone (83.43), haloxyfop-p-methyl (80.63), trifloxystrobin + tebuconazole (71.23), nicosulfuron (63.34), bispyribac sodium (55.39), pyraclostrobin + metiram (50.78) and spirotetramat (49.21). The most toxic chemicals for parasitism were, including acetamiprid with mean parasitism of 2.80, followed by spinetoram (2.83), abamectin (9.37) and fipronil (19.31).

b) Parasitism: 5-day-old residue.

Exposure of female parasitoids to 5 day-old residues demonstrated (Fig 8) that at maximum mean percentage parasitism (98.47) was observed with HaNPV, followed by haloxyfop-p-methyl (97.40), chlorantraniliprole (95.70), spiromesifen (94.80), myclobutanil (92.55) and chlorothalonil+procymidone (85.47). The most toxic chemical for parasitism was spinetoram, which led to 3.67% parasitism relative to controls, followed by acetamiprid (6.69%), abamectin (17.25%) and fipronil (28.74%).

c) Parasitism: 10-day-old residue

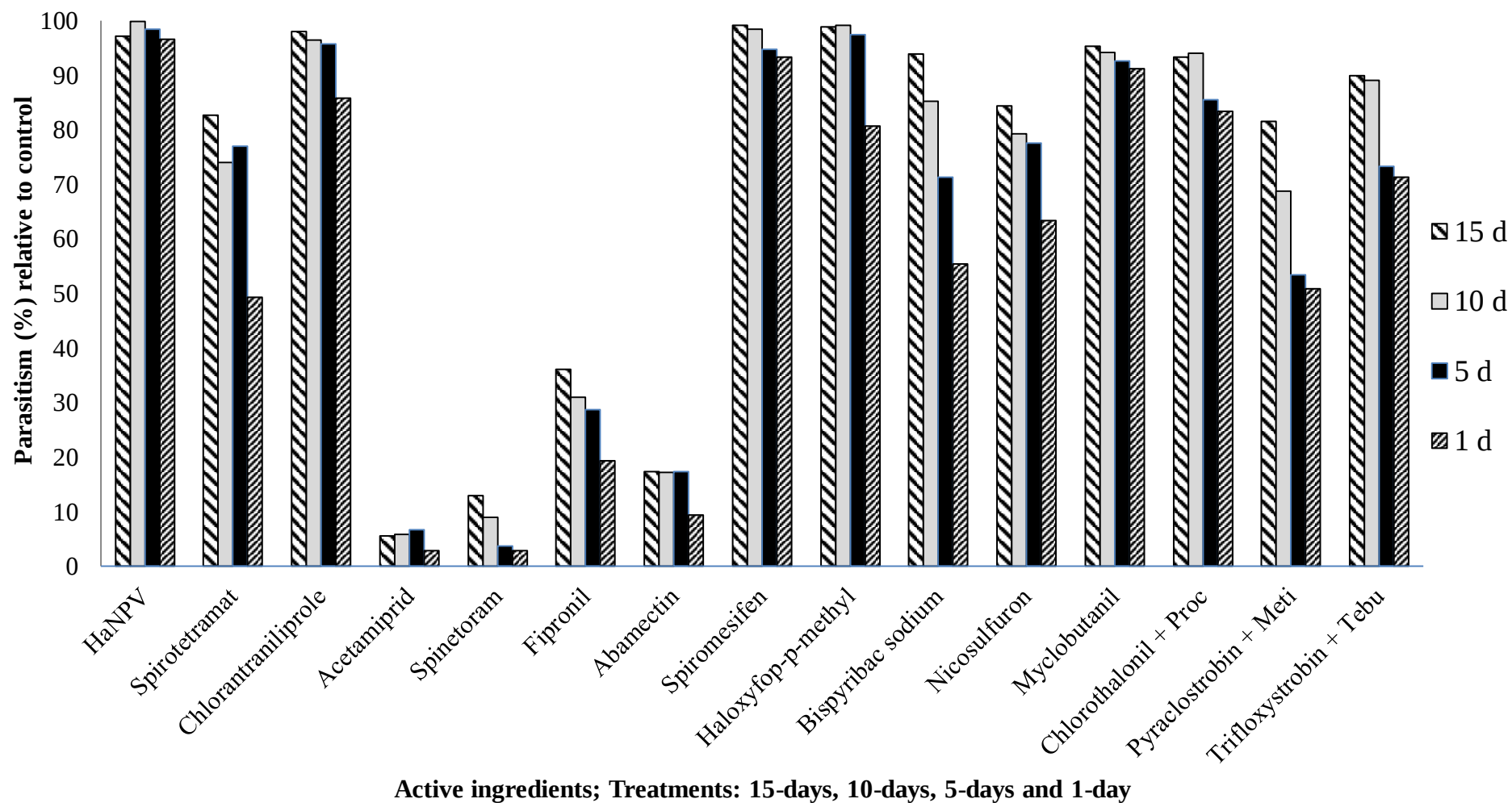
Females exposed to HaNPV-treated surfaces 10 days after application of pesticides (Fig 8) parasitized 99.82% of host eggs relative to control, followed by haloxyf-

op-p-methyl (99.12%), spiromesifen (98.38%), chlorantraniliprole (96.50%), myclobutanil (94.12%), chlorothalonil + procymidone (94.02%), trifloxystrobin + tebuconazole (89.10%), bispyribac sodium (85.23%), nicosulfuron (79.23%), spirotetramat (73.94%) and pyraclostrobin + metiram (68.77%). The most disruption effect on parasitism by *T. chilonis* was due to acetamiprid (5.84% parasitism), followed by spinetoram (8.93%), abamectin (17.15%) and fipronil (31.01%) relative to control.

d) Parasitism: 15-day-old residue

Females exposed to pesticide residues of 15 days age (Fig 8) revealed that the most harmful chemical to parasitism was acetamiprid, which yielded 5.59 percent parasitism, followed by spinetoram (12.98), abamectin (17.25) and fipronil (36.11). Spiromesifen resulted in 99.15% mean parasitism relative to control, followed by haloxyfop-p-methyl (98.80%), chlorantraniliprole (97.99%), HaNPV (97.16%), myclobutanil (95.27%), chlorothalonil + procymidone (93.34%), trifloxystrobin + tebuconazole (89.93%), nicosulfuron (84.34%), spirotetramat (82.60%) and pyraclostrobin + metiram (81.49%).

Fig. 8 Percent parasitism (mean) relative to control of host eggs (*S. cerealella*) by *T. chilonis* exposed to 1-, 5-, 10- and 15-days-old dried residues of pesticide for 24 h at field dose



4.9.4) Percentage reduction (mean) in parasitism over control by *T. chilonis* exposed in the different residual treatments, and IOBC/WPRS toxicity ranking

a) 1-day-old residue

The highest reduction (mean) in parasitism over control (Table 18) of the fresh *S. cerealella* eggs by the female *T. chilonis* exposed to the residue dried 1 day (24 h old residue) was 97.20 and 97.17% observed for acetamiprid and spinetoram, respectively, followed by abamectin (90.63%) and fipronil (80.69%). These aforementioned chemicals were ranked as moderately harmful for parasitism. Spirotetramat, bispyribac sodium, nicosulfuron and pyraclostrobin + metiram were ranked as slightly harmful for parasitism. While the remaining chemicals were all harmless for parasitism, including HaNPV and spiromesifen that caused lowest reduction in parasitism (3.48 and 6.71%, respectively), followed by remaining chemicals resulted in reduction in parasitism from 8.79 to 28.77% over control.

b) 5-day-old residue

Spinetoram caused highest reduction (mean) in parasitism over control by *T. chilonis* (Table 18), i.e., 96.33%, when females were exposed to the surface treated 5 days previously with spinetoram, followed by acetamiprid (93.11%) and abamectin (82.75%), and were all ranked as moderately harmful for parasitism, while pyraclostrobin + metiram and fipronil were ranked as slightly harmful for parasitism regarding exposure to 5-day-old residues. The remaining pesticides were all ranked as harmless for parasitism as they caused reduction in parasitism by wasps ranging from 1.53 to 28.66%, including HaNPV, which demonstrated the lowest mean reduction in parasitism over control: 1.53%, while bispyribac sodium showed 28.66% parasitism.

c) 10-day-old residue

The moderately harmful chemicals for parasitism were acetamiprid, which wa-

s the most toxic chemical for parasitism by the females exposed to 10-day-old dried residues among the tested chemicals (Table 18); led to 94.16% mean reduction in parasitism, followed by spinetoram (91.07%) and abamectin (82.85%). While slightly harmful chemicals for parasitism were pyraclostrobin + metiram and fipronil. The remaining pesticides were all harmless for parasitism: HaNPV that resulted in lowest reduction (mean) in parasitism (0.18%) by *T. chilonis* females, followed by the remaining pesticides resulted in reduction in parasitism from 0.88 to 26.06% over control.

d) 15-day-old residue

The exposure of female *T. chilonis* to 15 day-old-pesticide residues (Table 18) demonstrated that acetamiprid was the most harmful chemical for parasitism among the tested pesticides: led to highest reduction (mean) in parasitism (94.41%) over control, followed by the spinetoram (87.02%) and abamectin (82.75%) and these three chemicals were ranked as moderately harmful for parasitism by the exposed females. Fipronil was categorized as slightly harmful to parasitism. The remaining chemicals were all harmless for parasitism by *T. chilonis* as they led to reduction in parasitism from 0.85-18.51% over control.

4.9.5) Age-wise residual effects of pesticides on percent reduction in parasitism over control by *T. chilonis* exposed to the dried residues, and IOBC/WPRS toxicity ranking

The residual age-wise effects of pesticides on parasitism by *T. chilonis* exposed to the 1-, 5-, 10-, and 15-day-old residue showed (Table 18) that among the tested pesticides, the most toxic chemical for parasitism was acetamiprid, resulting in percentage reduction (mean) in parasitism over control by *T. chilonis* from 94.20 to 97.20 in all residual treatments, followed by spinetoram (97.17, 87.02) and abamectin

(90.63, 82.75) in 1-day- and 15-day-old residual treatments, respectively, All these four chemicals were moderately harmful for parasitism in all four residual treatments.

Fipronil led to 80.69% (moderately harmful) and 63.89% (slightly harmful) reduction in parasitism in 1- and 15-days residual treatments, followed by pyraclostrobin + metiram (49.22%: slightly harmful) and (18.51%: harmless) in 1- day and 15-day residual treatments, respectively. The slightly harmful chemicals to parasitism were spirotetramat, bispyribac sodium, and nicosulfuron resulted in (50.79, 44.61, 36.66%, respectively: slightly harmful) and (17.40, 6.18, and 15.66%, respectively: harmless) reduction in mean parasitism in 1-day and 15-day residual treatments, respectively. The remaining chemicals were harmless for parasitism by the wasp in all of the four residual treatments.

Table 18. Percentage reduction (mean) in parasitism of *S. cerealella* by *T. chilonis* exposed to 1-, 5-, 10- and 15- days- old residues of pesticides, and evaluation of toxicity of pesticides (IOBC/WPRS ranking)

R S.no	Pesticides	Age-wise percent reduction (mean) in parasitism and pesticides ranking								Mean redu (%)
		15 days	Class	10 days	Class	5 days	Class	1 day	Class	
1	HaNPV	2.84	1	0.18	1	1.53	1	3.48	1	2.01
2	Spirotetramat	17.40	1	26.06	1	23.05	1	50.79	2	29.33
3	Chlorantraniliprole	2.01	1	3.50	1	4.30	1	14.28	1	6.02
4	Acetamiprid	94.41	3	94.16	3	93.31	3	97.20	3	94.77
5	Spinetoram	87.02	3	91.07	3	96.33	3	97.17	3	92.90
6	Fipronil	63.89	2	68.99	2	71.26	2	80.69	3	71.21
7	Abamectin	82.75	3	82.85	3	82.75	3	90.63	3	84.74
8	Spiromesifen	0.85	1	1.62	1	5.20	1	6.71	1	3.59
9	Haloxyp-p-methyl	1.20	1	0.88	1	2.60	1	19.37	1	6.01
10	Bispyribac sodium	6.18	1	14.77	1	28.66	1	44.61	2	23.55
11	Nicosulfuron	15.66	1	20.77	1	22.42	1	36.66	2	23.88
12	Myclobutanil	4.73	1	5.88	1	7.45	1	8.79	1	6.72
13	Chlorothalonil + Procymidone	6.66	1	5.98	1	14.53	1	16.57	1	10.93
14	Pyraclostrobin + Metiram	18.51	1	31.23	2	46.57	2	49.22	2	36.38
15	Trifloxystrobin + Tebuconazole	10.07	1	10.90	1	26.76	1	28.77	1	19.12

Ranking: class 1= harmless ($E < 30\%$); 2 = slight harmful ($30 \leq E \leq 79\%$); 3 = moderately harmful ($79 < E \leq 99\%$); 4 = harmful ($> 99\%$)

Chapter 5

DISCUSSION

There is limited information available on the effects of many of the newer pesticides on *Trichogramma* spp (Khan *et al.*, 2015). The International Organization of Biological Control has established guidelines for the examination of direct effects of pesticides on natural enemies (Hassan *et al.*, 1991). Therefore, testing pesticides against beneficials generally emphasizes only direct contact effects and usually does not consider possible sublethal or residual effects (Croft, 1990). Similarly, limited sources of information are available addressing the effects of pesticides on the immature stages of *Trichogramma chilonis*, because the IOBC sequential scheme for testing side-effects of pesticides recommends the testing of the most susceptible life stage i.e., adult (Sattar *et al.*, 2011). Furthermore, sublethal effects of pesticides on *Trichogramma* have been limited to very few studies and are related to parasitism (Delpuech *et al.*, 1998).

The pesticides used in the current studies (Table 1) were relatively novel chemistries compared to organophosphates and pyrethroids, and more than half were relatively to highly selective, and were evaluated as have very limited adverse impact on acute parasitoid survival, emergence and parasitism. The pesticides tested against *T. chilonis* are commercial formulations including both active ingredient and inert material. Some of the products used have 2 active ingredients. Therefore, evaluation of the effect of such pesticides needs to be done with awareness that the inert material may also add to the effect of the active ingredient included in the formulation.

The current studies are helpful to growers using *T. chilonis* mass releases for

management of lepidopterous pests, since they can help in product selection, selection of appropriate time of pesticide application to control the pests or timing of mass releases in order to avoid the beneficial and to lessen effects on the overall parasitoid performance, as part of strategies to increase both quality and production as well as safeguard natural enemies, particularly *Trichogramma*, in the agro-ecosystem and our environment.

In the current studies, the pesticides tested in the laboratory experiments were evaluated for: 1) adult emergence from hosts (*S. cerealella*) treated with the chemicals in egg, larval, and pupal stages and subsequent parasitism by the emerged female parasitoids, 2) parasitism of the previously treated host eggs (no-choice test), and 3) adult survival and subsequent parasitism by the females when exposed to residues. Overall results of our data suggest that most of these products may be effectively integrated with biological control in IPM programs, particularly, where *Trichogramma* or other similarly sensitive parasitoids are important.

In the current laboratory work, the timing of pesticide exposure relative to pre-imaginal development (egg, larvae, and pupae) did not adversely affect emergence of the adult *T. chilonis* or parasitism by the female *T. chilonis* emerged from the treated host eggs for most of the chemicals used in the experiments (Table 19). However, acetamiprid, fipronil and abamectin demonstrated significant adverse effects on emergence of adults as well as on the parasitism by the female wasps emerged from the treated eggs relative to the three developmental stages as well as type of doses. It was discovered that aforementioned toxic chemicals have reduced toxicity for emergence as well as parasitism, from the egg stage toward pupal stage, when treatments shifted from the 2x toward treating at 0.5x dose. Spinetoram also severely affected the emer-

gence regarding all used doses and treated stages, but showed reduced toxicity for emergence as harmful at 2x through slightly harmful at 0.5x dose in the egg treatment. Stage dependent toxicity for emergence occurs due to some insecticides: the immature stage within the host eggs at the time of exposure can determine the effect of pesticides on emergence (Cônsoli *et al.*, 1998; Varma and Singh, 1987).

Similarly, the type of chemical as well as type of dose affected the parasitism of previously treated host eggs by female parasitoids for more than half of the chemicals used in the current research work (Table 15, 21): spinetoram, fipronil, abamectin and spiromesifen were harmful at 2x dose, however, the tested chemicals demonstrated reduced toxicity for parasitism of the treated eggs at both x and 0.5x doses compared to at 2x dose, nevertheless more than half of the chemicals used at field dose were harmless for parasitism by the females exposed to the treated eggs.

Furthermore, comparison of parasitism effects of previously treated host eggs at the three treated doses with the parasitism effects of the untreated host eggs by the female parasitoids emerged from the host eggs treated at the three doses (Table 21) when parasitoids were in immature stages also demonstrated the fact that acetamiprid, fipronil and abamectin were the most toxic products among the tested pesticides for both type of parasitism. However, they also showed reduced toxicity through declining doses (from 2x through 0.5x dose). Some of the used pesticides including spirotetramat, haloxyfop-p-methyl, trifloxystrobin + tebuconazole, myclobutanil and spiromesifen were toxic for parasitism when the host eggs were previously treated at 2x dose, nevertheless, the same chemicals had no adverse effects on the parasitism by the females emerged from the eggs treated at the same dose regarding all immature stages (except myclobutanil in the larval treatment) due to some sort of protection from these

pesticides to the young stage inside by the egg shell.

In the residual toxicity trial, acute toxicity and physiological selectivity of different pesticides for parasitism were evaluated at field dose through residual contact effects on parasitoids (Table 20). A high degree of variation in effect was found among pesticides. The pesticides with low toxicity for adult survival were also found harmless or slightly harmful for parasitism by the females exposed to the treated surface. However, it was found that the most toxic products in the present residual studies were actamiprid, fipronil, abamectin and spinetoram. These chemicals maintained their toxicity level for adult survival as well as parasitism by the exposed female parasitoids through all four residual treatments, while the remaining products showed reduced toxicity or were low toxic for survival of adults as well as parasitism by wasps through all treatments.

Comparison of effects of pesticides used in the current experiments on emergence and on adult survival demonstrated that some of the chemicals found harmless for emergence at three used doses regarding all immature stages (Table 19), including spiromesifen (except 2x dose in the egg treatment), haloxyfop-p-methyl, bispyribac sodium, pyraclostrobin + metiram and trifloxystrobin + tebuconazole, were found moderately toxic for adult survival, when adults were exposed for 24 h to glass surface (Table 20) treated with the same chemicals at the field dose 24 h after treatment (1-day's residual treatment), however, they showed reduced/low toxicity in the remaining residual treatments. This is due to the fact that adult *Trichogramma* are generally vulnerable to most broad-spectrum insecticides compared to immature stages developing within a host eggs and generally well protected from the most toxic compounds (Bull and House 1983; Bull and Coleman, 1985; C nsoli *et al.*, 1998; Suh *et al.*, 2000).

Similarly, comparison between the parasitism by the female wasps emerged from the host eggs treated at the field dose regarding all immature stages with parasitism by females exposed to the surface also treated at the field dose (Table 21) demonstrated that generally acetamiprid, fipronil and abamectin were found the most toxic chemical for both type of parasitism among the tested products. However, pyraclostrobin, spirotetramat and bispyribac sodium were harmless for parasitism of the untreated host eggs by the females emerged from treated immature stages, while the same chemicals were slightly harmful for parasitism of untreated host eggs when the female wasps were exposed to treated surface in the 1 day's residual treatment, however, showed reduced toxicity as harmless for parasitism in the remaining residual treatments (except pyraclostrobin in the 5-and 10-day's residual treatments). Nicosulfuron was slightly harmful for parasitism by the females exposed to the dried residues of 24 h (1-day-old) but the same chemical demonstrated as harmless for parasitism by the females emerged from the host eggs treated at all doses regarding all stages (except 2x dose in the egg treatment). The reason may be the high level of protection of immature stages from the sublethal effects of pesticides within the eggs by the egg shell from such chemicals.

Moreover, in the current studies, comparison of parasitism effects of previously treated host eggs at field dose with the parasitism effects of the untreated host by the female parasitoids exposed to the treated surface at the same dose (Table 21) demonstrated the fact that the chemicals acetamiprid, spinetoram, fipronil and abamectin were more inimical to parasitism of the untreated host eggs in the residual studies compared to parasitism of the previously treated host eggs. The reason may be that in the residual treatment, the early toxicity from the contact with dried residues severely

affected the parasitism potential of the exposed females before touching the host eggs.

The high level of tolerance of the immature stages of the wasps could be made possible by the chorion of the host eggs. (Sattar *et al.*, 2011). The chorion of *S. cerealella* eggs is composed of four layers (Bastos *et al.*, 2006). However, the first layer is covered by the unevenly distributed mucous layer (thickness ranging from 0.13 to 0.26 μm). According to Bastos *et al.*, 2006 the four layers followed are 1) the first chorionic layer (20 nm), 2) the lamellate exochorion (4.00–4.27 μm), 3) the trabecular layer (0.43–0.53 μm), and 4) the endochorion (13.33 nm).

Adults are the key parasitoid life stage for pest suppression, and as compared to immature stages, the adult stage of parasitoids is exposed to pesticide residues more readily because of its mobility (Khan *et al.*, 2015). Several means of adults exposure to pesticides, including contact with wet and dry pesticide residues on foraging substrates, on host eggs and, for translocated pesticides, through plant food resources: nectar (Khan *et al.*, 2015).

Although the translaminar and translocation properties of some of the insecticides make them available in host plant tissues as a control for pests, surface residues tend to disappear quickly, making them safer for natural enemies in the field (Iwata *et al.*, 1985, Hoy and Cave, 1985) as compared to the longer residual effects of pesticides on other than plant surfaces, such as glass surface under laboratory conditions. Understanding the direct effects of insecticide residues on adult *T. chilonis* is required to develop appropriate guidelines for timing releases of these beneficial wasps.

Solely laboratory testing is not sufficient to decide for selectivity of the pesticides. Therefore, screening of pesticides against natural enemies requires step-wise as-

essment, moving from the laboratory to the field, with adequate consideration of both direct and sublethal effects (Croft, 1990) to more accurately determine pesticide effects on parasitoids under natural conditions. However, the IOBC Working Group has postulated that pesticides found harmless to a particular beneficial insect in laboratory tests are likely to be of low risk to the populations in the field (Bigler and Waldburger, 1994; IOBC/WPRS, 1994). Testing pesticides effects against natural enemies in the laboratory is essential, at least as a screening step toward studies under field conditions (Klein *et al.*, 1992). Moreover, Amano and Haseeb *et al.* (2001) described laboratory testing as advantageous because one can readily obtain results urgently needed for large number of pesticides. Nevertheless, one must be careful, if the results of tests in the laboratory are to be extrapolated to the field, as results in laboratory may not be reflected under actual field conditions, because they may overestimate mortality of organisms by the residues (Amano and Haseeb, 2001).

It is concluded that our studies have some drawbacks: we only evaluated the effects of pesticide residues on the survival of adults/parasitism on glass surface and did not evaluate effects from exposure to foliar residues (field studies): the dry residue on the glass surface of the vial does not account for possible influences that plant surfaces may have on pesticide residues, such as absorption (Wang *et al.*, 2012). Therefore, further work is needed to evaluate the residual toxicity of these pesticides on plant surfaces as well as their potential sub-lethal effects. Similarly, we have evaluated short term effect of pesticides: adult survival, and parasitism of the survived female wasps exposed to the residues, however, these are not the only matter, as the residual exposure of natural enemies to some insecticides affects the reproductive potential of their offspring (Jenkins and Isaacs, 2007), and also long-term effects of exposure to the

pesticides led to cumulative sublethal effects on behavior of natural enemies (Khan *et al.*, 2015). This should not be ignored in future studies. However, these studies are helpful for effective integration of *T. chilonis* in the short term with many of the pesticides studied here (Khan *et al.*, 2015).

In the current studies, pesticides found slightly harmful in specific or moderately harmful in general for emergence of the parasitoids or parasitism by the females either emerged from the treated host eggs or exposed to dried residues or the previously treated host eggs in the laboratory are suggested for further studies under field conditions to evaluate more accurate effects of pesticides under natural conditions.

There may be different results of pesticide effect on life parameters (survival or parasitism) that depend on whether exposure occurs in an immature or adult stage. Orr *et al.* (1989) proposed that the developmental stage of parasitoids at the time of chemical treatment determines the time allowed for pesticide degradation before the emergence of parasitoids.

There occur great differences in biological control agents' fauna between cropping systems as well as within a crop grown in different areas (Brunner *et al.*, 2001). Therefore, selectivity of the pesticides for an agro-ecosystem could not be decided by testing one or several different types of natural enemies. Instead, evaluation of selectivity should remain within an individual pest management system, as each system is unique (Brunner *et al.*, 2001).

Natural enemies demonstrate different responses to the pesticides tested in the laboratory or under field conditions, i.e., the differences being found in the selectivity of pesticides in natural conditions or while using natural hosts in the bioassays may be

based on the differences in the egg morphology of hosts or parasitoid performance (Bastos *et al.*, 2006). Natural hosts, for example, can present a greater number of aeropyles (openings in the eggs) than laboratory hosts. The susceptibility of the parasitoids developing inside the host eggs is expected to increase due to the presence of aeropyles in the eggs of natural hosts (Bastos *et al.*, 2006).

Furthermore, the variability in the results for pesticides tested in the laboratory or under field conditions may be due to the host eggs received a large amount of the chemicals, when dipped in the pesticide solution in the laboratory, comparatively to lower amounts of pesticides received under field conditions, and many eggs could avoid the chemicals such as those oviposited on the underside of the leaves that may not be exposed to the pesticides (Saber, 2011).

In addition, under field conditions, pesticides might have less adverse impact comparatively to in the laboratory, because natural enemies can take advantage of natural shelters and avoid treated areas. Moreover, sunlight degradation plays an important role in the field by reducing the impact of pesticides on the parasitoids observed in the laboratory (Wang *et al.*, 2012). In the field, continuous fluctuation in temperature affect the natural enemies, as temperature is the most important factor affecting biological parameters; e.g., reproduction type, parasitism (fecundity), duration of development, emergence rate, and the longevity of insects (Stern and Bowen, 1963; Butler Júnior and López, 1980; Harrison *et al.*, 1985; Noldus, 1989; Pratissoli, *et al.*, 2005).

The time of spraying (pre- or post-treatment) had different consequences to parasitoid survival: exposure of the female to the previously treated host eggs was more detrimental to parasitoid development and emergence than treatment of the parasitized hosts: treating hosts after parasitization avoids the deterrent effects and mortality

that the products may cause on adult, affecting parasitism rates (Hohmann, 2010).

5.1) Comparison of Experimental Pesticide Effects on Emergence of *T. chilonis* with Previous Literature

In the current studies, various pesticides showed stage-as well as dose-wise variability in their effects on percentage emergence of *T. chilonis* from the treated host eggs compared to controls. There is very limited literature available on the toxicity of the tested pesticides on the immature stages of *Trichogramma* spp regarding emergence. Nevertheless, the adverse impacts of some of the chemicals used in the current experiments were evaluated on the emergence of parasitoid from treated eggs, larvae, and pupae in previous studies and are outlined below.

In the current studies, abamectin was moderately harmful for emergence of *T. chilonis* at field dose (x), 2x and 0.5x doses treated against eggs and larvae of the parasitoids (Table 3, 5) but showed reduced toxicity as slightly harmful at all the doses treated against the host eggs, when the parasitoids were in pupal stage (Table 7). Similar observations to the current result of laboratory studies were made by C nsoli *et al.* (1998), who reported that abamectin is harmful to slightly harmful for *T. chilonis* emergence, when treated against different immature stages. Carvalho *et al.* (2003) also found abamectin as the only insecticide among the tested chemicals, which adversely affected emergence of *T. pretiosum* from host eggs treated, when parasitoids were in egg, larval and pupal stages.

The toxicity of abamectin for emergence in the current studies was also supported by observations made by Hewa-Kapuge *et al.* (2003), who tested seven different insecticides and reported that abamectin was toxic for emergence of *T. chilonis*. Mou-

ra *et al.* (2006) also tested abamectin (sprayed) on host eggs (*Anagasta kuehniella*) and found abamectin slightly harmful to larvae, but moderately harmful to pupae of egg parasitoid *T. pretiosum*.

Hussain *et al.* (2010) also evaluated abamectin effects on immature development of parasitic wasp *T. chilonis*, and confirmed the effect of abamectin observed in the current experimental work, i.e., concluded that abamectin had significantly adversely affected emergence of parasitoids from the host eggs of *S. cerealella* treated when parasitoids were in egg, larvae, pre-pupae, early pupae and pupae.

The evaluation of spinetoram (spinosyn) in the present studies demonstrated the chemical as harmful and moderately harmful for emergence of *T. chilonis* at 2x and x doses, respectively, but was slightly harmful for emergence at 0.5x concentration regarding egg treatment (Table 3). However, spinetoram was moderately harmful for emergence at all treated doses, when *T. chilonis* was treated in the larval and pupal stages (Table 5, 7, respectively).

Although there is no previous studies on the effect of spinetoram on the emergence of *Trichogramma*, however, the toxicity of spinetoram for the emergence of *T. chilonis* in the current studies was supported by Nevarez *et al.* (2009) who determined severe effects of spinosad (also belonging to spinosyn group), similar to spinetoram, on the adult emergence of *T. platneri* (Nagakartti), *T. exiguum* (Pinto & Platner) and *T. pretiosum* Riley. While according to studies of Wise *et al.*, 2010, the same chemical had no adverse impact on the emergence of *T. minutum* from treated host eggs. Bueno *et al.* (2008) tested spinosad under laboratory conditions against eggs, larvae and pupae of *T. pretiosum* and found the chemical as harmful for emergence of minute parasitoids.

Hussain *et al.* (2010) had tested and found spinosad as adversely affecting the emergence of *T. chilonis*, when host eggs of *S. cerealella* were treated containing egg, larvae, pre-pupae, early pupae and pupae of the egg parasitoids. Suh *et al.*, 2000 found that spinosad (LC₅₀) was among the most toxic compounds and adversely affected emergence of *T. exiguum* from *Helicoverpa zea* (Boddie) host eggs, when exposed at larval, prepupal, or pupal stages. Hussain *et al.* (2012) evaluated toxicity of some new insecticides including spinosad under laboratory conditions against immature stages of *T. chilonis*. Spinosad resulted in the lowest emergence regardless whether parasitoids were treated in the egg, larval or pupal stage.

Acetamiprid was moderately harmful for emergence at 2x dose, but slightly harmful at both x and 0.5x doses in the egg treatment (Table 3). In the larval treatment (Table 5), acetamiprid was rated slightly harmful at both 2x and x doses, while harmless for emergence at 0.5x dose. When pupal stage was treated (Table 7), acetamiprid was moderately harmful, slightly harmful and harmless for emergence of *T. chilonis* at each 2x, x and 0.5x dose, respectively.

The toxic effects of acetamiprid on emergence in the current studies are supported by Hewa-Kapuge *et al.* (2003), who tested seven different insecticides and reported that acetamiprid was toxic for emergence of *T. chilonis*. Moura *et al.* (2006) also treated (sprayed) host eggs (*Anagasta kuehniella*) with acetamiprid and found acetamiprid as harmless to larvae, but was slightly harmful to pupae of *T. pretiosum*.

Current studies also revealed fipronil as harmful for emergence at all three doses in the egg treatment (Table 3), while being rated as harmful at 2x, moderately harmful at x and slightly harmful for emergence of *T. chilonis* at 0.5x dose regarding larval treatment (Table 5). Fipronil was slightly harmful at 2x and was demonstrated har-

harmless for emergence at both x and 0.5x doses regarding pupal treatment (Table 7).

The remaining pesticides were found harmless for emergence of the tiny wasp at all the doses treated against the three immature stages (Table 3, 5, 7) except spiromesifen, which was slightly harmful (31.87%) for emergence, when parasitoids were treated at 2x dose in the egg stage.

HaNPV is a microbial insecticide, and was evaluated as harmless for emergence in the current studies, as Sagheer *et al.* (2008) described that microbial pesticides are highly effective, safe, and ecologically acceptable. Nevertheless, there was found no previous studies of the same product against *Trichogramma*. However, the current result is supported by studies with other microbial insecticide such as Gandhi *et al.* (2005), who described that emergence success of the *T. chilonis* was not influenced by the treatment of bacterium *Pseudomonas fluorescens*. Furthermore, Nasreen *et al.* (2004), who treated *T. chilonis* in the late pupae in the host eggs of *S. cerealella* by several insecticides under semifield conditions, also found *B. thuringiensis* (microbial insecticide) as relatively safe for emergence of the *T. chilonis*. Furthermore, studies conducted on the lethal and sublethal effects of microbial insecticides have generally reported that Bt- insecticides are harmless to *Trichogramma* (Salama and Zaki 1985; Ruberson and Tillman 1999; Vieira *et al.*, 2001).

Chlorantraniliprole was found harmless for emergence of *T. chilonis* in the current laboratory studies, which was confirmed by Hussain *et al.* (2012), who evaluated toxicity of chlorantraniliprole under laboratory conditions and demonstrated that the same chemical resulted in maximum emergence of *T. chilonis* from the host eggs treated after 8 days of parasitism and showed minimum effect on emergence of the tiny wasp from 1, 3, 5 and 7 days old parasitized treated eggs.

In the present study, trifloxystrobin + tebuconazole and pyraclostrobin were also found harmless for emergence of *T. chilonis*. The previous studies demonstrated fungicides as generally harmless for emergence of *Trichogramma* (Hagley and Laing, 1989; Sterk *et al.*, 1999; Jalali and Singh, 1993; Vieira *et al.*, 2001), particularly, when sprayed on pupae (Hassan 1994). Similarly, the result obtained by the Carmo *et al.*, 2010, also supports that fungicides, including trifloxystrobin, pyraclostrobin and tebuconazole demonstrated no significant difference in emergence of *Telonomus remus* compared to control and were categorized as harmless to immature stages of the same egg parasitoid.

In the current laboratory work, myclobutanil was found harmless for emergence of immature stages of *T. chilonis*, a result supported by Bueno *et al.* (2008), who tested myclobutanil under laboratory conditions and found the same chemical harmless for emergence of *T. pretiosum*, when parasitoids were treated in all three immature stages. The data for effect of chlorothalonil + procymidone on emergence regarding both larval and pupal stages (Table 4, 6, respectively) are not presented due to fungus attack on the parasitized cards.

5.2) Comparison of Tested Pesticide Effects on Parasitism by *T. chilonis* in the Current Studies with Previous Literature

The current studies on pesticide effects on parasitism (disruption of parasitism) by the females emerged from the host eggs treated with different pesticides when parasitoids were in egg, larval and pupal stages showed variability regarding type of pesticides, dose and treated life stages.

Acetamiprid was discovered under current research work as harmful and mod-

erately harmful for parasitism at both x and 0.5x doses, respectively, regarding egg treatment (Table 9), while the same chemical showed decrease in toxicity as slightly harmful and harmless for parasitism at x and 0.5x doses, respectively, in the larval treatment (Table 11). Acetamiprid also demonstrated reduced toxicity as moderately harmful at 2x but slightly harmful for parasitism at both x and 0.5x doses, when parasitoids were treated in pupal stage within host eggs (Table 13).

Toxicity in the current studies due to abamectin and fipronil was decreased from harmful and moderately harmful for parasitism, respectively at 2x, to moderately and slightly harmful for parasitism, respectively, at both of lower doses, regarding pupal treatment (Table 13). Similarly, fipronil was slightly harmful for parasitism at x and 0.5x doses in the larval treatment. Literature is not available on effect of fipronil on parasitism by parasitoids, while toxicity due to abamectin in the current experimental studies, was strongly supported by Carvalho *et al.* (2003), who found that treatment of the host eggs by abamectin, when parasitoids were in pupal stage, led to significantly reduced parasitism by the emerged female *T. pretiosum*.

The remaining pesticides were found harmless for parasitism by *T. chilonis* emerged from the host eggs treated at all three doses when parasitoids were in the three immature stages. HaNPV, a microbial pesticide, was found harmless for the parasitism by *T. chilonis* in the current research work, supported by Vianna *et al.*, 2009, that *B. thuringiensis* (microbial insecticide) was harmless for parasitism by *T. pretiosum*. Moreover, according to Gandhi *et al.* (2005): the bio-pesticides were less harmful for parasitism by parasitoids than synthetic chemicals. Parasitism success of the *T. chilonis* was not influenced by the treatment of bacterium *Pseudomonas fluorescens* (Gandhi *et al.*, 2005).

Spirotetramat was rated as harmless for parasitism by *T. chilonis* in the present study in a line of support by Bruck *et al.*, 2009, that spirotetramat is harmless to *T. cryptophlebiae* in citrus, moreover, according to Moens *et al.*, 2012, limited research has been conducted to assess the side effects of spirotetramat on natural enemies.

There are extremely few previous studies on the effect of herbicides and fungicides on *Trichogramma*. In the current studies, nicosulfuron and chlorothalonil + procymidone showed increased toxicity for parasitism from harmless at x dose to slightly harmful at 2x dose regarding egg treatment (Table 9), while the said chemicals were found harmless for parasitism at all used doses, when larvae and pupae were treated (Table 11, 13, respectively). Myclobutanil demonstrated reduced parasitism as slightly harmful at 2x dose to harmless at the x dose tested against larvae (Table 11), while the same chemical revealed as harmless for parasitism at all used doses, when tested against egg and pupal stages (Table 9, 13, respectively).

The current study also demonstrated that fungicides including trifloxystrobin + tebuconazole and pyraclostrobin were harmless for parasitism by *T. chilonis* emerged from treated host eggs, as similar result were obtained by the Carmo *et al.*, 2010, that trifloxystrobin, tebuconazole and pyraclostrobin had no significant difference in parasitism by *Telonomus remus* compared to control and were categorized as harmless for adults.

Some of the previous studies on assessment of experimental pesticide effects on other natural enemies than *Trichogramma* showed similar effects for *Trichogramma* in the current studies. The present result revealed that pyraclostrobin + metiram, and chlorothalonil + procymidone (except 2x dose in egg treatment) have no adverse impact on parasitism by *T. chilonis* emerged from treated host eggs, supporting Peter-

sen, 1995, who concluded that metiram had no significant effect on percent reduction in egg production and hatchability, and further concluded that chlorothalonil and procymidone had no adverse impact on parasitism in the rove beetle *Alleochara bilineata*. Similarly, haloxyfop-p-methyl was disclosed in the present research as slightly harmful to harmless for parasitism, also supported by Peterson, 1995 that haloxyfop-R has been found safe to egg production (%) of beetle *Alleochara bilineata*.

While data on parasitism for abamectin regarding egg and larval treatments (Table 2, 4, respectively) is not available, may be one of the reason of very low emerged adults: $\leq 7.60\%$ at all treated doses in egg treatment, and $\leq 4.34\%$ at both 2x and x doses, while 17.20% at 0.5x dose in the larval treatment. Fipronil also have demonstrated no parasitism, when parasitoids were treated at egg stage due to very low adults emerged ($\leq 0.68\%$) at all treated doses, however, when tested against larvae, fipronil led to complete failure of emergence at 2x dose. Spinetoram showed no parasitism may be due to very low emergence of adults (0.66% and 17.20%) at 2x and x doses, respectively, when treated in egg stage, while caused adult emergence $\leq 11.59\%$ in the larval and $\leq 6.36\%$ in the pupal treatment (Table 6) at all treated doses. The data for effect of chlorothalonil + procymidone on parasitism regarding both larval and pupal stages are not presented due to fungus attack on the parasitized cards.

5.3) Comparison of Effect of Tested Pesticides on Emergence of Adults and Parasitism by Female *T. chilonis* Emerged from the Treated Immature Stages.

5.3.1) Comparison of pesticide effects regarding egg treatment.

Several chemicals caused variable effects of toxicity for emergence of adults as well as disrupting parasitism by the female *T. chilonis* (Table 19) emerged from the

host eggs treated, when parasitoids were in egg stage. Infact, comparatively to parasitism, these chemicals were found more toxic for emergence of adults. Acetamiprid showed greater toxicity for emergence of parasitoids compared to parasitism: slightly harmful at both 0.5x and x doses for emergence, but was harmful and moderately harmful for parasitism at x and 0.5x doses, respectively.

Similarly, spiromesifen was slightly harmful for emergence at 2x dose (Table 3), but was harmless for parasitism at the same dose. Nicosulfuron and chlorothalonil + procymidone were harmless for emergence regarding all treated doses; however, they showed increased level of disruption for parasitism from harmless at x dose to slightly harmful at 2x dose. The remaining pesticides did not vary in their effect on emergence or parasitism: they were found harmless for emergence of adults as well as parasitism by female *T. chilonis* at the three doses treated against the parasitoids regarding all immature stages.

5.3.2) Comparison of pesticide effects on emergence and parasitism regarding larval treatment.

Some of the pesticides tested against the larvae of *T. chilonis* demonstrated variable effects of toxicity for emergence of adults and disruption of parasitism by the female *T. chilonis* (Table 19) emerged from the treated host eggs. Myclobutanil was harmless for emergence at all treated doses but was found slightly harmful for parasitism at 2x dose for the females emerged from the treated host eggs. Acetamiprid demonstrated same level of toxicity for emergence as well as parasitism: slightly harmful at x dose, but was harmless at 0.5x dose. The remaining chemicals, which were not affected by the type of treated dose, were harmless for emergence as well as for parasitism at the three treated doses.

5.3.3 Comparison of pesticide effects on emergence and parasitism regarding pupal treatment.

There occurred variability in the effects of several chemicals on emergence of adults and parasitism by the female *T. chilonis* (Table 19) emerged from the host eggs treated, when parasitoids were in the pupal stage. Abamectin was categorized as slightly harmful for emergence at all three doses but was found harmful for parasitism at 2x dose, while moderately harmful for parasitism at both x and 0.5x doses. Acetamiprid was harmless, slightly harmful and moderately harmful for emergence at each 0.5x, x and 2x doses, respectively, however acetamiprid was ranked slightly harmful for parasitism at both 0.5x and x doses, while moderately harmful for parasitism at 2x dose. Fipronil was harmless at both 0.5x and x doses, but was slightly harmful for emergence at 2x dose. Nevertheless, toxicity of fipronil for parasitism by wasps was also decreased from moderately harmful at 2x to slightly harmful at both x and 0.5x doses. The remaining chemicals which were found harmless for parasitism (Table 13), were also found harmless for emergence (Table 7) of wasps at the three doses.

5.4) Comparison of Pesticide Effects on Parasitism of the Treated Host Eggs by *T. chilonis* in the Current Studies with Previous Literature (No-Choice Test)

Very limited studies were conducted by previous researchers to assess the effect of pesticides on the parasitism of the previously treated fresh host eggs by the females *T. chilonis*. The results on effect of pesticides as well as doses on parasitism of previously treated eggs by *T. chilonis* in the no-choice test demonstrated that parasitism was affected by 10 chemicals (Table 14). Spirotetramat, trifloxystrobin + tebuconazole, and myclobutanil showed reduced toxicity (disruption of parasitism) for parasitism: as slightly harmful at 2x dose, while harmless at both x and 0.5x doses. Acetam-

iprid as well as haloxyfop-p-methyl experienced reduced toxicity for parasitism by parasitoids from slightly harmful at both 2x and x doses through harmless at 0.5x dose.

Spinetoram, abamectin and fipronil demonstrated the same level of reduced toxicity for parasitism from moderately harmful at 2x through slightly harmful for parasitism at both x and 0.5x doses, while spiromesifen was moderately harmful at both 2x and x doses, but slightly harmful for parasitism at 0.5x dose. The remaining chemicals were all harmless for parasitism of previously treated host eggs by parasitoids regarding three used doses.

The toxicity of abamectin for parasitism by adults in the current research work were supported by Vianna *et al.*, 2009, who described considerable reduction in parasitism rate by two populations of *T. pretiosum* from Brazil of the eggs of *Anagasta kuehniella* previously immersed in different solutions of abamectin.

5.5) Comparison of the Experimental Pesticide Effects on the Adult Survival of *T. chilonis* in the Current Studies with Previous Literature on Adult Mortality

The exposure of randomly selected adults in the glass vial to the surface treated with the different pesticides for 24 h, and to different residual treatments (1-, 5-, 10-, and 15-days-old residue) at their respective field recommended doses were evaluated for residual effects of pesticides, which demonstrated variable effects of different pesticides and residual treatments on adult survival, and showed that some of the chemicals inflicted high mortality to the adult *T. chilonis* within 24 h of exposure.

The current research work demonstrated that four chemicals-fipronil, abamectin, spinetoram and acetamiprid, were harmful for survival of adult *T. chilonis* (acute toxicity) regarding all residual treatments. The high toxicity of these chemicals were

supported by Khan *et al.* (2015), who concluded that fipronil, abamectin and spinetoram inflicted > 90%, while acetamiprid induced 83.9% mortalities of adults of *T. pretiosum* exposed to the treated cotton leaves within 24 h of post treatment at their respective field doses. The toxicity of spinetoram in the current studies was also supported by Saljoqi *et al.* (2012), who studied different concentrations of spinosad (0.2, 0.15, 0.1, 0.05, and 0.01%) against adult *T. chilonis*, and found all concentrations caused 100% adult mortality of parasitoids within 15 minutes. Similarly, Sattar *et al.* (2011) reported 100% mortality of *T. chilonis* adults by spinosad- (spinosyn) treated host eggs or tomato leaves, which is comparable to the result of 100% mortality of adult *T. chilonis* induced by spinetoram (spinosyn) in the current laboratory studies.

Fipronil exhibited high toxicity (harmful) for adult survival of *T. chilonis* regarding all treatments in the current research work was also supported by Balanca and Visscher (1997), who described similar results in previous studies demonstrating acute toxic effects of fipronil on adult insects belonging to the orders Coleoptera, Hymenoptera and Diptera, even at very low doses.

Moura *et al.* (2006) supported toxicity of abamectin and acetamiprid as harmful to *T. chilonis* in the current studies, and described abamectin harmful for adult (residue test on glass plates) *T. pretiosum*, while acetamiprid was determined as moderately harmful for adults (acute toxicity) of same parasitoid.

The harmful activity of abamectin against imaginal *T. chilonis* in the present laboratory work was further supported by Hussain *et al.* (2010) evaluation of the effects of the exposure of adult *T. chilonis* to insecticides including abamectin that resulted in 18.48% adult survival after 4 hours of exposure, however, after 24 h of adults exposure, abamectin led to 100% mortality of adults parasitoids. Brunner *et al.*, 2001

conducted experiments to assess the impacts of topical application of abamectin on the egg parasitoid *T. platneri* and found the chemical was highly and acutely toxic to adults of the parasitoid but showed no residual toxicity after 24 h of application on the minute wasp.

The encouraging results with stated compatible pesticides on adult stage of *T. chilonis* obtained in the current studies are supported by some previous studies with *Trichogramma* and some natural enemies. Chlorantraniliprole and spirotetramat were evaluated and found as low toxic to *T. chilonis* regarding adult survival in the current research work was confirmed by Preetha *et al.* (2009) that chlorantraniliprole inflicted no significant mortality at field rates against the adult stage of *T. chilonis*. Moreover, Khan *et al.*, 2015 also supported the current results that both chlorantraniliprole and spirotetramat induced < 10% mortality to adult *T. pretiosum* exposed to treated leaves of cotton with in 24 h of post treatment. Martinou *et al.* (2014) tested and found that chlorantraniliprole led to < 25% mortality of the adult generalist predator *Macrolophus pygmaeus* and was ranked as harmless to adults. Bruck *et al.* (2009) reported spirotetramat safe to *T. cryptophlebiae* (Nagaraja) adults in citrus.

Very limited literature is available on the effect of fungicides and herbicides on *Trichogramma*. The fungicides likewise exhibited good selectivity to adult *T. chilonis* in the current studies, supported by previous nontarget work with other natural enemies, i.e., myclobutanil was found harmless for adult survival of *T. chilonis* in the current studies, as supported by Khan *et al.* (2015), who observed myclobutanil as harmless/low toxic to adult *T. pretiosum* (acute toxicity) when exposed to treated cotton leaves within 24 h of exposure, moreover, James and Coyle (2001), who also observed no acute toxicity of myclobutanil against several species of predatory mites and

Coccinellids. These assertions of our data are also supported by the work of Udayagiri *et al.* (2000), in which a variety of fungicides (including myclobutanil and benomyl) caused no acute toxicity to the hymenopteran egg parasitoid *Anaphes iole* Girault. Jepsen *et al.* (2007) similarly noted that fungicides have been found to inflict little acute toxicity on insects, although low levels of mortality have been observed in some cases.

Pyraclostrobin + metiram and spiromesifen were all moderately harmful only in the 1 day treatment, but were low toxic for adults in the remaining residual treatments. Pyraclostrobin and spiromesifen were evaluated by Khan *et al.*, 2015 as low toxic to adult *T. pretiosum* (acute toxicity) within 24 h of exposure: induced < 10% adult mortality of *T. pretiosum*. Bielza *et al.* (2009) also concluded that spiromesifen was harmless for adults of the parasitoid *Eretmocerus mundus* Mercet, and the predator *Orius laevigatus* (Fieber).

Trifloxystrobin + tebuconazole were moderately harmful in the 1-and 15-day treatments, while toxicity for adults was low in the 5-and 10-day residual treatments. The same mixture of chemicals was discovered as low toxic to adult survival of *T. pretiosum* within 24h of exposure to the cotton leaves by Khan *et al.* (2015). Petersen, 1995 assessed tebuconazole toxicity against adult female rove beetle *Aleochara bylin-eata* with mated single females exposed to treated (FRC dose) sand in a glass cell and the fungicide tebuconazole was found to have no measurable effects on rove beetle mortality. Thus based on tebuconazole harmlessness, it may be possible that pyraclostrobin and myclobutanil are similarly harmless, at least in acute lethal effects.

Bispyribac sodium was indicated as moderately harmful in the 1-and 5-day treatments, but toxicity decreased over time: became low toxic for adult (acute toxicit-

y) in both 10-day and 15-day treatment. While haloxyfop-p-methyl was moderately harmful only in the 1-day treatment, but were low toxic for adults in the remaining residual treatments. Although, previous studies were not found for the remaining chemicals regarding toxicity to adults *Trichogramma*, nevertheless, they were all low toxic for adult survival in all the four residual treatments tested here.

5.6) Comparison of the Experimental Pesticide Effects on Parasitism by Female *T. chilonis* Exposed to the Treated Surface with the Previous Studies

There are very limited sources of literature available on the effect of pesticides on the parasitism of fresh untreated host eggs (*S. cerealella*) by *Trichogramma* exposed to treated surface in the glass vial. The current studies demonstrated that the most toxic chemicals for parasitism were acetamiprid, spinetoram and abamectin, as they were moderately harmful for parasitism by females exposed to treated surface of glass vials for 24 h in all the four residual treatments. Fipronil showed reduced toxicity as moderately harmful in the 1-day treatment, while slightly harmful for parasitism in the 15-day treatment.

The toxicity of acetamiprid and abamectin for parasitism by exposed females was strongly supported by similar observations carried out by Moura *et al.* (2006) that acetamiprid and abamectin treated surfaces reduced the parasitism efficiency of *T. pretiosum* females exposed to the surfaces, and were ranked as moderately harmful and harmful for parasitism, respectively.

Pyraclostrobin + metiram were harmless for parasitism in the 15-day residual treatment, while only slightly harmful in the remaining residual treatments. Spirotetramat, bispyribac sodium, and nicosulfuron were slightly harmful in the 1-day treatment.

t, but were harmless for parasitism in the remaining treatments. The remaining chemicals were harmless for parasitism by the females exposed for 24 h in all of the four residual treatments. Nevertheless, literature is not available for these chemicals regarding effect on parasitism by the exposed female *Trichogramma*.

5.7) The Effects of Some of Experimental Pesticides on the Target Pests

The body of literature regarding target pest efficacy for all pesticides evaluated in the present study is highly variable for the respective products. Nevertheless, their efficacy against target pests is outlined below.

Abamectin and emamectin benzoate belong to the avermectin class of insecticides, and products of this class have been reported to provide excellent control of corn ear worm, *H. Zea*, in foliar application with an LC₉₀ value of 0.002 µg/ml (White *et al.*, 1997). However, resistance to avermectins also has been reported. For example, the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), exhibited high resistance ratios (at LC₅₀) for abamectin and milbemectin (Sato *et al.*, 2005).

The recommended rate of fipronil (4%) was found to be very effective used against *Hieroglyphus* spp., the most destructive pests of rice, maize, wheat and sugarcane in Pakistan. Treatment of the various stages of *H. perpolita* (Uvarov), *H. oryzivorus* Carl and *H. nigrorepletus* I. Bolivar under laboratory conditions demonstrated that the early stage nymphs were more susceptible for insecticides than the later stage nymphs or the adults (Sultana and Wagan, 2011).

Fipronil applied at the rate of 0.6 g a.i./ha in the field successfully suppressed outbreaks of adult grasshoppers, including *Oedaleus senegalensis* Krauss and *Acrotylus blondeli* Saussure, with 47% mortality obtained in 2 days and 91% in 10 days (Bal-

anca and Visscher, 1997). In laboratory and field tests, low doses of fipronil (5-20 g a.i. ha⁻¹) effectively controlled the brown locust, *Locustuna purdalina* (Walker), the African migratory locust, *Locusta migratoria migratorioides* (Reiche & Fairmaire), and the desert locust (Butler and Du Preez, 1994; Kriel *et al.*, 1994; Megenasa and Muinamia, 1994; Price *et al.*, 1994). Fipronil also effectively reduced populations of the Colorado potato beetle *Leptinotarsa decemlineata* (Say) in another study (Shi *et al.*, 2012).

Acetamiprid (20% a.i) at 250 g/1000 L, caused 40% adult mortality of the predatory coccinellid, *Oenopia conglobata* L., but 87.5% adult mortality of the common pistachio psyllid, *Agonoscyta pistaciae*, 24 h after treatment (Kabiri and Amiri-Besheli, 2012). Spinosad belongs to the same group as spinetoram, and they provide effective management of lepidopteran pests such as species of the genera *Spodoptera* and *Helicoverpa* in many greenhouse and outdoor crops (William *et al.*, 2004; Penagos *et al.*, 2005; Pineda *et al.*, 2007; Santis *et al.*, 2012).

Literature is not available on the effectiveness of HANPV against *H. armigera*, however, Shahid *et al.* (2003) reported that biopesticides based on fungus *Metarhizium anisopliae* and bacteria *B. thuringiensis* have effectively suppressed stem borer and rice leaf folder populations in the laboratory and field.

Chlorantraniliprole was highly toxic for bollworm *He. Zea*, fall armyworm *Spodoptera frugiperda* (J.E. Smith), and tobacco budworm *Heliothis virescens* (F.), in all three laboratory bioassay procedures: insecticide treated diet, topical application, and adult vial test (Temple *et al.*, 2009). Chlorantraniliprole also effectively controlled all populations of *Chilo suppressalis* (Walker) at maximal LC₅₀=18.70 ng per larva (Jia *et al.*, 2011).

Spirotetramat has demonstrated outstanding control of sucking insect pests, including whiteflies, aphids, scales (soft and armoured scales), mealy-bugs, psyllids, and selected thrips species in vegetables, cotton, soybean, pome and stone fruit, grapes, hop, citrus, nut trees, and banana in laboratory and greenhouse assays, as well as in semi-field and field trials (Bruck *et al.*, 2009). Khaliq *et al.* (2014) evaluated acephate 75%, spirotetramat 22.23%, and spinetoram 11.70% at field doses of 2.5g/l, 1.25ml/l, and 0.4ml/l, respectively, found they caused significant reductions (45-70%) in onion thrips (*Thrips tabaci*) populations.

Spiromesifen treatment (5 mg l⁻¹) caused 40% adult mortality of *Bemisia tabaci* (Gennadius) and also reduced fecundity of adult female *B. tabaci* by more than 80%, and egg fertility was almost zero (Kontsedalov *et al.*, 2008). Spiromesifen at a rate of 96, 112, 114, and/or 149 g a.i./ha, was highly toxic to nymphs of the whitefly, *B. tabaci* biotype “B”, on both melons and collards, causing approximately 100% mortality to young nymphs (first and second instars).

At these same rates, spiromesifen was moderately toxic for old nymphs (third and fourth instars), but not toxic for eggs (2.8–6.3% on melon and 2.9–6.4% on collard) and only slightly toxic for adults (24 h toxicity), 4.5–15.1% on melon and 25.1–37.0% on collard). Potentially, spiromesifen may be used in the integrated pest management program for whitefly control and to overcome pest resistance in vegetables and other field crops (Liu, 2004).

Fungicides such as pyraclostrobin and tebuconazole can successfully control a variety of important plant pathogens such as, *Quambalaria eucalypti*, the agent causing eucalypt leaf spot (Ferreiraa, 2008). Chlorothalonil decreased the level of infection by the mite-pathogenic fungus *Neozygites floridana* in corn (Brandenburg and

Kennedy, 1982). However, fungicides also may adversely affect biological control in cropping systems.

5.8) Use of the Current Research-Based Knowledge to Facilitate Successful Integration of *Trichogramma* with Tested Compatible Pesticides

The most widely used arthropod biological control agents in augmentation programs belong to Trichogrammatidae (Li, 1994). *Trichogramma* species are polyphagous egg parasitoids of caterpillar pests belonging to the order Lepidoptera (Pintureau, 1990). They are augmented successfully on a large scale on corn, cotton, sugarcane, fruit tree and vegetable crops in more than 50 countries (Hassan, 1993; Smith, 1996; Antoon *et al.*, 2006). Most releases are to control corn borers, sugarcane borers and cotton bollworm (Knutson, 2005). These wasps have several advantages as natural enemies, including relative ease of rearing, and fact that they kill their host in the egg stage before it causes feeding damage (Hassan, 1982; Bigler, 1984).

Although our bioassays were conducted under relatively controlled laboratory conditions, nevertheless, we are hopeful that our findings provide good picture of likely trends in the field. Although, in the field, adverse impacts of pesticide may differ from those in the laboratory due to several factors, including differential spray coverage, and differential parasitoid behavior as compared to in the laboratory (Udayagiri *et al.*, 2000). Nevertheless, laboratory tests are essential at least as a screening step toward pesticide (Klein *et al.*, 1992) to determine which type of pesticide should be incorporated in further studies under semi-field and field conditions to decide properly about the fate of the chemical in agro-ecosystem.

Furthermore, we obtained some valuable information concerning the pesticid-

es tested in the present studies against *T. chilonis* that is useful for selection of appropriate pesticides, doses, timing of the sprays and timing of release of *Trichogramma* to minimize the potential negative impacts of harmful pesticides on egg parasitoids of genus *Trichogramma* in important crops, vegetables and orchard, while at the same time managing insect pests.

The pesticides found harmless in the current studies were recommended for integration with *T. chilonis* in IPM based on: 1) beneficial conservation strategy, and 2) maintenance of smooth functioning of the parasitoid. The pesticides found slightly and moderately harmful for the studied parameters of *T. chilonis* will require further research to evaluate more precise impacts under varying field conditions in order to implement successful integration of used pesticides with *Trichogramma* to control lepidopteran pests. The uses of pesticides found harmful for this parasitoid are recommended to be stopped in the future, because of severe adverse impacts on the adult survival, emergence, and beneficial performance (parasitism) of the *T. chilonis*.

5.9) Management of Pests and Integrated Use of Compatible Pesticides with *Trichogramma*: Case Studies

There are several situations where *Trichogramma* are used to manage pests belonging to the order Lepidoptera in vegetables and crops, including grapes (Glenn and Hoffmann, 1997), tomatoes in greenhouses (Shipp and Wang, 1998), tomatoes in the field (Consoli *et al.*, 1998) and sugar cane (Greenberg *et al.*, 1998). *Trichogramma* is used effectively as a natural enemy against the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae), throughout Europe (e.g. Mertz *et al.*, 1995) and North America (Andow *et al.*, 1995).

Trichogramma are also used to control *H. armigera* on a variety of crops in India (Romeis and Shanower, 1996) and on sweet corn in Australia (Scholz *et al.*, 1998). They are used in nonfood crops such as cotton (Naranjo, 1993) and to provide foliage protection in forests (Bai *et al.*, 1995). *Trichogramma* are even also used to overcome problems related with lepidoperons in stored grain, where *T. evanescans* and *T. embryophaga* attack the stored grain pests *E. kuehniella* and *E. elutella* (Scholler *et al.*, 1996).

Solely biological control by native populations of *Trichogramma* is not sufficient to suppress the pests from reaching damaging levels in most crop production systems (Knutson, 1998). Therefore, an integration of *Trichogramma* and pesticides is required for successful control of lepidopteron pests. There are some case studies outlined below briefly related to integration of pesticides with *Trichogramma* in biological control for rice, cotton, plum orchard and sweet corn.

5.9.1) Integrating insecticides and *Trichogramma ostriniae* to control European corn borer in sweet corn: Economic analysis

Gardner *et al.*, (2011) conducted a series of field (plot) experiments in 2003 and 2006–2008 to compare the relative economic impacts of *T. ostriniae* (Pang et Chen) alone and combined with insecticidal control for controlling European corn borer *Ostrinia nubilalis* (Hübner) in sweet corn. The plots were treated with the herbicides S-metolachlor, atrazine, and mesotrione prior to planting. An initial experiment in 2003 compared *T. ostriniae* alone against insecticide alone, and a second set of experiments conducted over 3 years (2006–2008) compared (1) insecticide alone; (2) no insecticide, no *T. ostriniae* (untreated check); (3) *T. ostriniae* alone: 1x; and (4) *T. ostriniae* + insecticide (integrated).

In 2007 and 2008, a fifth treatment was added consisting of three approximately weekly releases of *T. ostriniae* (*T. ostriniae*: 3x). Parasitism of *O. nubilalis* eggs was higher in plots receiving *T. ostriniae*; *O. nubilalis* eclosion was lower with *T. ostriniae*; there was no interaction of *T. ostriniae* and insecticide on parasitism, *O. nubilalis* eclosion, or total *O. nubilalis* larvae at harvest time.

Partial crop budgets were conducted for each treatment. The highest sweet corn ear damage was reported in untreated (control) in three of the 4 years. Ear damage after a single release of *T. ostriniae* was not statistically different than using insecticides alone. In two of the three years, the integration of *T. ostriniae* and insecticides was found to generate the largest increase in economic benefit. The second best result for profitability was demonstrated by only using insecticide. When comparing a single release of *T. ostriniae* to integration with the insecticide, the latter provided a better combination of efficacy and profitability.

5.9.2) Integration of Some Biopesticides and *Trichogramma chilonis* for the Sustainable Management of Rice Leaf Folder *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae)

Muhammad Sagheer *et al.* (2008) conducted experiments on the experimental farms of the nursery of super basmati (rice) of University of Agriculture, Faisalabad (Pakistan) during 2006-2007. *T. chilonis* was reared on the eggs of *S. cerealella* in laboratory for augmentative release. The parasitized egg-pasted cards of *T. chilonis* were applied in the farms under different treatments (75000, 100000, 125000, 150000 eggs) to check their efficacy against rice leaf folder *Cnaphalocrocis medinalis* (Guenee) based on data collected on the basis of damaged/folded leaves.

The release was made either with no pesticide application or coincident with the release, the field was sprayed with biopesticides neem oil (botanical insecticide) and microbial insecticide *B. thuringiensis* (Bt). The experiments were carried out to check the wasp's efficacy against *C. medinalis*, i.e., evaluate the effective dose of *T. chilonis* for a successful management of the rice leaf folder, with no pesticide application, and also to study the synergistic effect of *Trichogramma* sp. and biopesticides for the sustainable control of this pest in rice.

The results of the experiment related to biopesticides shows that neem and Bt gave good results and demonstrated minimum post treatment increase in infestation, 6.087 and 4.093%, respectively. The results of the experiment regarding the integration of the best treatments of the *Trichogramma* egg releases and bio-pesticides shows that minimum increase in post treatment infestation (1.373%) was recorded from those plots which were treated with *Trichogramma* + Bt + Neem, while the control treatment showed maximum increase in post treatment infestation of *C. medinalis* (8.053%).

Thus, the study concluded that 1) bioinsecticides, neem, and Bt affect the pest insect, and 2) these insecticides and egg parasitoid *Trichogramma* can be successfully integrated to enhance its bio-efficacy against *C. medinalis*. The use of these biopesticides may play a more prominent role in integrated pest control programmes in future.

5.9.3) Cotton insect pest control on a small farm: an approach of successful biological control using *Trichogramma*

Almeida R.P. de (2001) reported using a combination of inundative release of *T. pretiosum*, cultural and chemical controls in order to keep the pest densities includ-

ing cotton leafworm *Alabama argillacea* and other insect pests in cotton below the economic threshold. In inundative releases, 100,000 reared pupae of *T. pretiosum* in laboratory were released weekly per ha. Fifteen release devices of *Trichogramma* were installed. The egg parasitism of *Alabama argillacea* (cotton leafworm) by *T. pretiosum* was assessed by counting the number of parasitized and unparasitized eggs on all leaves of twenty plants on weekly basis. In total twelve samples were done. The first cotton leafworm eggs and cotton aphid colonies were observed at the 14th day of planting.

The insecticides diflubenzuron 25 WP (12.5 g a.i./ha), monocrotophos 400 EC (120.0 g a.i./ha) and endosulfan EC 350 (525 g a.i./ha) and betacyfluthrin 125 CS (7.5 g a.i./ha) were sprayed, respectively, for the cotton leafworm, the cotton aphid and the cotton boll weevil, until the economic threshold established for each insect pest was reached. These insecticides were applied using a knapsack sprayer.

The first release of *T. pretiosum* resulted in high level of mean parasitism of cotton leafworm: 82%. The insecticide diflubenzuron was used at the 21st day after planting to avoid the increase of the cotton leafworm population (larvae < 15 mm). These two measures led to the reduction of the cotton leafworm larvae to 0% level. The negative effects on the natural enemies were precluded by adopting strategy to use half dose of the insecticide monocrotophos.

The efficient control of the cotton leafworm (integrated use of pesticides with *Trichogramma*) made it possible to reduce the number of chemical applications by 55-70% (from 10-15 applications to 4.5 applications) compared to using solely chemical control. This led to an increase in other natural enemy populations (Coccinellidae, Chrysopidae and Syrphidae). Garcia-Roa (1991) stated that the biological control due

to *Trichogramma* resulted in valuable economic and ecological advantages to the cotton crop: the reduction of pesticides use by at least 50% led to the biological balance re-established in the agro-ecosystem.

SUMMARY

The pesticides tested in the current studies were insecticides, miticides, herbicides, and fungicides. Fifteen pesticides were tested against egg stage, while, 14 pesticides were tested against both larvae and pupae of *T. chilonis* to evaluate their effects on emergence of parasitoids from the treated host eggs of *S. cerealella*. Twelve pesticides were evaluated for their effects on parasitism of *S. cerealella* eggs by female *T. chilonis* emerged from host eggs treated either in egg or larval stage; however, 13 pesticides were tested against parasitoid pupae to evaluate their effects on parasitism by the emerged females. Fifteen pesticides were evaluated for their effects on parasitism by the females exposed to previously treated host eggs of *S. cerealella*. Eleven chemicals were assessed to determine their residual effects 1, 5, 10 and 15 days after treatments on survival of adult *T. chilonis* by exposure of adults for 24 h to the glass surface treated with pesticides. While 15 pesticides were evaluated for their residual effects on parasitism by the females exposed to the treated surface for 24 h in the aforementioned 4 residual treatments.

The pesticides were tested at field (x), 2x and 0.5x doses by dipping methods to determine their effects on the emergence, and parasitism by the *T. chilonis* emerged from the treated eggs, and on the parasitism of the previously treated host eggs. Residual effects on both adult survival and parasitism by the females exposed to the dried residues were also evaluated only at field dose. Although, the pesticides used in the current research work in laboratory demonstrated variability not only regarding dose and life stage but also showed variable effects on different parameters, i.e. emergence, parasitism, and residual toxicity. Nevertheless, most of the products (more than half) studied, were evaluated as harmless to *T. chilonis* regarding aforementioned paramet-

ers. The results are summarized below.

6.1) Emergence of *T. chilonis* Relative to Control from Treated Host Eggs

6.1.1) Effect of pesticides on emergence when *T. chilonis* treated in egg stage

Fipronil was the most toxic chemical for emergence (rated harmful), while abamectin was moderately harmful for emergence of *T. chilonis* at all the three doses regarding egg treatment (Table 19). Spinetoram was slightly harmful at 0.5x dose, but was moderately harmful for emergence at both field (x) and 2x doses. Acetamiprid was slightly harmful for emergence at both 0.5x and x doses, but moderately harmful at 2x dose. Spiromesifen was categorized as slightly harmful for emergence only at 2x treatment. The remaining pesticides were harmless for emergence of parasitic wasps from host eggs treated at each of three doses.

6.1.2) Effect of pesticides on emergence when *T. chilonis* treated in larval stage

Abamectin and spinetoram were both moderately harmful for emergence of *T. chilonis* based on x dose (Table 19), while acetamiprid was slightly harmful at x and 2x doses, but was harmless for emergence at 0.5x dose. Fipronil demonstrated slightly harmful, moderately harmful and harmful at 0.5x, x and 2x doses, respectively. The remaining pesticides were harmless for emergence of wasps at all used doses.

6.1.3) Effect of pesticides on emergence when *T. chilonis* treated in pupal stage

Spinetoram (the most toxic) and abamectin were ranked as moderately and slightly harmful (Table 19) for emergence of *T. chilonis*, respectively, at all used doses. Acetamiprid demonstrated reduced toxicity for emergence as moderately harmful, slightly harmful and harmless for emergence at 2x, x and 0.5x doses, respectively.

Fipronil was harmless at both 0.5x and x doses, but revealed as slightly harmful for emergence at 2x treatment. The remaining chemicals were ranked as harmless for emergence of parasitoids from host eggs treated at all three doses.

6.1.4) Stage-wise effects of pesticides on emergence from treated host eggs

Fipronil demonstrated as harmful for emergence of *T. chilonis* at the three doses in egg treatment (Table 19), where as toxicity declined in the larval (from harmful to slightly harmful) and pupal (slightly harmful to harmless) treatments at 2x through 0.5x doses. Abamectin was moderately harmful for emergence in both egg and larval treatments but was slightly harmful for emergence in the pupal treatment regarding all used doses.

Spinetoram showed reduced toxicity as harmful to slightly harmful at 2x through 0.5x dose regarding egg treatment, but showed as moderately harmful for emergence at the three doses treated against larvae and pupae. Acetamiprid revealed reduced toxicity through doses as moderately and slightly harmful for emergence at 2x and 0.5x doses, respectively, when wasps were treated at both egg and pupal stages. While showed as slightly harmful for emergence at the said doses in the larval treatment. The remaining pesticides were found harmless for emergence by the wasps when treated in the three immature stages except spiromesifen, which was slightly harmful for emergence, when 2x dose of the chemical was used against egg stage of parasitoids.

6.2) Parasitism of *S. cerealella* Eggs by *T. chilonis* Emerged from Treated Host Eggs

6.2.1) Effect of pesticides on parasitism when treated in egg stage

Acetamiprid was rated (Table 19) as harmful and moderately harmful for para-

sitism by female *T. chilonis* emerged from the host eggs (*S. cerealella*) treated at both x and 0.5x doses in the egg stage, respectively. While nicosulfuron and chlorothalonil + procymidone showed increase in toxicity for parasitism from harmless at x dose to slightly harmful at 2x dose. The remaining pesticides were harmless for parasitism by *T. chilonis* at all used doses.

6.2.2) Effect of pesticides on parasitism when treated in larval stage

Myclobutanil was deemed (Table 19) slightly harmful for parasitism at 2x, but harmless at the x dose. While acetamiprid showed slightly harmful for parasitism at both 2x and x doses, but harmless at 0.5x dose. Fipronil was slightly harmful for parasitism at both x and 0.5x doses. The remaining chemicals were not affected by the type of dose used and were harmless for parasitism at all used doses.

6.2.3) Effect of pesticides on parasitism when treated in pupal stage

Disruption of parasitism due to abamectin (Table 19) decreased from harmful at 2x to moderately harmful at both of x and 0.5x doses. Fipronil and acetamiprid demonstrated same level of toxicity as moderately harmful at 2x, but slightly harmful at both x and 0.5x doses. The remaining pesticides were harmless for parasitism by wasps at three doses used against the pupae of parasitoids.

6.2.4) Stage-wise effects of pesticides on parasitism regarding immature stages

Female *T. chilonis* emerged from the host eggs treated in egg, larval and pupal stages demonstrated (Table 19) that acetamiprid was rated as harmful and moderately harmful for parasitism at x and 0.5 x doses, respectively, in the egg treatment; however, showed reduced toxicity as slightly harmful at x dose, while harmless for parasitism at 0.5x dose in larval treatment, but showed slightly harmful for parasitism at

both x and 0.5x doses, when parasitoids were treated at pupal stage in the host egg. Nicosulfuron and chlorothalonil + procymidone were harmless at x dose, while slightly harmful for parasitism at 2x dose regarding egg treatment; however, they demonstrated reduced toxicity as harmless for parasitism at all the doses used against larvae and pupae.

Myclobutanil demonstrated as harmless for parasitism at all doses used against all three immature stages except as slightly harmful for parasitism at 2x dose in the larval treatment. Fipronil was categorized as slightly harmful for parasitism at both x and 0.5x doses used against larvae. However, fipronil and abamectin demonstrated reduced toxicity from moderately harmful and harmful for parasitism, respectively, at 2x dose to slightly harmful and moderately harmful for parasitism, respectively, at both x and 0.5x doses, in the treatment of pupae.

Data regarding effects of abamectin on disruption of parasitism at all treated doses, while fipronil only at 2x dose, by the females emerged from host eggs treated in the egg and larval treatments are not available because no adult wasps emerged from the egg and larvae treated with these two chemicals at their said doses. The remaining pesticides were found harmless for parasitism by *T. chilonis* at the three doses used against all immature stages of *T. chilonis*.

6.3) Parasitism of Previously Treated Host Eggs by *T. chilonis* (No-Choice test)

6.3.1) Effect of pesticides on parasitism of previously treated host eggs

Pesticide assessment in a no-choice test for their effects on parasitism (Table 21) of the previously treated host eggs (*S. cerealella*) by *T. chilonis* revealed that spir-otetramat, trifloxystrobin + tebuconazole and myclobutanil observed reduced toxi-

icity for parasitism by female parasitoids, being rated as slightly harmful at 2x dose but harmless at x dose. Acetamiprid and haloxyfop-p-methyl experienced reduction in toxicity for parasitism, i.e., both deemed slightly harmful at both 2x and x doses but were harmless for parasitism at 0.5x dose. Abamectin, spinetoram and fipronil were moderately harmful for parasitism at 2x dose but toxicity reduced to slightly harmful at both x and 0.5x doses. While spiromesifen rated as moderately harmful at both 2x and x doses, and slightly harmful for parasitism at 0.5x dose. The remaining chemicals were all harmless for parasitism of previously treated host eggs by *T. chilonis* regarding all three used doses.

6.7) Mortality of Adult *T. chilonis* Based on Residual Toxicity

6.4.1) Residual effects of different-aged pesticide residues on adult survival

Evaluation of mortality of adult *T. chilonis* in response to exposure of adults for 24 h to a glass surface treated with different pesticides (Table 20) at their respective field doses (Table 1) demonstrated that fipronil, followed by spinetoram, acetamiprid and abamectin were highly toxic for adult survival of *T. chilonis* in all the four residual treatments (1-, 5-, 10- and 15-day-old residues). While HaNPV and chlorantraniliprole demonstrated low toxicity for adult parasitoid survival in all the four residual treatments.

The exposure of adult wasps to surfaces treated with spiromesifen, haloxyfop-p-methyl and pyraclostrobin + metiram after 24 h of treatment (1-day-old residue) showed these chemicals as moderately toxic for survival of the adults; however the same chemicals showed low toxicity for adult survival of *T. chilonis*, when adults were exposed in the remaining residual treatments. Bispyribac sodium was moderately tox-

ic, when adults were exposed to both 1-day and 5-days-old residues, while toxicity declined, i.e., low toxic for adult survival of wasps, when adults were exposed to both 10- and 15-day-old residues. Trifloxystrobin + tebuconazole revealed moderate toxicity for adult survival of *T. chilonis*, when adults were exposed to both 1- and 15-day-old residues, but were low toxic, when exposed to both 5- and 10-days-old residues.

6.8) Parasitism of Host Eggs by *T. chilonis* Based on Residual Toxicity

6.5.1) Effect of pesticides on parasitism regarding exposure to residues

The female *T. chilonis* exposed in the four residual treatments (1-, 5-, 10- and 15-day-old residues) demonstrated (Table 20) that the most toxic chemicals were acetamiprid, spinetoram and abamectin; all ranked as moderately harmful for parasitism in all residual treatments. Fipronil was rated moderately harmful for parasitism by the females exposed to 1-day-old residue but was slightly harmful for parasitism, when females were exposed in the remaining treatments. Pyraclostrobin + metiram were harmless for parasitism by females exposed to 15-day-old residues, but were slightly harmful for parasitism, when females were exposed to 1-, 5-, and 10-day-old residues.

Spirotetramat, bispyribac sodium and nicosulfuron were slightly harmful for parasitism, when the females were exposed to 1-day-old residue, but were harmless for parasitism, when the females were exposed to the remaining residual treatments. The remaining chemicals were harmless for parasitism by female *T. chilonis* exposed to the residues of pesticides of all four ages.

6.9) Effects of Pesticide on Parasitism of Host Eggs by *T. chilonis* at Field Dose Through Different Ways of Exposure

The three ways of parasitism of *S. cerealella* eggs by *T. chilonis*: 1) by female-

s emerged from treated host eggs, 2) by females exposed to previously treated host eggs, and 3) of fresh untreated host eggs by females exposed to dried residues, at field dose, demonstrated (Table 21) that acetamiprid and spinetoram showed reduced toxicity for parasitism (disruption of parasitism) from moderately harmful based on residual treatments, to slightly harmful for parasitism of previously treated host eggs. Furthermore, acetamiprid also showed decrease in toxicity as slightly harmful for parasitism regarding both larval and pupal treatments.

Fipronil showed as slightly harmful for parasitism based on both larval and pupal treatments and residual exposure of adults. Abamecin showed decrease in toxicity from moderately harmful based on all residual treatments and treatment of pupae, to slightly harmful for parasitism by the females exposed to the treated eggs. Spiromesifen and haloxyfop-p-methyl demonstrated as harmless for parasitism regarding all treated stages and residual treatments, but showed increase in toxicity as moderately and slightly harmful for parasitism, respectively, by females exposed to the previously treated eggs.

Pyraclostrobin was harmless for parasitism, when females were exposed either to previously treated eggs or by females emerged from the treated immature stages, but showed increase in toxicity as slightly harmful for parasitism, when females exposed to surface treated with the same chemicals in all four residual treatments. The remaining pesticides were all harmless for parasitism at field dose in all three aforementioned ways of exposure of host eggs except female exposed to 1-day-old residue of spirotetramat, bispyribac sodium and nicosulfuron.

Table 19. Ranking of toxicity of pesticides for the emergence of *T. chilonis*, as well as parasitism of *S. cerealella* eggs by female emerged from host eggs (*S. cerealella*) treated at the three doses regarding treatment of all immature stages (IOBC/WPRS toxicity ranking)

S. no	Pesticides	Emergence									Parasitism								
		Egg stage			Larval stage			Pupal stage			Egg stage			Larval stage			Pupal stage		
		2x	x	0.5x	2x	x	0.5x	2x	x	0.5x	2x	x	0.5x	2x	x	0.5x	2x	x	0.5x
1	HaNPV	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	Spirotetramat	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	Chlorantraniliprole	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4	Acetamiprid	3	2	2	2	2	1	3	2	1	4	4	3	2	2	1	3	2	2
5	Spinetoram	4	3	2	3	3	3	3	3	3	-	-	-	-	-	-	-	-	-
6	Fipronil	4	4	4	4	3	2	2	1	1	-	-	-	-	2	2	3	2	2
7	Abamectin	3	3	3	3	3	3	2	2	2	-	-	-	-	-	-	4	3	3
8	Spiromesifen	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	Haloxyp-methyl	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	Bispyribac sodium	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	Nicosulfuron	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1
12	Myclobutanil	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1
13	Chlorotha + Procy	1	1	1	-	-	-	-	-	-	2	1	1	-	-	-	-	-	-
14	Pyraclo + Metiram	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	Trifloxy + Tebuco	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Ranking: class 1= harmless ($E < 30\%$); 2 = slightly harmful ($30 \leq E \leq 79\%$); 3 = moderately harmful ($79 < E \leq 99\%$); 4 = harmful ($> 99\%$)

Table 20. Ranking of toxicity of pesticides for the adult survival and parasitism of *S. cerealella* eggs by *T. chilonis* exposed to 1-, 5-, 10-, and 15- day-old residues of pesticides on glass surface (toxicity rankings: Brunner *et al.* (2001), and IOBC/WPRS)

S.no	Pesticides	Adult mortality				Parasitism by survived females			
		1 days	5 days	10 days	15 days	1 day	5 days	10 days	15 day
1	HaNPV	1	1	1	1	1	1	1	1
2	Spirotetramat	-	-	-	-	2	1	1	1
3	Chlorantraniliprole	1	1	1	1	1	1	1	1
4	Acetamiprid	3	3	3	3	3	3	3	3
5	Spinetoram	3	3	3	3	3	3	3	3
6	Fipronil	3	3	3	3	3	2	2	2
7	Abamectin	3	3	3	3	3	3	3	3
8	Spiromesifen	2	1	1	1	1	1	1	1
9	Haloxypop-p-methyl	2	2	1	1	1	1	1	1
10	Bispyribac sodium	2	2	1	1	2	1	1	1
11	Nicosulfuron	-	-	-	-	2	1	1	1
12	Myclobutanil	-	-	-	-	1	1	1	1
13	Chlorothalonil + Procymidone	-	-	-	-	1	1	1	1
14	Pyraclostrobin + Metiram	2	2	1	1	2	2	2	1
15	Trifloxystrobin + Tebuconazole	2	1	1	2	1	1	1	1

Brunner *et al.* (2001) adults (Corrected mortality) toxicity ranking: 1 for low toxic ($E < 20\%$); 2 for moderate toxic ($20 < E < 70\%$); 3 for highly toxic ($E > 70\%$), where E in this case stand for adult mortality. **IOBC/WPRS Ranking (Parasitism effects):** class 1= harmless ($E < 30\%$); 2 = slightly harmful ($30 \leq E \leq 79\%$); 3 = moderately harmful ($79 < E \leq 99\%$); 4 = harmful ($> 99\%$).

Table 21. Ranking of toxicity of pesticides for parasitism of host eggs of *S. cerealella* by *T. chilonis* of the three different ways based on all used doses, treated stages, and residual treatments (IOBC/WPRS toxicity ranking)

S. no	Pesticides	Parasitism of untreated host eggs based on emergence									Parasitism of prev. treated host eggs (no-choice test)			Parasitism of untreated host eggs at field dose based on residual toxicity			
		Egg			Larval			Pupal			2x	x	0.5x	1-day	5-days	10-days	15-days
		2x	x	0.5x	2x	x	0.5x	2x	x	0.5x							
1	HaNPV	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	Spirotetramat	1	1	1	1	1	1	1	1	1	2	1	1	2	1	1	1
3	Chlorantranilipr	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4	Acetamiprid	4	4	3	2	2	1	3	2	2	2	2	1	3	3	3	3
5	Spinetoram	-	-	-	-	-	-	-	-	-	3	2	2	3	3	3	3
6	Fipronil	-	-	-	-	2	2	3	2	2	3	2	2	3	2	2	2
7	Abamectin	-	-	-	-	-	-	4	3	3	3	2	2	3	3	3	3
8	Spiromesifen	1	1	1	1	1	1	1	1	1	3	3	2	1	1	1	1
9	Haloxyp-meth	1	1	1	1	1	1	1	1	1	2	2	1	1	1	1	1
10	Bispyribac sodi	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1
11	Nicosulfuron	2	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1
12	Myclobutanil	1	1	1	2	1	1	1	1	1	2	1	1	1	1	1	1
13	Chloro + Proc	2	1	1	-	-	-	-	-	-	1	1	1	1	1	1	1
14	Pyraclo + Metir	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	1
15	Triflox + Tebu	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1

IOBC/WPRS Ranking: class 1= harmless ($E < 30\%$); 2 = slightly harmful ($30 \leq E \leq 79\%$); 3 = moderately harmful ($79 < E \leq 99\%$); 4 = harmful ($> 99\%$).

CONCLUSION

Most of the pesticides tested/evaluated were harmless for *T. chilonis* regarding emergence, parasitism and residual toxicity. Abamectin, spinetoram, fipronil and acetamiprid were considered the most harmful products, nevertheless, they also demonstrated variable effects on the said life parameters. The current results are helpful for integration of chemical control with *T. chilonis* for successful management of lepidopteran pests.

7.4) Emergence of and Parasitism by *T. chilonis*

7.1.1) Emergence of adults from treated host eggs

The pesticide effects on emergence of *T. chilonis* from the treated host eggs (*S. cerealella*) demonstrated that fipronil was harmful for emergence at all treated doses in the treatment of egg stage, but was moderately harmful and harmless for emergence at field doses (x) when the parasitoids were treated in larval and pupal stages, respectively. Abamectin was moderately harmful for emergence of *T. chilonis* at the three doses when egg and larvae of parasitoids were treated, but only slightly harmful for emergence of parasitoids when the wasps were treated at all doses in the pupal stage. Spinetoram and acetamiprid were moderately and slightly harmful for emergence by tiny parasitoids at x dose, respectively, treated against all immature stages.

The remaining pesticides- HaNPV, spirotetramat, chlorantraniliprole, haloxyfop-p-methyl, bispyribac sodium, nicosulfuron, myclobutanil, chlorothalonil + procyimidone (only for egg treatment), spiromesifen (except 2x dose in the egg treatment) pyraclostrobin + metiram and trifloxystrobin + tebuconazole -were evaluated as harmless for emergence of *T. chilonis* regarding all three doses treated against the three

immature stages. Nevertheless, the result on emergence for chlorothalonil + procymidone was only obtained for egg stage, and spiromesifen was slightly harmful for emergence at 2x dose in the egg treatment.

7.1.2) Parasitism by females emerged from treated host eggs

Pesticide effects on parasitism of the untreated fresh host eggs of *S. cerealella* by the female *T. chilonis* emerged from the treated host eggs (*S. cerealella*) showed that acetamiprid was harmful for parasitism by females at x dose when the females emerged were treated at egg stage, but demonstrated low toxicity as slightly harmful for parasitism at the same dose in the treatments of larvae and pupae. Abamectin was moderately harmful for parasitism at x and 0.5x doses in the pupal treatment. Fipronil and acetamiprid were slightly harmful for parasitism both of at x and 0.5x doses by females emerged from host eggs treated when parasitoids were at larval and pupal stage.

The pesticides tested and found harmless for parasitism at all the three treated doses against all the three life stages were HaNPV, spirotetramat, spiromesifen, chlorantraniliprole, haloxyfop-p-methyl, bispyribac sodium, pyraclostrobin + metiram, myclobutanil (except 2x dose in larval treatment), nicosulfuron (except 2x dose in egg treatment) and chlorothalonil + procymidone (except 2x dose in egg treatment) and trifloxystrobin + tebuconazole.

7.5) Parasitism of Previously Treated Host Eggs by Females

The results on parasitism of previously treated fresh host eggs of *S. cerealella* by female *T. chilonis* based on no-choice test showed that spiromesifen was moderately, while spinetoram, fipronil, acetamiprid, abamectin and haloxyfop-p-methyl were

slightly harmful for parasitism at their respective field (x) doses.

The remaining pesticides were all evaluated as harmless for parasitism of previously treated host eggs when treated at field doses: HaNPV, spirotetramat, chlorantraniliprole, nicosulfuron, bispyribac sodium, myclobutanil, chlorothalonil + procymidone, trifloxystrobin + tebuconazole and pyraclostrobin + metiram.

7.6) Adult Mortality and Parasitism Based on Residual Toxicity

7.3.3) Adult mortality

The corrected percent adult mortality (mean) of *T. chilonis* corrected for control, based on 24-hour exposure of randomly selected adults to treated surfaces in the glass vial at their respective field-recommended doses in the different residual treatments (1-, 5-, 10-, and 15-days old) were evaluated, which demonstrated that fipronil, spinetoram, acetamiprid and abamectin were highly toxic for adult survival of *T. chilonis* regarding all four treatments.

Spiromesifen, haloxyfop-p-methyl and pyraclostrobin + metiram were rated as moderately toxic when the adults were exposed to 1-day-old residue; however, they demonstrated reduced toxicity as low toxic to adult survival of *T. chilonis* regarding remaining residual treatments. Bispyribac sodium was rated as moderately toxic for adult survival when adult parasitoids were exposed to 1-and 5-day-old residues, but exhibited low toxicity for survival of adult wasps exposed to 10- and 15-day-old residues. Exposure of adults to 1-and 15-day-old residues of trifloxystrobin + tebuconazole demonstrated the chemical mixture as moderately toxic for adult survival of wasps, but was ranked as low toxic when exposed to both of 5-and 10-day-old residues. While HaNPV and chlorantraniliprole demonstrated as low toxic for adult survival of

T. chilonis when the adults were exposed to all four aged residues.

7.3.4) Parasitism based on residual toxicity

Pesticides were evaluated at field doses for their effects on parasitism of fresh untreated host eggs of *S. cerealella* by female *T. chilonis* exposed for 24 h to the treated glass surface, which demonstrated that the most toxic chemicals for parasitism were acetamiprid, spinetoram and abamectin: moderately harmful for parasitism in all four residual treatments (1-, 5-, 10- and 15-day- old residues). Fipronil was moderately harmful when females were exposed to 1- day- old residue, but was slightly harmful for parasitism in the remaining residual treatments.

Pyraclostrobin + metiram were harmless for parasitism by females when exposed to 15-day-old residue, while slightly harmful for parasitism at the remaining three residual treatments. The exposure of adult female parasitoids to the surface treated 1 day previously with spirotetramat, bispyribac sodium and nicosulfuron demonstrated that these chemicals were slightly harmful for parasitism; however, the same chemicals were ranked as harmless for parasitism by *T. chilonis* in the remaining residual treatments.

The remaining pesticides- HaNPV, chlorantraniliprole, spiromesifen, haloxyfop-p-methyl, myclobutanil, chlorothalonil + procymidone and trifloxystrobin + tebucanazole- were harmless for parasitism by *T. chilonis* exposed in all the four treatment.

RECOMMENDATIONS AND SUGGESTIONS

The pesticides tested and found harmless for emergence and parasitism or exhibiting low toxicity to adult survival could be integrated with *T. chilonis* for effective control of lepidopteran pests. The recommendations/suggestions based on the result outputs of evaluation of emergence, parasitism, and residual toxicity of wasps in response to treatment with pesticides are described below.

8.1) Emergence of *T. chilonis* from Treated Host Eggs

HaNPV, spirotetramat, chlorantraniliprole, haloxyfop-p-methyl, bispyribac sodium, nicosulfuron, myclobutanil, pyraclostrobin + metiram, trifloxystrobin + tebuconazole, chlorothalonil + procymidone and spiromesifen (except 2x dose) were tested and found harmless for *T. chilonis* at all three doses regarding all stages treated in addition to fipronil which is also recommended only at x dose in the treatment of pupae, and are recommended for integration with *T. chilonis* in the agro-ecosystem.

Nevertheless, both spiromesifen and fipronil (both slightly harmful for emergence) need further evaluation at 2x dose under field conditions in the egg and pupal treatment, respectively. Acetamiprid as slightly harmful for emergence requires further field studies at x dose against pupae, and also at both x and 2x doses against larval stage of parasitoids. Abamectin (slightly harmful) requires further evaluation at all three doses treated against the pupae of *T. chilonis* under field conditions.

8.2) Parasitism by the Female *T. chilonis* Emerged from the Treated Host Eggs

The field-dose based pesticide effects on parasitism of the untreated fresh host eggs of *S. cerealella* by female *T. chilonis* emerged from host eggs treated when parasitoids were in egg, larval and pupal stages showed, that HaNPV, spirotetramat, chl-

orantraniliprole, spiromesifen, haloxyfop-p-methyl, bispyribac sodium, pyraclostrobin + metiram, trifloxystrobin + tebuconazole, nicosulfuron, myclobutanil and chlorothalonil + procymidone were all harmless for parasitism by female *T. chilonis*, and are recommended for integration with *T. chilonis* regarding all immature stages.

Fipronil and acetamiprid were slightly harmful for parasitism by the females emerged from treated both larval and pupal stage at field dose, and therefore need further studies under field conditions regarding these two stages. 2x dose demonstrated that nicosulfuron and chlorothalonil + procymidone were slightly harmful for parasitism by the females emerged from host eggs treated when parasitoids were in the egg stage, and acetamiprid and myclobutanil were slightly harmful for parasitism regarding treatment of larval stage, and they need further studies under field conditions for their respective stages. The remaining chemicals found harmless at x dose were also harmless at this stage and are recommended to integrate with *T. chilonis* at 2x dose when the field doses of such chemicals are not working properly to control the target pests in agro-ecosystem, provided the pesticide label rates are not exceeded.

8.3) Parasitism by *T. chilonis* of Previously Treated Host Eggs

Effects of pesticides at field dose (x) on parasitism of treated fresh host eggs of *S. cerealella* by *T. chilonis* based on the no-choice test showed that acetamiprid, spinetoram, fipronil, abamectin and haloxyfop-p-methyl were slightly harmful for parasitism and will require further studies under field conditions to properly decide about their integration with parasitoid in the agro-ecosystem. While remaining pesticides as harmless for parasitism are recommended for integration with the *T. chilonis*.

Pesticide effects on parasitism of fresh host eggs treated at 2x dose demonstra-

ted that five chemicals (spirotetramat, trifloxystrobin + tebuconazole, myclobutanil, acetamiprid and haloxyfop-p-methyl) were slightly harmful for parasitism by *T. chilonis*, and these chemicals require further studies under field conditions to properly decide if they should be integrated with *Trichogramma* in the agro-ecosystem, provided when the field doses of such chemicals are not properly working to control target pests. However, HaNPV, chlorantraniliprole, bispyribac sodium, pyraclostrobin + metiram, nicosulfuron and chlorothalonil + procymidone were all harmless for parasitism of treated host eggs, and can be integrated with wasps even at 2x dose. Furthermore, one should be careful that the 2x dose should be within the range of allowable rate on the pesticide label, which may not be much above the recommended field rate

8.4) Mortality of Adult *T. chilonis* Exposed to Treated Surface

a) 1-day-old residue

The corrected percent adult mortality (mean) for control of *T. chilonis* based on 24-h exposure at their respective field doses demonstrated that only two chemicals, chlorantraniliprole and HaNPV, were rated as low toxic for survival of *T. chilonis* adults exposed to 1-day-old residues, and these can be integrated with release of *T. chilonis* simultaneously in the agro-ecosystem. The moderately toxic chemicals for adult survival were spiromesifen, haloxyfop-p-methyl, bispyribac sodium, pyraclostrobin + metiram and trifloxystrobin + tebuconazole, which candidates for further testing under field conditions to determine their actual toxicity under natural conditions.

b) 5-day, 10-day and 15-day-old residues

The corrected (%) adult mortality (mean) for *T. chilonis* based on exposure to each 5-day, 10-day and 15-day-old residues for 24 h demonstrated that chlorantraniliprole, HaNPV, haloxyfop-p-methyl, pyraclostrobin + metiram and spiromesifen were

comparably benign and were ranked as low toxic to adults (acute mortality) in all three residual treatments. Similarly, trifloxystrobin + tebuconazole was mistakenly found moderately harmful for adult survival in the 15 days treatment due to might be some error during experimental set up, but the same chemical otherwise demonstrated as low toxic in the remaining treatments. All the said chemicals were recommended for integration with adult parasitoids provided the release of adults should be carried out atleast 5 days after treatments except chlorantraniliprole and HaNPV to avoid the harmful impacts of pesticides on the beneficial wasps. However, bispyribac sodium was moderately harmful in the 5-day-old treatment and need further testing under field conditions, while the same chemical was harmless in the remaining two treatments.

8.5) Parasitism by the Female *T. chilonis* Exposed to Treated Surface

a) 1-day-old residue

Pesticides were evaluated for their effects on parasitism of fresh untreated host eggs of *S. cerealella* by the females of *T. chilonis* exposed for 24 h to the treated surface of 1-day-old residue demonstrated that pyraclostrobin + metiram, spirotetramat, bispyribac sodium and nicosulfuron were slightly harmful for parasitism by *T. chilonis*, and will require further field studies to decide properly whether or not to integrate them with *T. chilonis*. While HaNPV, chlorantraniliprole, spiromesifen, haloxyfop-p-methyl, myclobutanil, chlorothalonil + procymidone and trifloxystrobin + tebuconazole were harmless for parasitism by female *T. chilonis*, and are recommended for integration with the release of *T. chilonis* simultaneously, or after release to successfully control caterpillar pests in agro-ecosystems.

b) 5-day-, 10-day- and 15-day-old residue

The pesticide effects on parasitism by the exposed females to 5-day-, 10-day-

and 15-day-old residues demonstrated that fipronil was slightly harmful for parasitism in all three residual treatments. While pyraclostrobin + metiram demonstrated reduced toxicity as harmless in 15 days treatment comparatively to as slightly harmful for parasitism at the remaining residual treatments. The slightly harmful products will require further field studies to decide properly to integrate with *T. chilonis* for optimal results. The chemicals found harmless for parasitism by the exposed females in all the three treatments were HaNPV, spirotetramat, chlorantraniliprole, spiromesifen, haloxypop-p-methyl, bispyribac sodium, nicosulfuron, myclobutanil, chlorothalonil + procymidone and trifloxystrobin + tebuconazole. All these chemicals are advised for integration with the *T. chilonis*.

However, in order to more effectively integrate both chemical and biological control strategies in agro-ecosystem, where *Trichogramma* plays a key role to manage lepidopteran pests, requires further sublethal studies on *T. chilonis* evaluating pesticides' effects on sex ratio, longevity, foraging behaviors of the adult wasps, and field studies should also be carried out to determine pesticides' effects on the different biological parameters of this important parasitoids under natural conditions.

Furthermore, laboratory/field studies are required to assess the effects of the recommended pesticides (harmless for *T. chilonis*) on other natural enemies including parasitoids, predators, and pathogens, in order to get fruitful results of more highly effective and comprehensive integration of pesticides with natural enemies in the particular agro-ecosystem under IPM program.

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