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Side effects of insecticides used for management of *Tuta absoluta* Meyrick (Lepidoptera: Gelechidae) on the biocontrol agent *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae)

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Abstract

Trichogramma brassicae is one of the most important egg parasitoids of many pests including South American tomato pinworm, *Tuta absoluta* (Lepidoptera: Gelechiidae). In agrosystems, the function of parasitoids can be affected by the application of insecticides. To use biological control agents and pesticides simultaneously in pest management, it is necessary to know the potential effect of pesticides on biological control agents. In this study, the lethal and sublethal effects of four insecticides (flubendiamide, emamectin benzoate + lufenuron, thiocyclam, and spinosad) frequently used to control *T. absoluta*, were investigated on its parasitoid *Trichogramma Brassicae* Bezdenko (Hymenoptera: Trichogrammatidae), under controlled laboratory conditions. The results showed that spinosad with an LC_{50} value of 0.73 mg a.i./ L was highly toxic against adults of *T. brassicae* in comparison to flubendiamide (4.09 mg a.i./ L), emamectin benzoate + lufenuron (4.17 mg a.i./ L), and thiocyclam (9.59 mg a.i./ L). Furthermore, key demographic parameters such as net reproductive rate (R_0), intrinsic rate of increase (r_m), mean generation time (T), and doubling time (DT) were significantly decreased after exposure of *T. brassicae* to the LC_{30}s of spinosad, thiocyclam, flubendiamide, and emamectin benzoate + lufenuron compared to the control. In the light of our results, the four insecticides should be used cautiously in *T. absoluta* integrated management programs. While the thiocyclam can be combined with the *T. brassicae,* the flubendiamide and emamectin benzoate + lufenuron are not recommended in the presence of this parasitoid. The use of spinosad should be avoided concomitantly with the release of *T. brassicae*.

Keywords Biological control, Egg parasitoid, Chemical insecticides, Toxicology, South American tomato pinworm

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Background

The South American tomato pinworm, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), which is native to South America, emerged as a major invasive insect pest of tomato plants worldwide (Desneux et al. 2011; Biondi et al. 2018). *T. absoluta* can cause up to 100% crop loss if not effectively controlled (Desneux et al. 2010; Mansour et al. 2018).

Initially, insecticides were used to control the *T. absoluta* larvae, but this pest has developed resistance to commonly used insecticide-active substances (Haddi et al. 2012, 2017; Roditakis et al. 2015; Guedes et al. 2019). Therefore, new biological and chemical control options such as reduction in pesticide use via incorporation of less toxic active ingredients and sustainable pest management strategies including biological control and sex pheromone-based biotechnical control have been adopted to limit the severe crop damages caused by *T. absoluta* larvae (Desneux et al. 2022; Colmenárez et al. 2022).

Chemical and biological control are two effective and common methods used against invasive pest species (Ragsdale et al. 2011; Paini et al. 2016; Soares et al. 2019b; Zang et al. 2021; Han et al. 2022). Insecticides are generally applied at a concentration that kills the target insect pests (Wang et al. 2019; Ullah et al. 2019; Khan et al. 2021; Hafeez et al. 2022). However, abiotic and biotic factors may produce spatiotemporal changes in concentrations, affecting the intended doses/concentrations (Desneux et al. 2005). These changing concentrations/doses (sublethal/low lethal) cause potential sublethal effects on the life-history traits of exposed arthropods (Guedes et al. 2016; Xu et al. 2022; Gul et al. 2021; Shi et al. 2022; Guo et al. 2023). These sublethal effects are generally negative (Desneux et al. 2007) although positive effects may occur e.g. through hormesis (Guedes et al. 2016; Wang et al. 2022). Besides, the extensive use of chemical insecticides potentially induces multiple side effects on non-target insects including pest insect's natural enemies, on environment and human health (Desneux et al. 2007; Biondi et al. 2012a; Lu et al. 2012; Guedes et al. 2016; Soares et al. 2019a; Akhtar et al. 2021).

Several biological control agents, such as *Trichogramma* spp. egg parasitoids and predatory mirid bugs have been studied and used to control *T. absoluta*, with about 160 species being reported as natural enemies of this pest (Desneux et al. 2010; Biondi et al. 2018; Mansour and Biondi 2021; Zhang et al. 2021). *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) is the only species in Iran that is reared to a limited extent, and thus has potential for application in Integrated Pest Management (IPM) programs in Iran (Nozad-Bonab et al. 2021). The parasitism rate of *T. brassicae* ranges from 9.13% in the tomato greenhouse (Lalegani et al. 2014) to 53.89% in laboratory conditions (Ahmadipour et al. 2015) suggesting a potentially important role of this parasitoid in the reduction of the pest population. The parasitism rates under field conditions still need to be confirmed as the performance of *T. brassicae* in the field may be affected by many factors like temperature, release point, host species, and rearing conditions.

In most cases, natural enemies may not completely control a pest, and their use needs to be integrated with insecticide applications. In IPM programs, the chemical control should be selective to the target pest species, and harmless or with the least negative effects on non-target, beneficial organisms such as parasitoids (Wang et al. 2019). Research on the effects of insecticides on *Trichogramma* spp. wasps, particularly in controlling the tomato leaf miner *T. absoluta*, is crucial due to the pest's significant impact on tomato production. The continuing studies aim to address the challenges of insecticide resistance and to enhance the efficacy of biological control methods, which are increasingly necessary as reliance on chemical pesticides raises environmental and health concerns (Abdel-Razek et al. 2019; Nozad-Bonab et al. 2021). Some of the effective and widely used insecticides against *T. absoluta* (Nozad-Bonab et al. 2021), initially considered safe, were reported to have contrasting potential side effects on natural enemies. For example, the frequently used bioinsecticide spinosad has been shown to cause sublethal effects in many parasitoids (Biondi et al. 2012a). Furthermore, emamectin benzoate was reported very toxic to *Trichogramma chilonis* Ishii, 1941 (Sattar et al. 2011) while emamectin and flubendiamide were considered to present a high degree of compatibility with egg parasitoid releases (Gallego et al. 2019). Such contrasting findings have raised concerns about the real impact of these insecticides on non-target organisms and combining them with biological control agents needs to be further investigated.

This study evaluated the lethal and sublethal effects of four insecticides (flubendiamide, emamectin benzoate + lufenuron, thiocyclam, and spinosad), on *T. brassicae* wasp, as an important parasitoid of *T. absoluta* under controlled laboratory conditions. These insecticides were selected based on their common usage in local agricultural practices for managing *T. absoluta* and their varying modes of action.

Methods

Insecticides

The insecticides used were formulated products containing flubendiamide (Takumi®, 20% WG, Nihon Nohyaku, Japan), emamectin benzoate + lufenuron (Proclim Fit®, 50% WG, Syngenta, Switzerland), thiocyclam hydrogen oxalate (Evisect®, 50% SP, Nippon Kayaku, Japan) and spinosad (Tracer®, 24% SC, Dow Elanco, England). A general description of the tested insecticide formulations is presented in (Table 1).

Rearing of the Trichogramma brassicae wasp

Cylindrical plastic containers (25 cm in diameter and 40 cm high) were used to rear *Trichogramma* wasps. To

Table 1 General information on tested insecticide formulations

Action Ingredients (AI)/Trade name	Supplier	Mode of action[1]	Chemical group	Concentration	Formulation[2]	Dose
Flubendiamide/ Takumi®	Nihon Nohyaku	Ryanodine receptor modulators	Diamide	20%	WG	0.2 gr/L
Emamectin benzoate + lufenuron/ Proclim Fit®	Syngenta	Glutamate-gated chloride channel (GluCl) allosteric modulators Inhibitors of chitin biosynthesis affecting CHS1	Avermectins Benzoylureas	50%	WG	0.25 gr/L
Thiocyclam hydrogen oxalate/ Evisect®	Nippon Kayaku	Nicotinic acetylcholine receptor (nAChR) channel blockers	Nereistoxin analogues	50%	SP	1 gr/L
Spinosad/ Tracer®	Dow Elanco	Nicotinic acetylcholine receptor (nAChR) allosteric modulators	Spinosyns	24%	SC	0.25 ml/L

[1] Mode of action: IRAC (2021). [2]Formulation: WG: water–dispersible granules, SP: soluble powder, SC: suspension concentrate

provide ventilation, a circular opening was made on the upper surface of the containers and closed with a net. On the side of the containers, a paper strip impregnated with a thin layer of honey was placed to feed the wasps. The parasitoids were offered 100 eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) every 24 h. The rearing containers were kept in a growth chamber at 25 ± 1 °C, 65 ± 5% RH, and a 16 h:8 h L:D photoperiod. Parasitized eggs were used for bioassays, rearing, and population maintenance.

Determination of the concentration–response curves of insecticides against adults of T. brassicae

Preliminary experiments were performed to determine the range of the lowest and highest concentrations that caused respectively 20% and 80% mortality. Then, six intermediate logarithmic concentrations were used to determine the concentration–response curves for each insecticide. A stock solution of each insecticide was initially prepared, and serial dilutions were made from this stock solution. The insecticidal solutions (2 ml) were applied to McCarthy glass tube (40 ml) and left to dry for 30 min before introducing the wasps. Distilled water was used as a control. For each insecticide's concentration, three replicates (tubes) were performed, with 20 wasps used per replicate (Jafari et al. 2015). Wasps' mortality in each treatment was recorded at 24 h. The mortality rate was assessed in relation to the control group. The wasps were fed on honey solution (10%) during the experiment.

Effects of sublethal exposure to insecticides on demographic parameters of *T. brassicae*

To assess the effects of exposure to sublethal concentration of insecticides on the demographic parameters of *T. brassicae*, the inner walls of McCarthy glass tubes (40 ml) were impregnated with 2 ml of the LC_{30} concentrations of insecticides and left to dry for 30 min as described above.

One hundred female (< 24 h old) wasps were placed in groups of 10 individuals per McCarthy glass tube (40 ml). After adding 10 male wasps to each tube, wasp couples were allowed to mate for 24 h (Rafiee-Dastjerdi et al. 2009). Ten (10) mated females were placed inside each treated glass tube. After 24 h of exposure to the LC_{30} concentrations, 25 female wasps from the surviving insects were randomly selected and transferred individually to tubes. A one cm^2 card with 150 host eggs was offered daily to each female wasp until the death of the parasitoid. After 24 h, the egg cards were removed and kept in separate glass tubes to check for parasitism. Honey solution (10%) was provided to the wasp during the experiment. The glass tubes were stored under the aforementioned climatic conditions until the emergence of adults. A completely randomized design was employed, consisting of five treatments, which included various insecticides and a control group. The experiment was conducted with three replications. The number of parasitized eggs, non-parasitized eggs, and survival of adult male and female insects was monitored until the death of all treated females.

Statistical analysis

The Probit method was used to analyze the data of toxicological bioassay. In most cases, the mortality of the control treatment was zero or very low and negligible. Collected data of reproductive, biological and demographic parameters after females' exposure to sublethal concentration (LC_{30}) were used to construct a life table for each treatment using Carry's method (Carey 1993).

Data of Gross Reproduction Rate (*GRR*), Net Reproduction Rate (R_0), Intrinsic Rate of Increase (r_m), Mean Generation Time (*T*) and Doubling Time (*DT*) were separated using the least significant difference (LSD) test. All statistical analysis were performed with SAS software (SAS Institute 2002).

Insecticide classifications according to IOBC

The mortality caused by each product was categorized according to toxicological classes proposed by the International Organization for Biological Control (IOBC) working group 'Pesticides and Beneficial Organisms' (Hassan 1992). The insecticides were assigned to one of the following four categories: harmful (˃ 99% mortality), moderately harmful (80–99% mortality), slightly harmful (30–79% mortality) and harmless (< 30% mortality) (Table 2).

Results

Lethal effects of insecticides on *T. brassicae*

Based on the LCs of each insecticide, spinosad and thiocyclam caused significantly different mortalities compared to the other insecticides (Table 3). The spinosad presented the lowest LC_{50} and LC_{90}, and was considered as the most toxic to adults of *T. brassicae*. Flubendiamide and emamectin benzoate + lufenuron caused similar mortality to the parasitoid wasp being 5.6 and 5.7-fold less toxic than spinosad when the LC_{50s} were compared. Thiocyclam was the least toxic among the tested insecticides and presented about 13-fold less lethality to *T. brassicae* compared to spinosad. Furthermore, based on the caused mortalities, and according to the classification of the International Organization of Biological Control (IOBC), spinosad can be classified as harmful, emamectin benzoate + lufenuron and flubendiamide were classified as moderately harmful, while thiocyclam was classified as harmless to *T. brassicae*.

Table 2 The IOBC system for classifying the adverse effects of pesticides on non-target arthropods (Hassan 1992)

Class	IOBC category	%Mortality	
		Laboratory studies	Other study types
Class 1	Harmless	< 30%	< 25%
Class 2	Slightly Harmful	30–79%	25–50%
Class 3	Moderately Harmful	80–99%	51–75%
Class 4	Harmful	> 99%	> 75%

Sublethal effects of insecticides on the longevity and reproductive outputs of *T. brassicae* females

The effects of sublethal exposure to the four insecticides LC_{30} concentrations on fertility, fecundity, and female longevity of *T. brassicae* adults are shown in Fig. 1. Generally, all tested insecticides had significant (LSD: df = 4, P < 0.001) adverse effects on these biological parameters of *T. brassicae* decreasing both the number of eggs laid per female and the percentage of egg hatching compared to the control. Furthermore, spinosad had the greatest adverse effect on the reproduction (Fig. 1A) and fertility (Fig. 1B) of *T. brassicae* while the thiocyclam had the least negative effect among the tested insecticides. Similar trends were observed for female wasps' longevity (Fig. 1C), although no significant effect on the lifespan of *T. brassicae* adult insects was found between the treatments of thiocyclam and flubendiamide and between spinosad and emamectin benzoate + lufenuron. Such declining in longevity could be verified through the age-specific survival curves (l_x) and age-specific regeneration curves (m_x) of *T. brassicae* females under the influence of insecticides (Fig. 2). Overall, mortality was lower at early ages and progressively increased at later ages for all treatments (Fig. 2).

Sublethal effects of insecticides on population growth parameters of *T. brassicae*

Life table parameters of *T. brassicae* after sublethal exposure (LC_{30}) to insecticides are given in Table 4. The gross (*GRR*) and net reproduction (R_0) rates of insecticide-exposed females were significantly (LSD:

Table 3 Toxicity of spinosad, emamectin benzoate + lufenuron, thiocyclam and flubendiamide insecticides to adults of *Trichogramma brassicae* wasp

Insecticides	Slope ± SE	χ^2	Lethal Dose [mg a.i/L]			Classification
			LC_{30} (95%FL)	LC_{50} (95%FL)	LC_{90} (95%FL)	
Spinosad	2.26 ± 0.37	37.92	0.43 (0.54–0.29)	0.73 (0.85–0.60)	2.71 (4.60–2.03)	Harmful
Emamectin benzoate + Lufenuron	2.92 ± 0.42	47.34	2.76 (3.22–2.17)	4.17 (4.68–3.67)	11.46 (16.90–9.14)	Moderately Harmful
Thiocyclam	4.60 ± 0.68	45.87	7.37 (8.14–6.29)	9.59 (10.30–8.81)	18.2 (23.35–15.77)	Harmless
Flubendiamide	2.48 ± 0.39	40.90	2.51 (3.03–1.83)	4.09 (4.67–3.48)	13.4 (22.05–10.21)	Moderately Harmful

The classification was made according to IOBC ranking (Hassan 1992)

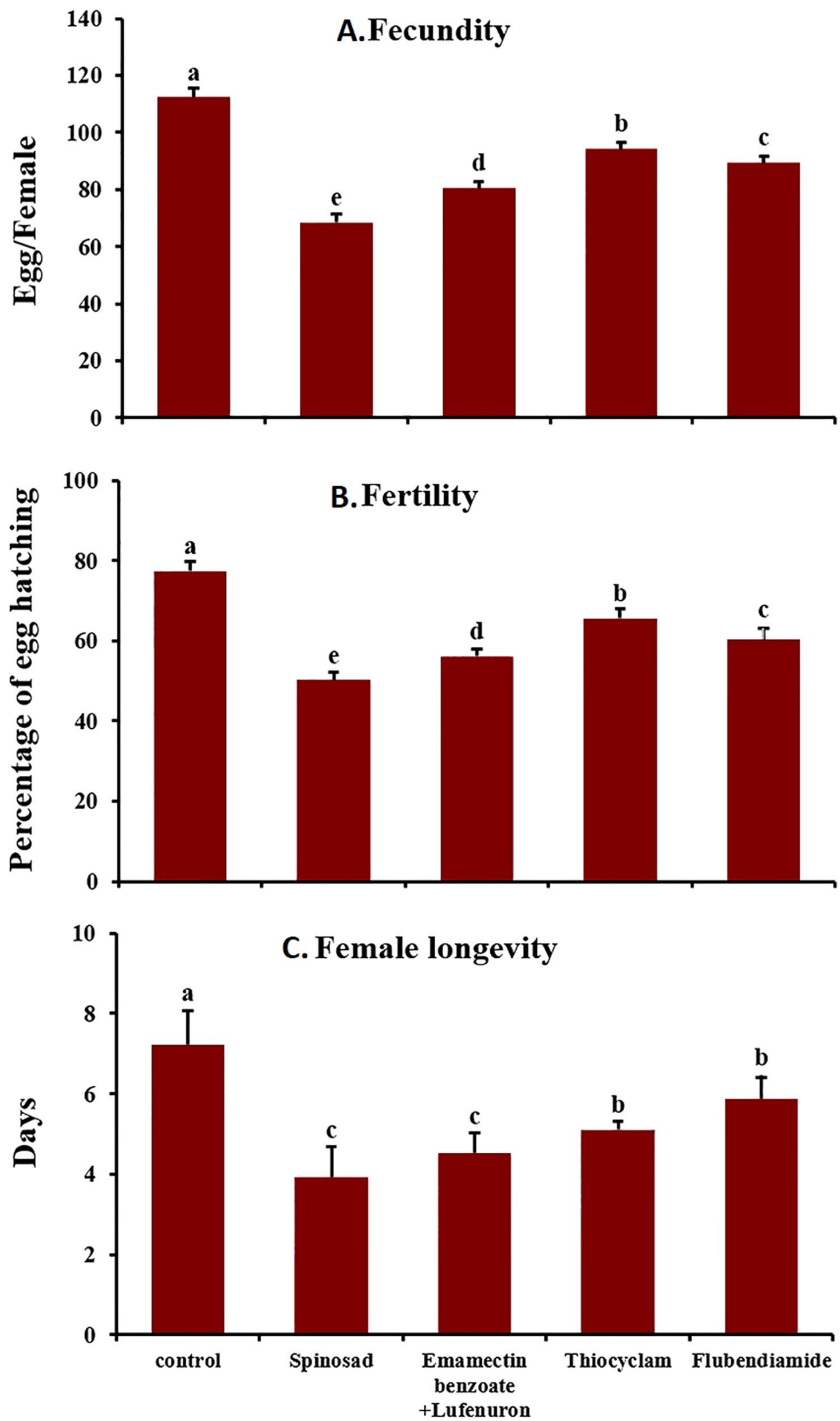

Fig. 1 Effects of exposure to LC_{30}s of spinosad, emamectin benzoate + lufenuron, thiocyclam, and flubendiamide insecticides on the biological parameters of the parasitoid wasp *Trichogramma brassicae*

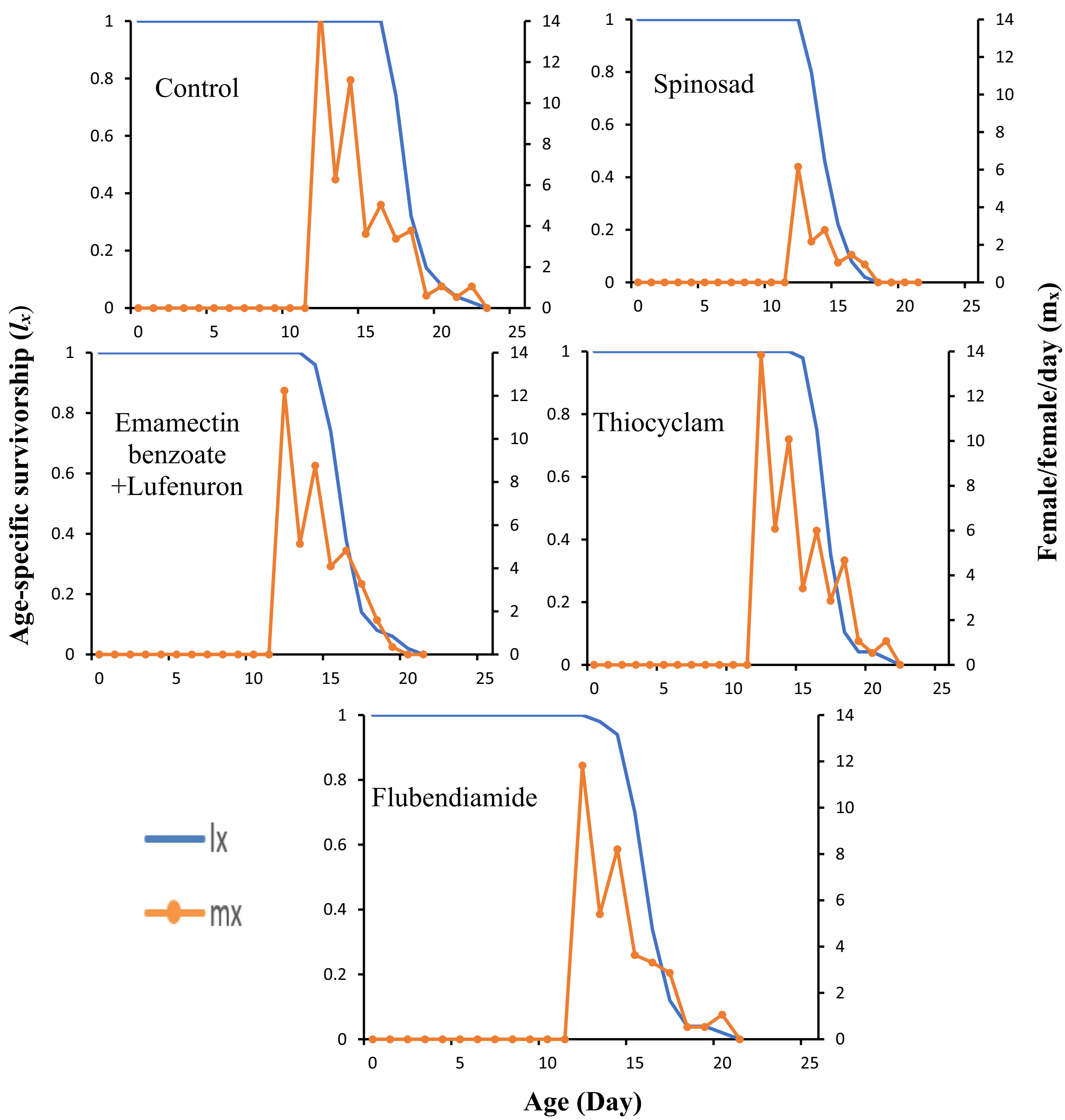

Fig. 2 Age-specific survival (l_x) and age-specific regeneration (m_x) curves for *Trichogramma brassicae* unexposed (control) and exposed to LC_{30}s of spinosad, emamectin benzoate + lufenuron, thiocyclam and flubendiamide insecticides. The l_x and m_x curves are presented in blue and yellow, respectively

df = 4, P < 0.001) lower than in the control with spinosad presenting the lowest values of both *GRR* and R_0. The lowest mean generation time and the highest mean doubling duration of the wasp population were obtained also for females exposed to sublethal concentration of spinosad (Table 4).

Discussion

The use of insecticides such as emamectin benzoate, spinosad, thiophenoxide, and flubendiamide is still a frequent practice for controlling the South American tomato pinworm moth (Lietti et al. 2005). However, due to the larval feeding behavior, such a control strategy may show limited effectiveness (Cherif et al. 2013) and

Table 4 Population demographical parameters of the parasitoid wasp *Trichogramma brassicae* exposed to the sublethal LC_{30} concentrations of different insecticides (Mean ± SE)

Parameter	Control	Treatment			
		Spinosad	Emamectin benzoate + Lufenuron	Thiocyclam	Flubendiamide
Gross Reproduction Rate (*GRR*)	52.43 ± 0.074a	19.62 ± 0.048e	37.96 ± 0.117d	48.50 ± 0.076b	41.41 ± 0.049c
Net Reproduction Rate (R_0)	42.64 ± 0.036a	10.55 ± 0.041e	29.96 ± 0.178d	39.08 ± 0.054b	33.51 ± 0.078c
Intrinsic Rate of Increase (r_m)	0.242 ± 0.005a	0.171 ± 0.001e	0.202 ± 0.002d	0.228 ± 0.001b	0.221 ± 0.002c
Mean Generation Time (*T*)	15.49 ± 0.002d	13.82 ± 0.001e	16.76 ± 0.001a	16.18 ± 0.002b	16.15 ± 0.001c
Doubling Time (*DT*)	2.85 ± 0.004e	4.059 ± 0.002a	3.42 ± 0.001b	3.06 ± 0.001d	3.17 ± 0.001c

Different letters indicate statistical difference between the means of the treatments using the LSD test ($P < 0.05$)

need to be applied simultaneously with other approaches especially using biological control agents like the parasitoids of the Trichogrammatidae family. Moreover, such a combination of control methods requires an assessment, understanding, and mitigation of the potential negative effect of chemical control on natural enemy populations. Consequently, only selective insecticides that effectively control pests while presenting low toxicity to biological control agents should be recommended for use. Thus, in the present study, the lethal and sublethal effects of four insecticides on the biological and reproductive outputs of *T. brassicae*, as a natural enemy of South American tomato pinworm, were assessed revealing pronounced differential effects among the tested compounds.

Spinosad showed higher mortality in *T. brassicae* than other insecticides and in sublethal exposure (LC_{30}) spinosad and emamectin benzoate + lufenuron had the greatest adverse effects on the parasitoid's biological parameters, reproduction, and life table. The lowest intrinsic rate of population growth of *T. brassicae* was recorded in spinosad treatment while the toxicity and sublethal effects of thiocyclam and flubendiamide were moderate.

Spinosad is a natural neurotoxic insecticide produced by fermentation of an actinomycete. Spinosad is an allosteric modulator that targets nicotinic acetylcholine receptors (nAChRs) (Martelli et al. 2023). Exposure of insects to spinosad results in hyperexcitation of the insect nervous system starting with initial involuntary muscle contractions and tremors, followed by paralysis and death (Geng et al. 2013). Although spinosad was initially classified as an environmentally and toxicologically reduced risk pesticide, its impact on natural enemies and pollinators (Miles et al. 2002; Mayes et al. 2003; Besard et al. 2011; Biondi et al. 2012b) has been frequently assessed and its high toxicity to various species of *Trichogramma* (Suh et al. 2000; Bueno et al. 2008; Hussain et al. 2010; Parsaeyan et al. 2020) has been previously reported. However, the extent of spinosad effects on parasitoids are less predictable, and depends on the parasitoid species and application methods (Tillman et al. 2000; Smith et al. 2024).

Previous researches have shown that spinosad can cause more than 50% mortality in *T. brassicae* within six hours of treatment, and it significantly reduces various biological parameters and reproductive rates of the parasitoid (Thubru et al. 2018; Parsaeyan et al. 2020). However, it has also been noted that spinosad can be relatively safe to *T. brassicae* after 15 days of application, making it a potential option for the management of lepidopteran pests in cruciferous ecosystems, with minimized chances of parasitoid exposure if used 15 days before parasitoid release (Thubru et al. 2018). Furthermore, spinosad has even been suggested to enhance the performances of *T. brassicae* (Smith et al. 2024).

Emamectin benzoate + lufenuron is a mixture of two active ingredients. The first is an avermectin analog which is highly effective against lepidopterans by activating chloride channels and decreasing neuron excitability. This leads larvae to stop feeding, becoming paralyzed, and dying within 3–4 days. The second, lufenuron, is an insect growth regulator (IGR) acting as chitin synthesis inhibitor, influences larvae's development and causes infertile eggs, causing insects to fail to complete their moults (El-Sheikh 2015). The lethal effects of this insecticide (LC_{50} = 4.17 mg a.i./L) on *T. brassicae* are close to the flubendiamide (LC_{50} = 4.09 mg a.i./L), however, the first's sublethal effects are greater.

The effects of emamectin benzoate and lufenuron on different *Trichogramma* species have been studied before to some extent. Emamectin benzoate has been found to have adverse effects on *Trichogramma*, causing significant mortality and reducing various biological parameters and reproductive rates of the parasitoid (Sattar et al. 2011; Nozad-Bonab et al. 2021).

Lufenuron has also been reported to have toxic effects on *T. chilonis*, being classified as "slightly harmful" and exhibiting significantly higher levels of toxicity against the larval stage of the parasitoid (Sattar et al. 2011).

Therefore, both Emamectin benzoate and Lufenuron have been shown to have negative impacts on *T. brassicae*, which is important to consider in integrated pest management strategies.

Flubendiamide is an organofluorine insecticide that acts as a ryanodine receptor modulator (RyRs). It is the first product from the family of 1,2-diamides insecticides, and exerts its insecticidal effect by activating the intracellular non-voltage-gated calcium channel (RyRs, ryanodine receptors) in the endoplasmic reticulum of insects (El Qacemi et al. 2019).

Flubendiamide based insecticides were considered non-harmful to both *T. brassicae* and *T. evanescence* wasps and are good candidates to be incorporated into IPM programs in combination with biological agents (Ashtari et al. 2019). Moreover, flubendiamide was found to be the most selective and with low impact on the development, survival, and fecundity of *T. chilonis* whereas spinosad, closely followed by emamectin benzoate severely curtailed adult survival and fecundity and to a lesser degree, the development of immature stages inside host eggs (Sattar et al. 2011).

Thiocyclam is a nereistoxin analog insecticide acting as a Nicotinic acetylcholine receptor (nAChR) channel blocker. It is a broad-spectrum insecticide used to control sucking and chewing pests on a variety of crops. Thiocyclam was found to be the most harmful insecticide for immature stages of *T. brassicae* and *T. evanescens* parasitoids, compared to tetraniliprole, chlorantraniliprole, and lufenuron and was found to cause significant mortality in *T. brassicae* at five days post-treatment (Ashtari 2022).

According to the classification of the International Organization of Biological Control (IOBC), spinosad can be classified as a harmful (Class 4) insecticide, and emamectin benzoate + lufenuron and flubendiamide were classified as a moderately harmful (Class 3) insecticides, while thiocyclam was classified as harmless (Class 1) to *T. brassicae* (Hassan 1992). Ashtari et al. (2019) have also reported that flubendiamide has less toxicity and negative impacts on the life table parameters of two *Trichogramma* species. Therefore, after additional field studies, flubendiamide and thiocyclam could be considered for use along with this biological control agent in integrated management programs of South American tomato pinworms. In addition, further studies should aim to assess more thoroughly the multifaceted side effects of pesticides on parasitoids at demographic levels, e.g. using the TWOSEX-MSChart method (Chi et al. 2022a, 2022b, 2023).

Our results showed that thiocyclam is the only insecticide that has low toxicity against the parasitoid, *T. brassicae* while the emamectin benzoate + lufenuron and flubendiamide were moderately harmful. These findings may be valuable in controlling *T. absoluta* combining thiocyclam with *T. brassicae.* However, it should be noted, that the findings of a small-scale laboratory experiment may not be generalizable to natural conditions. Laboratory studies help select pesticide compounds for studies under natural field conditions. Therefore, future studies should be performed under natural conditions to confirm the potential of integrating thiocyclam in *T. absoluta* targeted IPM programs. The emamectin benzoate + lufenuron and flubendiamide also must be evaluated under semi and open-field conditions to reassess their potential selectivity to the parasitoid species used in *T. absoluta*.

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Author contributions
All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Salma Papari. The first draft of the manuscript was written by Salma Papari, AbuFazel Dousti, Majid Fallahzadeh and Nazila Saghaei. Critical revision of the manuscript was done by Khalid Haddi and Nicolas Desneux and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript and agreed on its publication.

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Consent for publication
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Competing interests
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