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Original article

Toxicity of neem seed extract and different insecticides on *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae)

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ABSTRACT

Background and objectives: Trichogramma chilonis is an important biological control agent (parasitoid) that kill the host species before they damage the crops. However, insecticide sprayed after *T. chilonis* release significantly alter its efficacy. The acute toxicity risks of neem seed extract and commonly used insecticides for the control of lepidopteran insects are unknown for *T. chilonis*. The main objective of this study was to assess the toxicity of four insecticides, i.e., buprofezin, lufenuron, methoxyfenozide, pyriproxyfin and neem seed extract to *T. chilonis*.

Methods: Egg card and dipped surface bioassays were used to test the toxicity insecticides, i.e., lufenuron (2 ml/liter), methoxyfenozide (2 ml/liter), buprofezin (5 g/liter), pyriproxyfin (5 ml/liter) and neem seed extract (2 ml/liter). Egg cardboards prepared from the parasitized eggs of *Sitotroga cerealella* collected at 1st, 3rd, 5th, 7th, and 8th day after oviposition were dipped in the respective concentrations of inleticides and neem seed extract. The dipped cardboards were dried and emergence of *T. chilonis* was recorded. Similarly, dipped surface bioassay was conducted to observe the mortality of *T. chilonis* at 4 and 24-h after exposure.

Results: The applied insecticides exhibited varying degree of toxicity against *T. chilonis.* Lufenuron and neem seed extract proved least toxic, methoxyfenozide and buprofezin were moderately toxic and pyripoxyfen exhibited the highest toxicity during the study. Similarly, lufenuron and neem seed extract proved less toxic for the adults, buprofezin was moderately toxic, and methoxyfenozide and pyripoxyfen exhibited the highest toxicity in the dipped surface assay after 4 h of exposure. All insecticides proved highly toxic for the adults 24 h after exposure. The results reveled that lufenuron and neem oil are relatively safe for *T. chilonis*.

Conclusions: The tested insecticides and neem seed oil exhibited varying degree of toxicity against *T. chilonis*. The overall toxicity was classified as; pyriproxyfen > methoxyfenozide > buprofezin > lufenuron > neem seed extract. Therefore, neem seed extract and lufenuron are safer for *T. chilonis* compared to the rest of the insecticides included in the study.

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1. Introduction

Trichogramma (Hymenoptera: Trichogrammatidae) are polyphagous parasitoid wasps, distributed globally and play an important role in the management of several lepidopterous pests (Cadapan, 1986; Khan, 2020; Mahankuda and Sawai, 2020). They target the eggs of host species; thus, emerging adults are parasitized (Khan, 2022, 2020; Sont et al., 1999). Trichogrammatids are excessively used for the management of *Helicoverpa armigera* Hübner, *Scirpophaga incertulas* Walker, and *Plutella xylostella* Linnaeus in rice, vegetables, and cotton crops (Jalali et al., 2014; Kurtuluş and Kornoşor, 2015; Lei et al., 2021). In Pakistan *Trichogramma* is effectively used for the management of sugarcane borer (Hamid et al., 1998; Hussain et al., 2012). *Trichogramma* are used as egg parasitoids in the biological control programs (Preetha et al., 2018; Singhamuni et al., 2015). These species are easy to rear, and their production is economically profitable (Preetha et al., 2010, 2018). *Trichogramma* is frequently in the Indian sub-continent for effective management of *H. armigera* (Lingathurai et al., 2015).

Trichogrammatids are beneficial biological control agents in the egg parasitoid group (Sarwar and Salman, 2015). They have been successfully used for biological control in > 50 countries of the world. *Trichogramma* are released on ~ 32 million ha annually; however, the insecticides used for the management of harmful pests significantly reduce their efficacy (King and Dunkin, 1986; Mahankuda and Sawai, 2020; Thangavel et al., 2018). Biological control with *Trichogramma* coupled with chemical control is not an attractive option due to harmful effects linked with the pesticides (Saber, 2011).

Insect growth regulators (IGRs) attack specific biochemical systems that are unique to insects and safe to non-target organisms, i.e., other arthropod species and mammals (Cadapan, 1986; Jalali et al., 2016; Zengxin et al., 2021). Bio-rational approaches would play a key role in reducing the risks associated with pest management tactics, including pesticides (Horowitz et al., 2020). The IGRs are effective at lethal and sublethal concentrations and used for the effective results on physiological parameters of exposed insect species (Sadeghi et al., 2021). The IGRs significantly influence pupil and larval weight (Yin et al., 2008), fecundity, developmental time (Mahmoudvand et al., 2011), egg size, hatching (Guo et al., 2013), adult endurance (Mahmoudvand et al., 2011), pupal ratio, adult development (Sial and Brunner, 2010) and other biological parameters (Ahmad et al., 2012).

Over time, excessive use of synthetic insecticides has resulted in serious problems, including evolution of insecticide resistance, insecticide-induced resurgence of insect pests, adverse effects on non-target organisms, i.e., parasitoids, predators, honeybees, pollinators, birds, cattle, and human (Arif et al., 2020; Naeem et al., 2022, 2021c, 2021a, 2021b). In addition, phytotoxicity, environmental pollution, and excessive increase in the cost of pesticides have necessitated alternative, effective and biodegradable pest control materials with greater selectivity (Cherif et al., 2021; Mohapatra and Shinde, 2021; Subandi et al., 2017). For these reasons, natural pesticides are often preferred over synthetic ones. Among several options, neem (*Azadirachta indica*) has evoked a great deal of interest because of its bio-efficacy and biodegradability (Ahmad et al., 2012; Preetha et al., 2018). However, the safety of neem-based pesticides to non-target organisms has been the subject of some controversy. Although sufficient data are available on the bioactivity of neem against insect pests, there have been few detailed studies on the effects of neem on parasitoids.

This study assessed the effect of four insecticides and neem seed extracts on *T. chilonis*. Insecticides were selected based on their potential use in the management of lepidopteron insects. The results of the study would help to devise integrated pest management practice for lepidopteron insects.

2. Materials and methods

2.1. Experimental site and details

This study was carried out in the Eco-toxicological laboratory, Ayyub Agricultural Research Institute (AARI) Faisalabad, Pakistan. Comparative toxicity of four insecticides and neem seed extract was tested against *T. chilonis* under laboratory conditions. Four insecticides, i.e., lufenuron, methoxyfenozide, buprofezin, pyriproxyfin and neem seed extract were included in the study. The experiment Research work was started in March 2019.

The experiment was conducted at 27 ± 1 °C temperature and 65 ± 5% relative humidity. The experiment was arranged according to completely randomized design and each treatment had three replications. The concentrations of the insecticides and neem extract were selected based on their recommended doses for the registered crops. The concentrations were lufenuron (2 ml/liter), methoxyfenozide (2 ml/liter), buprofezin (5 g/liter), pyriproxyfin (5 ml/liter) and neem seed extract (2 ml/liter). These insecticides are usually used to manage the pests of a lot of crops in the landscape.

Trichogramma chilonis culture was maintained on the eggs of *Sitotroga cerealella* under laboratory conditions at 25–27 °C and 70–80% relative humidity. The toxicity of different insecticides and neem seed extract on different life stages of *T. chilonis* was tested by egg card and dipped surface residue bioassays described below.

2.2. Egg card bioassay

Egg cards were prepared from parasitized *Sitotroga cerealella* eggs during various phases of immature *T. chilonis* and used in the egg card bioassays. *Sitotroga cerealella* eggs were exposed to *T. chilonis* for likely parasitism for 24 h and then egg cards were prepared from the exposed eggs (Khan, 2022, 2020). Five randomly chosen egg cards (each having 40 eggs) were dipped into the solutions of insecticides and neem seed extract for one second at 1st, 3rd, 5th, 7th, and 8th day after oviposition. Untreated cards were regarded as control. These days correspond to the life stages of *Trichogramma*, i.e., eggs (1d), larvae (3d), early pupae (5d), pupae (7d) and late pupae or one day before adult emergence (8d). Dipped egg cards were dried at ambient room temperature. Once dried, the egg cards were placed in a small glass Petri dish (5 cm diameter and 5 cm deep) and kept at 25 ± 2 °C temperature and of 60 ± 5% relative humidity until all healthy parasitoids emerged. Afterwards, the remaining eggs were examined under microscope. The percentage emergence of *T. chilonis* was computed for each treatment and used in the interpretation of the results.

2.3. Dipped surface residue bioassay

The insecticide residue bioassay was conducted in ventilate glass bioassay chambers (15 cm length and 4 cm diameter). Whatman no. 1 filter paper was dipped in the respective solutions of each treatment. The filter papers were then dried and put into the glass bioassays tube. The control tubes contained filter papers moistened with distilled water and then dried. Afterwards, the newly emerging adults were released in the tubes. The mortality in the bioassay tubes was recorded 4 and 24 h after the release of the adults. Percent mortality was computed and interpreted. Cardboard and dipped surface residue experiments were arranged according to completely randomized design and each treatment had three replications.

2.4. Data analysis

The collected data on emergence and mortality were analyzed by one way analysis of variance (ANOVA) (Steel et al., 1997). The normality in the data was tested which indicated that data had normal distribution (Shapiro and Wilk, 1965). The means were compared by Tukey's post hoc test at 95% probability level where ANOVA indicated significant differences. Based on means, toxicity was interpreted. The treatment means having higher emergence and low mortality was considered less toxic and vice versa. The statistical computations were done on SPSS statistical software version 20.0 (IBM Inc., 2012).

3. Results

The *T. achilonis* emergence from different aged-parasitized eggs was significantly altered by different insecticides and neem seed extract included in the study. Lufenuron proved least toxic for the 1-day old eggs with 25 % emergence. Overall, the emergence was 25, 23, 21, 23 and 19% the 1-day old egg cardboards dipped in lufenuron, methoxifenazide, pyriproxyfen, beprofezone and neem seed extract, respectively. The control treatment resulted in 94.5% emergence on 1st observation (Fig. 1).

Pyripoxifen was least toxic against *T. chilonis* with 39% emergence for 3-day old parasitoid egg. Overall emergence was 31, 29, 38, 39 and 27% for the eggs treated with pyriproxyfen, beprofezone, lufenuron, methoxifenazide and neem seed extract, respectively at 2nd observation (Table 1).

Pyripoxifen was comparatively less toxic against 5-day old parasitoid eggs of *T. chilonis* as it gave 47% emergence. The emergence from other insecticides, i.e., lufenuron, methoxyfenozide, buprofezin, pyripoxifen and neem seed extract was recorded 39, 43, 43, 47 and 35%, respectively.

Pyriproxifen proved the least toxic against 7-day old parasitoids egg with 49% emergence. The recorded emergence from methoxifenazide, beprofezone, neem seed extract and lufenuron was 47, 42, 36 and 35%, respectively.

Similarly, pyriproxifen proved the least toxic against 8-day old parasitoids egg with 59% emergence. The recorded emergence from methoxifenazide, beprofezone, lufenuron and neem seed extract was 56, 51, 44 and 43%, respectively.

The percentage mortality of *T. chilonis* adults after 4-hour exposure to lufenuron, methoxyfenozide, buprofezin, pyripoxifen and neem seed extract was recorded 48, 76, 52, 76 and 44% (Fig. 2). The mortality reached to 100, 100, 80, 80 and 96% after 24 h exposure to lufenuron, methoxyfenozide, buprofezin, pyripoxifen and neem seed extract, respectively (Table 2).

4. Discussion

Trichogramma has been used as important biological control agents in >50 countries of the world (Khan, 2022, 2020). These are released on ~32 million ha; however, excessive use of pesti-

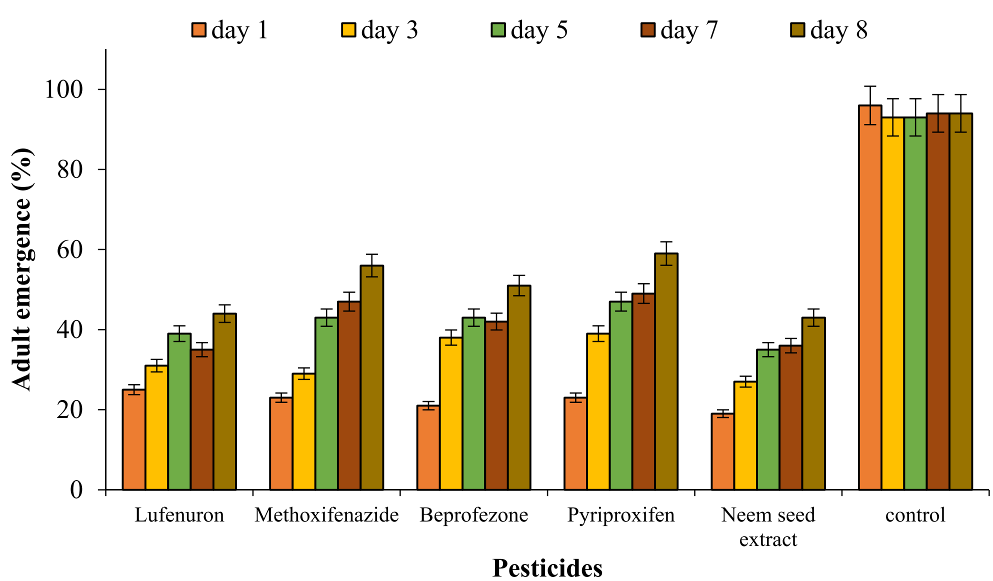

Fig. 1. The effectiveness of different insecticides and neem seed extract on *Trichogramma chilonis* emergence at 1, 3, 5, 7 & 8 days after post parasitism.

Table 1
The efficacy of different pesticides and neem seed extract on the emergence of *Trichogramma chilonis* from1, 3, 5, 7 & 8 days-old parasitized eggs.

	Parasitoid emergence (%)				
Treatments	1-day old eggs	3-day old eggs	5-day old eggs	7-day old eggs	8-day old eggs
Lufenuron	25.0 ± 1.06C	31.0 ± 1.31 AB	39.0 ± 1.02 BC	35.0 ± 2.83 D	44.0 ± 1.04B
Methoxifenazide	23.0 ± 0.45 AB	29.0 ± 2.12B	43.0 ± 0.91 AB	47.0 ± 1.04 AB	56.0 ± 0.41A
Beprofezone	21.0 ± 0.71 A	38.0 ± 1.28 A	43.0 ± 1.02 AB	42.0 ± 2.32 BC	51.0 ± 1.02 AB
Pyriproxifen	23.0 ± 1.31AB	39.0 ± 0.62 A	47.0 ± 0.62 A	49.0 ± 0.52 A	59.0 ± 0.58 A
Neem seed extract	19.0 ± 0.93 A	27.0 ± 2.01B	35.0 ± 2.07C	36.0 ± 2.91CD	43.0 ± 1.05B

The values represent the means ± SE (n = 3). The means followed by different letters are significantly different form each other (P < 0.01).

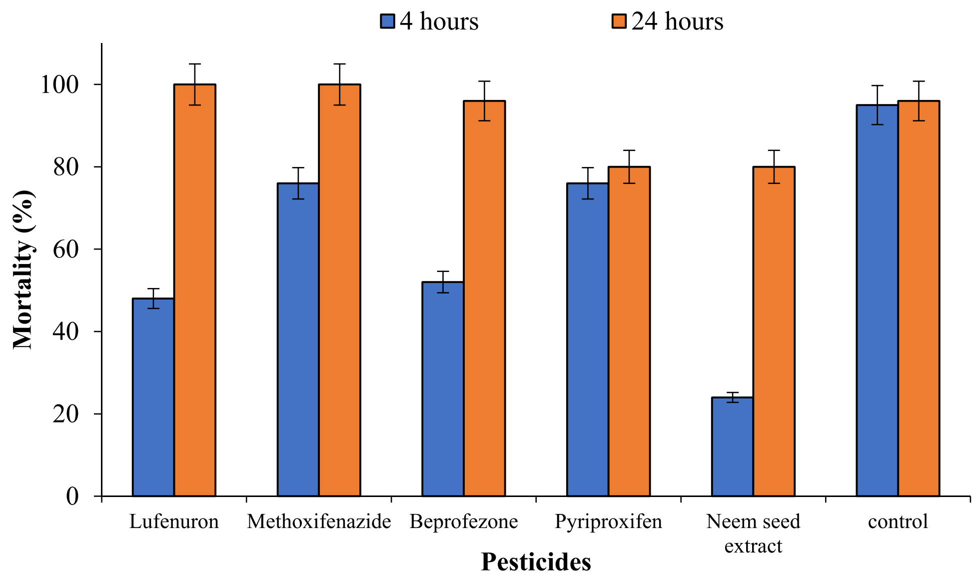

Fig. 2. Mortality of *Trichogramma chilonis* adults after 4 h-old parasitized eggs 24 h after exposure to insecticides residues.

Table 2
The efficacy of different pesticides and neem seed extract on *Trichogramma chilonis* and adult mortality at 4 and 24 h exposure to pesticides.

Exposure time (h)	Lufenuron	Methoxifenazide	Beprofezone	Pyriproxifen	Neem seed extract
	Mortality/%				
4	48.0 ± 1.02 AB	76.0 ± 0.32A	52.0 ± 1.26 AB	76.0 ± 0.39 A	24.0 ± 1.21B
24	100.0 ± 0.82A	100.0 ± 0.41 A	80.0 ± 0.62 A	80.0 ± 0.72 A	96.0 ± 0.62 A

The values represent the means ± SE (n = 3). The means followed by different letters are significantly different form each other (P < 0.01).

cides significantly lowers their efficacy (King and Dunkin, 1986). Biological and chemical control combination in IMP is unfit due to harmful effects of insecticides on *Trichogramma* (Saber, 2011). Therefore, this study assessed whether the frequently used insecticides exert negative impacts on different immature stages of *Trichogramma*. The tested insecticides significantly differed in their toxic effects indicating that the insecticides with lower toxicity could be combined with *Trichogramma* release in IPM.

Trichogramma eggs are used biological control program which increases their population and suppress harmful pests (Lei et al., 2021; Mahankuda and Sawai, 2020; Preetha et al., 2018; Singhamuni et al., 2015). *Trichogramma* speices can be reared easily and effectively control harmful pests (Khan, 2022, 2020; Preetha et al., 2010). *Trichogramma* are frequently used Indian sub-continent for the management of American bollworm (Lingathurai et al., 2015).

Combination of *T. chilonis* with various insecticides successfully controlled sucking pests (Sant et al., 2019; Thangavel et al., 2018; Wahengbam et al., 2018). The *T. chilonis* integrated with insecticides in Bathinda, India, reduced bollworm damage by 70.30% and improved cotton yields up to 44.50% compared to sole application of insecticides (Brar et al., 2002).

Trichogramma chilonis are affected by insecticides' spray which normally lower their efficacy. The oviposition of this species is significantly altered by the insecticides (Suzuki and Waller, 1984). *Trichogramma* wasps are tiny in size, belong to friendly insect fauna and used against lepidopterous insects in IPM for pest management in vegetables and field crops (Nagarkatti and Nagaraja, 1977). It is very destructive parasitoid (Subandi et al., 2017).

Trichogramma species usually attack on the eggs of ~200 species (Sattar et al., 2011). There and reared as the biological control agents and released in the target fields (Tarès et al., 2000). Their egg phases start in March and end during September. *Trichogramma chilonis* regulate their host eggs in July.

When their hosts complete their three generations then they continue to egg parasitized at the end of October, but its occurrence started from July onward, so their discharge adjusted as early as their oviposition started in the area and they depend on their native main temperature(Ullah et al., 2012). The applied insecticides exerted differential effects on the emergence and adult mortality of *T. chilonis*. Although plant extracts are regarded safe for the beneficial organisms, some stages of *T. chilonis* were significantly affected by the neem seed extract. However, it was least toxic to *T. chilonis* compared to the rest of the insecticides included in the study. The differences among insecticides can be explained with their chemical composition and use. Nevertheless, the applied concentration of the insecticides could be the other major reason for the differences among insecticides.

Khan (2020) reported that acetamiprid, spinetoram, fipronil and abamectin proved highly toxic against *T. chilonis*, while the remaining pesticides were less toxic and can be integrated with the parasitoid in agroecosystem.

The results of the current study warrant that neem seed extract could be incorporated in IMP due to its less toxicity to *T. chilonis*. However, the toxicity of neem seed extract should be determined for the target harmful pests before the inclusion of neem seed extract in IMP.

5. Conclusion

The results revealed that lufenuron and neem seed extract proved least toxic for *Trichogramma chilonis*; therefore, these could be used for the management of harmful pests and their use will had lower toxic effects on *T. chilonis* compared to the rest of the insecticides included in the study. Methoxyfenozide and buprofezin showed moderately toxicity, whereas pyripoxifen proved highly toxic for the emergence of *T. chilonis*. Lufenuron and neem oil were comparatively less toxic to adults after 4 h of exposure, buprofezin was moderately toxic, while methoxyfenozide and pyripoxifen were highly toxic to adults of *T. chilonis*. It is therefore recommended that lufenuron and neem seed extract could be used for the management of lepidopteran pests in various field crops.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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