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Research Article

Toxicity Potential of Different Azadirachtin against *Plutella Xylostella* (*Lepidoptera; Plutellidae*) and its Natural Enemy, *Diadegma* Species

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Abstract

The present study was conducted to assess different concentrations of azadirachtin against 3rd and 4th instar larvae of *P. xylostella* and to determine their effects on natural enemy, *Diadegma* sp. Four different concentrations (0.31, 0.50, 0.60 and 1%) of azadirachtin were evaluated in the choice and non-choice tests methods. In the choice test, the LC_{50} value for the 3rd instar larvae were 0.66, 0.41 and 0.37 µg/ml on day 1st, 2nd and 3rd, respectively. Similarly the LC_{50} values for the 4th instar larvae were 0.55, 0.34 and 0.34 µg/ml after 24, 48 and 72 hours of treatment, respectively. Descending order of toxicity for azadirachtin was observed in no choice method. The LC_{50} values for the 3rd instar larvae in no choice test were 0.63, 0.32 and 0.29 µg/ml on 24, 48 and 72 hours of treatment, respectively. LC_{50} value for the 4th instar larvae in no choice test were 0.52, 0.36 and 0.31 µg/ml on day 1st, 2nd and 3rd respectively. Maximum repellencies for 3rd (95%) and 4th instar larvae (90%) were noticed at 1% concentration. Highest mortalities of *Diadegma* sp were recorded for 1% concentration which started decreasing with decrease in tested concentrations. High LC_{50} values (4.91 and 1.63ppm) were recorded for *Diadegma* sp after 24 and 48h exposer time. Likewise, maximum adults emerged from pupae in lowest concentration 0.31% which increased with increase in concentration. Concluding, for better management of azadirachtin should be optimized with *Diadegma* sp.

Keywords: Biopesticide; Bio-rational; Dose-response; Eco-friendly; LC_{50}

Introduction

The diamondback moth, *Plutella xylostella* (L.) is one of the most serious and economically potential insect pests of cruciferous crops throughout world. Insecticides such as spinosad, tebufenozide, indoxacarb and emamectin benzoate are mostly applied for the control of this pest, but it has started developing resistance to these new insecticides [1,2].

Due to indiscriminant use of insecticides in developing countries, this pest has developed resistance to different groups of chemicals and adverse effects on natural enemies has also been noticed. Besides, negative impacts of these insecticides and strict government regulation have created interest in the preparation and use of botanical pesticides [3-6].

Azadirachtin is neem based insecticide derived from extracts of *Azadirachta indica* (Meliaceae). This product has played a vital role in crop protection in the last decade. This insecticide is very complex terpenoid, has been successfully used not only for the management of this pest, but against more than 400 species of insects. Azadirachtin has proved to be one of the most promising plant products for integrated pest management [7-11].

This compound can be used against insects as oviposition deterrent, anti-feedant, repellent, molting inhibitor, sterilant, growth retardant and preventing larvae from developing into adults [9,12,13].

Different azadirachtin concentrations have been used for the management of *P. xylostella* and other pest insects of cruciferous crops [14,15]. Neem based products can significantly reduce the pest damage on cauliflower and increase marketable head weight. Nonetheless, the efficacy of three new products of neem against *P. xylostella* has not been determined in USA [14].

Besides, this product has less toxic effect on natural enemies and environment [9,13]. Neem based insecticides are non-toxic to human and many beneficial insects, hence this product has regularly used many pest management programs [11,16,17].

Though there are many studies on crops regarding *P. xylostella* and *Diadegma* sp the main purpose of our study was to optimize the use of azadirachtin and *Diadegma* sp against *P. xylostella*. For these

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purpose different concentrations of azadirachtin were used to calculate percent mortality, larval repellency and its effect on its natural enemy in the laboratory.

Materials and Methods

Host: *Brassica oleracea italica*

Brassica oleracea italica, Broccoli green, produces 300-400 g (10-20 cm) large thick blue green heads on thin stalks. Full of nutrients and flavor, broccoli is rich in vitamins, high in fiber and low in cholesterol. These tasty vegetables prefer rich well drained soil and should be fed regularly during the growing season. They required plenty of organic matter during development and harvesting occurs after 60-85 days.

Fifteen days old broccoli Green seedlings were bought from a native nursery (Pohlmans Nursery) along with plant media. Media was transferred to 50 pots and a single broccoli seedling was then grown in each pot and were maintained at 25±2°C and 55±7% Relative Humidity (RH).

Rearing of *P. xylostella* in the laboratory

For stock of *P. xylostella* larvae used in this study, pupae were collected from the research field, University of Queensland, Gatton Campus, from broccoli plants. Pupae collected were then transferred to rearing cages in an insect rearing room on potted broccoli plants already raised in a controlled environment chamber of the glasshouse at School of Agriculture and Food Sciences, Gatton Campus. Rearing was maintained in a Controlled Environment (CE) room with photoperiods of 16:8 (light:dark) hours, temperature 25±2°C and 55±7% Relative Humidity (RH) at the School of Agriculture and Food Science, University of Queensland Gatton, using the modified rearing procedure of Talekar and Ying Lin [18]. Pupae were held in a CE room in wooden-framed net cages (45×45×45 cm) with cotton swabs containing a 10% sugar solution for the adult's egg laying in the cages for emerging adults in potted broccoli seedlings. They were kept caged until adults emerged and mated.

After oviposition, seedlings with eggs were removed from the cages and kept caged until the eggs hatched. Seedlings with newly-hatched larvae were transferred to large cages (120×60×60 cm) in the insect rearing room where they were kept until the larvae pupated. Pupae were then harvested and transferred to the laboratory, some for mating immediately, the rest kept at 4°C until needed to maintain the population

Plant insecticides

Azadirachtin extracts with 0.31%, 0.50%, 0.60% and 1% concentrations were provided by 'BioAust' Stafford Heights, Queensland. Neem trees produce azadirachtin, a chemical extracted from the seeds and leaves and possessing insecticidal properties, mostly used as a repellent for a broad spectrum of agricultural and household insects as well as considered more environmentally friendly than synthetic insecticides.

Repellency effect and developmental disruption (Larval Stage)

During preliminary screening in laboratory trials, third instars of *P. xylostella* were collected from insect rearing cages held in the insectary. The dipped leaf method was used in these experiments [19]. Leaf discs (3 cm in diameter) were dipped in 0.30%, 0.50%, 0.60% and 1% azadirachtin for 30 seconds, then dried for 2 hours in a fume cabinet and placed on filter paper in a plastic container (9 cm diameter; 6 cm height). Three larvae of the same age third instars were introduced in each leaf disc. During the insect rearing in this research, this stage usually occurred at day 5. Each concentration was replicated four times along with control. Larvae of *P. xylostella* were transferred to the extract soaked leaf discs with choice and no-choice options. For the choice method, three larvae of the 3rd instar were placed on treated and untreated/control leaf discs around a plastic container, while for the no-choice method, the treated leaf discs and controls were placed in separate plastic petri dishes.

Laboratory conditions for the experiments were maintained at 25°C±2 (temperature) and 54±10% (relative humidity). During the course of experiment, recordings were made for the following categories of effects:

- Repellency test (Choice and No choice method)
- Dose-response mortality

Extract efficacy and repellence effect against parasitoids of *Diadegma* sp Parasitoids of *P. xylostella*

Five adult parasitoids of *Diadegma* sp about 24 hours post emergence were placed in glass vials (15 cm in length; 1.5 cm in diameter) in a cold room at 5°C to slow down their active movement. Four different diluted concentrations of azadirachtin (0.30%, 0.50%, 0.60% and 1%) were assayed with 4 replications. Cut filter paper (10 cm in length; 1 cm in width) was dipped in the corresponding concentration suspensions and control for 30 seconds and were left to dry for 1 hour after which each was inserted into a glass vial. A streak of honey solution was applied to the inner side of each vial as a parasitoid food source and then the vial was plugged with a cotton pad. Mortality observations were made after 24 hours by counting the number of dead and alive parasitoids per concentration.

Data analysis

All the replicated data were statistically analyzed by using Analysis of Variance (ANOVA) technique appropriate for a Completely Randomized Design (CRD) by using a statistical software statistics 8.1 version. The significant means were separated by Fischer's protected Least Significant Difference (LSD) test after a significant F-test at α 0.05 level of probability [20]. The data were processed by probit analysis according to method described by using computer software developed by Chi [21,22].

Results

Percent mortality of *P. xylostella* larvae (Choice Method)

Based on a preliminary experiment, ascending mortality (30-95%) was observed with ascending concentrations (0.30%, 0.50%, 0.60% and 1%) in 3rd and 4th instar larvae after 24, 48 and 72 hours during choice experiments. For 3rd instar larvae, highest percent mortality was 60%, 75% and 95% for 1% concentration after 24, 48 and 72 hours respectively which were significantly higher than the rest of concentrations. Likewise, similar mortality was recorded for 4th instar larvae i.e., 75%, 85% and 95% after 24, 48 and 72 hours respectively (Figure 1). Azadirachtin concentration (1%) gave highest mortality.

However the order of toxicity of different azadirachtin concentrations was found to be 1% > 0.6% > 0.5% > 0.31%. The data showed (Figure 1) that the LC_{50} value decreased with increases in time of exposure. LC_{50} value after 1st day was 0.66 ppm which decreased to 0.41 and 0.37 ppm on 2nd and 3rd day respectively (Figure 2).

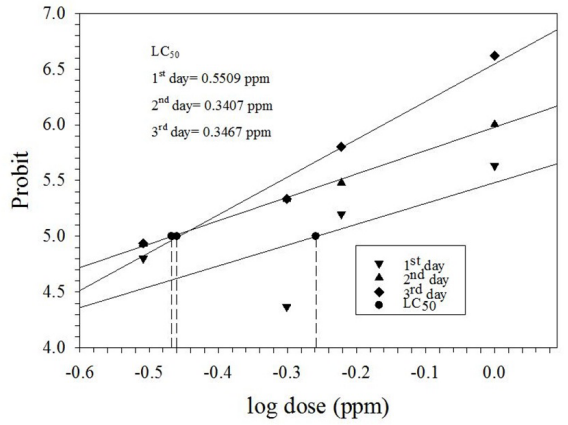

Figure 1: Graph showing the LC_{50} values of azadirachtin concentrations against 4th instar larvae of *P. xylostella* (L.) in choice test.

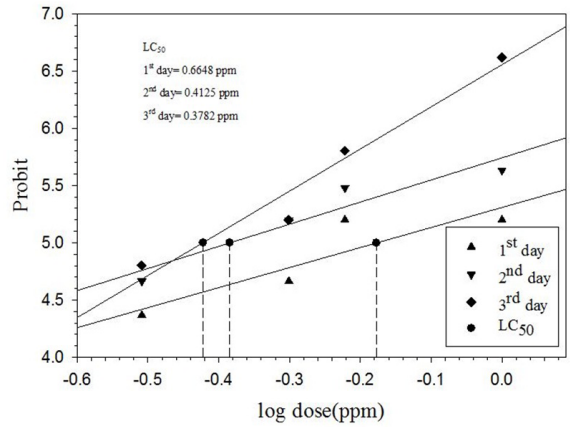

Figure 2: Graph showing the LC_{50} values of azadirachtin concentrations against 3rd instar larvae of *P. xylostella* (L.) in choice test.

It can be concluded that there was no significant difference among the azadirachtin concentrations (0.6% and 1%) efficacies against *P. xylostella*. The most commonly used concentrations for formulations of plant insecticides with contact effect is 0.5-1% (or 1-3 l/ha in the corresponding dosage) (Table 1). In the present study, it was further concluded that a 0.6-1% concentration would be the highest and appropriate dosage for the management of *P. xylostella* because no phytotoxic effects were found in the treated plants 24 hour after application of this extract.

Figure 2 shows percent mortality of *P. xylostella* from different azadirachtin concentrations in no choice methods. A range of concentrations gave 0-100% mortality for the 3rd and 4th instar larvae after 24, 48 and 72 hours. For 3rd instar larvae, highest percent mortalities were 75%, 85% and 100% for 1% concentration after 24, 48 and 72 hours respectively which were significantly higher than the rest of the concentrations (Figure 3). Likewise, similar results were recorded for 4th instar larvae i.e., 75%, 85% and 95% for 1% concentration after 24, 48 and 72 hours respectively (Figure 4). As can be seen from table 2, azadirachtin concentration (1%) gave the highest mortality. Nevertheless, the order of toxicity of different azadirachtin concentrations was found to be 1% > 0.6% > 0.5% > 0.31% (Table 2). Nonetheless, lowest percent mortality was observed for control array i.e., 0-10% for 3rd instar larvae and 0-5% for 4th instar larvae after 24, 48 and 72 hours. Similarly the LC_{50} values in figure 2 of azadirachtin concentrations were found on the decreasing trend with the increase time of exposer. Maximum LC_{50} values of azadirachtin concentrations in the no choice test on day 1st were 0.63 ppm which was decreased on day 2nd and day 3rd to 0.32 to 0.29 ppm respectively.

Concentration (%)	Percent Mortality of 3rd instar			Percent Mortality of 4th instar		
	24 h	48 h	72 h	24 h	48 h	72 h
0.31	30 b	40 b	45 c	45 b	50 b	55 b
0.5	40 ab	60 ab	60 bc	40 b	60 b	65 b
0.6	60 a	70 a	80 ab	55 ab	65 b	80 a
1	60 a	75 a	95 a	75 a	85 a	95 a
LSD value (0.05)	29.5	21.32	20.86	24.91	16.51	15.07

Table 1: Percent mortality of *P. xylostella* from different azadirachtin concentrations (Choice method).

Means in columns followed by different letters are significantly different at α = 0.05 level of significance by using Fischer's Protected LSD test.

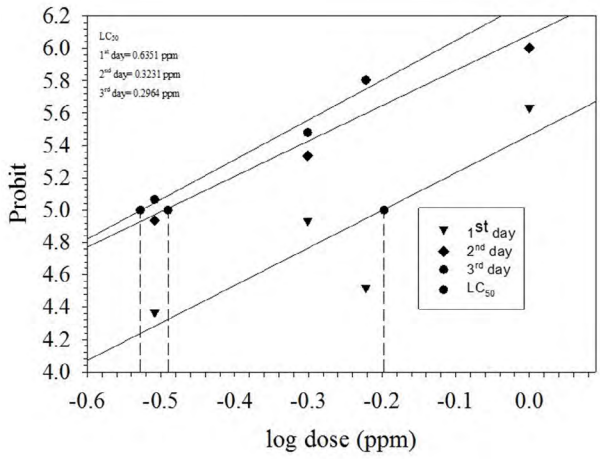

Figure 3: Graph showing the LC_{50} values of azadirachtin concentrations against 3rd instar larvae of *P. xylostella* (L.) in no choice test.

Repellency test

Maximum repellency (95%) was noticed for 3rd instar larvae at 1% concentration, which was at par with 0.6% concentration (90%) but significantly different from all other concentrations including control. Likewise, for 4th instar larvae, maximum repellency was observed at 1% concentration whilst all other concentrations including control

attracted maximum numbers of 4th instar larvae. Consequently, the repellency effects of all concentrations were significantly different from each other for both 3rd and 4th instar larvae of *P. xylostella*. Nevertheless, 0.6-1% concentration gave statistically non-significant results in both the cases (Table 3).

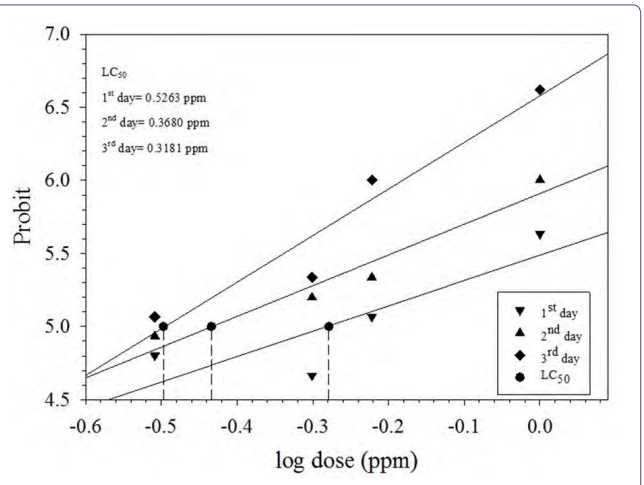

Figure 4: Graph showing the LC_{50} values of azadirachtin concentrations against 4th instar larvae of *P. xylostella* (L.) in no choice test.

Concentration (%)	Percent Mortality of 3rd instar			Percent Mortality of 4th instar		
	24 h	48 h	72 h	24 h	48 h	72 h
0	0 c	5 d	10 d	0 c	0 c	5 c
0.31	30 b	50 c	55 c	45 b	50 b	55 b
0.5	50 b	65 bc	70 bc	40 b	60 b	65 b
0.6	35 b	80 ab	80 b	55 ab	65 b	85 a
1	75 a	85 a	100 a	75 a	85 a	95 a
LSD value (0.05)	21.31	17.83	16.96	24.91	16.51	15.07

Table 2: Percent mortality of *P. xylostella* (No choice method) from different azadirachtin concentrations.

Means in columns followed by different letters are significantly different at α = 0.05 level of significance by using Fischer's Protected LSD test.

Concentration (%)	Repellency effect against 3rd instar larvae (%)	Repellency effect against 4th instar larvae (%)
0	0 c	0 d
0.31	60 b	40 c
0.5	65 b	75 b
0.6	90 a	85 ab
1	95 a	100 a
LSD value (0.05)	16.51	21.31

Table 3: Repellency effect (%) of different azadirachtin concentrations on the 3rd and 4th instars larvae of *P. xylostella* (L.)

Means in columns followed by different letters are significantly different at α = 0.05 level of significance by using Fischer's Protected LSD test.

Percent adult mortality of *Diadegma* sp

The effect of these different azadirachtin concentrations were also evaluated on *P. xylostella* natural enemy (larval parasitoid) i.e., *Diadegma* sp by exposing its adults for 24 and 48 hours. Maximum percent adult mortalities were noticed for 1% and 0.6% concentrations which were 17.50% and 15.00% respectively after 24 hours, whilst no mortality was observed for 0.31% concentration and control (Figure 5). Likewise, maximum percent mortality (27.50%) was noticed for 1% concentration which was significantly different from all other concentrations after 48 hour exposure to the treated leaves. Lowest percent mortalities were recorded for 0.31 and 0.5% (2.50 and 7.50% respectively) which were not far part with each other but differed significantly from 0.6, 1% and control (15.00%, 27.50% and 0.00%) (Table 4). The data in figure 5 showed that LC_{50} value on day 1st was 4.91 ppm which was reduced to 1.63 ppm on day 2nd.

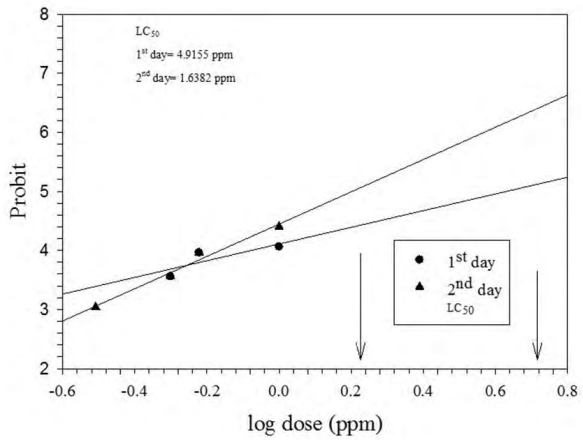

Figure 5: Graph showing the LC_{50} values of azadirachtin concentrations against adults of *Didegma* sp after 24 and 48 hours.

Concentration (%)r	Percent Mortality after 24 h (%)	Percent Mortality after 48 h (%)	Mean (%)
0.31	0.00 c	2.50 cd	1.25
0.5	7.50 b	7.50 c	7.5
0.6	15.00 a	15.00 b	15
1	17.50 a	27.50 a	22.5
Control	0.00 c	0.00 d	0
LSD value (0.05)	6.15	7.01	----

Table 4: Percent adult mortality of *Diadegma* sp after 24 and 48 hours exposure to various azadirachtin concentrations.

Means in columns followed by different letters are significantly different at α = 0.05 level of significance by using Fischer's Protected LSD test.

Percent adult emergence of *Diadegma* sp

Effect of the concentrations was also evaluated to discern the percent adult emergence of *Diadegma* sp after 24, 48 and 72 hours exposure. Maximum percent adult emergence was recorded in control (60.00%) which was significantly different from all other treatments, whilst the rest of the treatments did not give significant results after 24 hours. After 48 hours exposure, a high percent adult emergence

was observed in control and for 0.31% concentration i.e., 82.50% and 77.50% respectively which were at par but differed significantly from all other treatments, whilst the rest of the concentrations did not give significant results (Table 5). Likewise, higher number of adults emerged in 0.31% and control (90.00% and 97.50%) which were at par with each other but differed significantly from all other treatments after 72 hour of exposure to azadirachtin concentrations.

Concentrations (%)	Emergence after 24 h (%)	Emergence after 48 h (%)	Emergence after 72 h (%)	Mean (%)
0.31	35.00 b	77.50 a	90.00 a	67.5
0.5	30.00 b	40.00 b	60.00 b	43.33
0.6	27.50 b	35.00 b	57.50 b	40
1	22.50 b	27.50 b	52.50 b	34.17
Control	60.00 a	82.50 a	97.50 a	80
LSD value (0.05)	12.61	16.16	13.62	---

Table 5: Percent adult emergence of Diadegma sp from pupae after 24, 48 & 72 hours of exposure to different azadirachtin concentrations.

Means in columns followed by different letters are significantly different at α = 0.05 level of significance by using Fischer's Protected LSD test.

Discussion

Biorational control of insect pests is emerging as an important tool for pest management in an economically and ecologically sound way [23]. Azadirachtin has been proven as a natural insect growth regulator, repellent, anti-feedant, and feeding deterrent for many insects [24]. Present findings depicted that these different azadirachtin concentrations were toxic to all larval instars of *P. xylostella*, and all larvae died before pupation. Treated larvae died slowly, and the larvae were able to cause considerable foliage damage when older and larger instars were treated. Our findings are in accordance with findings of, who reported that azadirachtin-based insecticide-treated leaves can reduce survivorship in *lepidopteran* insects. This has been also reported in the case of *A. indica* and for *L. camara* [25-28]. In *A. indica* the insecticidal property is due to the triterpernoids, azadirachtin and salanin these triterpenoids are known for their antifeedant properties [28,29]. Once ingested, their effects are to prevent food utilization by susceptible insects and therefore mortality results from starvation.

Order of toxicity of different azadirachtin concentrations was found to be 1% > 0.6% > 0.5% > 0.31%. Additionally, time of exposure also increased the lethal and repellent activity of azadirachtin. The results are in accordance with the findings of as they reported insecticidal and repellent activities of plant parts increased with increase in dose and time. In the present study, it was further concluded that a 0.6-1% concentration would be the highest and most appropriate dosage for the management of *P. xylostella* because no phytotoxic effects were found in the treated plants after 24 hour application of this extract. Similarly, these compounds had lethal effects on diamondback moth larvae and neem oil reduced egg hatching and survival larvae of *H. armigera* [30,31]. Likewise, repellency increased with increase in concentration for 3rd instar larvae. Similarly, for 4th instar larvae, maximum repellency was observed at 1% concentration whilst all other concentrations including control attracted maximum number of 4th instar larvae.

Percent adult mortality of *Diadegema* sp

Similar pattern of mortality was noticed for *Diadegma* sp viz. insecticidal and repellent activities of plant parts increased with increase in dose and time. Similar results were reported by for adult *Diadegma semiclausum* exposed to aqueous neem seed extracts [32].

It is usually assumed that parasitoids life history (e.g., growth, survival, and adult emergence) respond directly to host properties such as body size [33,34]. A variety of studies has shown that host size influences parasitoid size and survival, and that parasitoid size is positively correlated with other fitness factors such as fecundity, mating capacity and longevity [35].

The *P. xylostella* feeding on broccoli plants treated with azadirachtin are smaller than those that have been feeding on control plants [36]. The development time of *Diadegma* sp would therefore be significantly prolonged if it were to wait for the host to develop a larger size. Results from this study confirmed that the time until cocoon formation of *Diadegma* sp is significantly longer if they have been feeding on *P. xylostella* that have been exposed to botanical compounds. This may indicate that there is some delay in development time of *Diadegma* sp feeding on larvae that have been exposed to the botanical pesticides, which would ensure that *P. xylostella* had increased sufficiently in size.

With further demonstration of their efficacies, these concentrations should enhance the management of *lepidopteran* pests in the vegetable agroecosystems because they do not persist in the environment, have unique modes of action, low mammalian toxicity, and may be potentially compatible with natural enemies. Azadirachtin extracts seem to be suitable for combination with biological control, as these extracts did not have a detrimental effect on longevity or behaviour of *Diadegma* sp, one of the most abundant parasitoid species found in the field in Australia.

Conclusion

Among four concentrations, 0.31, 0.50, 0.60 and 1% of azadirachtin product, lowest percent mortalities of 3rd and 4th instar larvae of *P. xylostella* were observed for 0.31% in choice and no choice method. The effects of various concentration of azadirachtin on adult emergence from pupae and adult mortality of *Diadegma* sp were also studied after 24, 48 and 72 hours exposure. Among all other concentrations, 0.31% was more effective for all the parameters studied for the management of *P. xylostella* and its associated natural enemy, *Diadegma* sp., under laboratory conditions. The use of plant extracts with insecticidal properties has the potential of reducing the effects of insect pests of agricultural crops. This research work can be of great importance to the resource-poor farming community in many areas of the developing world. The significant reduction in pest numbers on treated plants was an indication that they can be used as alternatives to chemical insecticides. Even though various pest species attacked the cabbage plants, *P. xylostella* caused the most serious damage and is the main cause of reduction in weight of cabbage heads.

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