Effect of Different Neem Extracts on Plutella Xylostella (Lepidoptera; Plutellidae) Under Laboratory Conditions

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Abstract

Effect of different Azadirachtin concentrations were studied on larval/pupal weight, oviposition, hatching and antifeedant effect on Plutella xylostella. The reductions in larval weight were 56.43-68.20% caused by 0.31, 0.5, 0.6 and 1% of azadirachtin, whilst pupal weight reduced by about 24.47-46.77%. The decrease in larval and pupal weight was significant among the different concentrations of azadirachtin. At all concentrations tested, mean egg laying was reduced significantly from 200.25 to 53.75 when compared to the control and mean egg hatch was significantly reduced from 271.5 in the controls to 18.75 in treated samples. Non significant maximum antifeedant index (AFI) were recorded for 1, 0.6 and 0.5% concentrations whilst the lowest was 0.31% in choice method for 3rd instar larvae. Fourth instar larvae almost showed similar trend to different concentrations of azadirachtin. In no choice method, maximum AFI for 3rd instar larvae were observed on 1% concentration which was significantly different from 0.31% concentration, however for 4th instar larvae, AFI showed non-significant trend.

Keywords: Plutella xylostella ; Azadirachtin ; concentrations.

Introduction

Broccoli is one of the most important vegetable crops in Australia and diamondback moth, Plutella xylostella L, is serious pest and played a vital role in curtailting the production of cruciferous crops in all major growing areas of the world [1]. Different insecticides are applied for the management of this pest. Losses due the infestation of P. xylostella have been estimated up to 90% in the areas of their severe outbreak [2]. Nonetheless, the economic losses due to this pest has been reported worldwide up to US$4- $5 billion [3]. Besides, US $1.0 billion is spent annually for the management of this pest globally [4]. P. xylostella is the first insect reported that have developed resistance to different insecticides [5] due to their genetic elasticity to chemical insecticides [6] and as result its control with insecticides is becoming more difficult [7].

Alternative method for efficient control of this pest may be natural plant extracts which are more effectively used for sustainable management of this pest [8]. These extracts comprised complex mixture of active compounds. Besides, there is less possibilities for pest to develop resistance against such mixture of chemicals [9, 10]. Product of neem tree, Azadirachta indica (Meliaeaceae), has been reported to contain a huge amount of chemicals compounds. Extract from the kernels and seeds have been reported to negatively influence the biology of many pests [2, 11, 12, 13, 14]. The most dynamic compound in the neem is ‘azadirachtin (AZA)’ which has been reported to generate various effects such as insecticidal activity, oviposition deterrent, growth retardant, molting inhibitor, antifeedant and sterilant etc [15]. Research studies have showed the usefulness of neem seed and kernels extracts for the effective management of P. xylostella throughout the world [16, 17, 18, 2, 19, 20]. However, the effect of leaf extracts on P. xylostella has been reported by few scientists. Keeping these points in view the present laboratory work was initiated to evaluate the effect of neem leaf extracts on development and survival of P. xylostella.

Materials and Methods

Host Raising of Broccoli, Brassica oleracea Italica, in Pot

Fifteen days old broccoli seedlings and pot mixture were moved to the Horticulture Section, School of Agriculture and Food Sciences (SAFS), University of Queensland, Gatton on July 8, 2014. Pot mixture transferred to the 50 pots and a single broccoli seedling in each pot. Pots were then transferred to Bay-8 inside the glasshouse and were kept at a distance of about 35 to 45 cm from each other. After transferring pots into the glasshouse, water was given to the young transferred broccoli seedlings through hand held hose. Broccolis were then observed regularly and water was provided as per plant requirement. These potted broccolis were then used for mass production of P. xylostella in insectary.

Rearing of P. xylostella in the laboratory

Stock population of P. xylostella larvae used in this study pupae were collected from broccoli (Brassica oleracea var Italica) crop in University of Queensland, Gatton Campus. To maintain stock culture of P. xylostella, pupae collected from the research field and transferred to rearing cages in insect rearing room on different potted broccoli plants already raised in a controlled environment of the glasshouse. Rearing was maintained in a controlled room at photoperiod of 16:08 (light: dark) hours, temperature 25±2°C and 55±07% relative humidity in the insect rearing room by using the modified rearing procedure of [21]. Pupae were held in lab in wooden-framed net cages (45 x 45 x 45 cm3) with cotton swabs containing a 10% sugar solution were available for the emerging adults and broccoli seedling for egg laying. They were kept caged until adults emerged and mated.

After females oviposited, seedlings with eggs were removed from
the cages and placed into different cages until the eggs hatched. The seedlings with newly-hatched larvae were transferred to big cages (120 x 60 x 60 cm3) 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 to be kept at 4°C until needed to maintain the population.

Plant Material

For the research, neem, Azadirachta indica insecticidal chemicals in 0.31, 0.50, 0.60 and 1% extracts dilutions were provided by Bio-Aust Stafford Heights Queensland 4053.

Effect on larval and pupal weight (mg)

To determine the effect of neem extracts on growth inhibition of P. xylostella larvae, ten third instars larvae in each treatment were exposed using the leaf dip test and the ultimate effect on larval and pupal weights (mg) was observed.

Effect on oviposition and hatching

From 60 days old broccoli seedling, leaves were detached and dipped into one of five different experimental concentrations. Each impregnated leaf was put in the small container which held some water to maintain the moisture. The detached leaves were put in a container (3 cm in diameter, 11 cm in height) together with a cotton swab moistened with 10% of honey solution. Two female and two male adults (3-4 days old) were released in the container and each treatment was replicated three times, giving a total of 60 adults used for these experiments. The total numbers of eggs deposited on the leaves were counted after 3 days and then the numbers of hatched eggs (F1) were recorded after 3 days.

Antifeedant Effect (Larval Stage)

Treated leaf discs (3 cm in diameter) with various concentrations were placed on a tray and left in a fume cabinet for 2 hours to evaporate any remaining solvents. For each treatment there were four leaf disc replicates; a total 12 same age larvae were used in each treatment. After being starved for 4 hours, the third instars of P. xylostella were exposed to the extracts impregnated leaf discs with choice and no-choice options. For the choice method, 3 larvae of the third instar were placed on treated and untreated/control leaf discs which were arranged around a plastic container, while for the no-choice method, the treated leaf discs and controls were placed in separate plastic containers (9 cm dia; 6cm height). After 24 hours, larvae were removed and faces brushed from the leaf discs, then the leftover leaf discs surfaces were placed on a piece of paper for digitizing the form of the leaf disc by using an Epson scanner at 300 dpi. After that, damage areas were measured using a computer programme, Image J.4.1 (open sources program on http://rsb.info.nih.gov/j)). The antifeedant index (AFI) for the choice method was calculated by means of the equations (C-T)/(C+T) * 100, and for the no-choice method (C-T)/C*100%, where C and T (mm2) denoted the consumed area of control and treated discs respectively [22].

Data Analysis

All the replicated data were statistically analyzed by using analysis of variance (ANOVA) technique appropriate for completely randomized design (CRD) [23] by using a statistical software “Statistics 8.1” version. The significant means were separated by Fischer’sprotected least significant difference test (LSD) after a significant F-test at a 0.05 level of probability.

Results and Discussion

Effect on larval and pupal weight

A dose dependent decrease was observed in larval and pupal weights (Table-1). In general, as the concentration of neem extracts increased, larval and pupal weight decreased. For the larval stage, there was about 56.43-68.20% reduction in weight caused by 0.31, 0.5, 0.6 and 1% concentrations. For the pupal stage, the weights were reduced by about 24.47-46.77%. The decrease in larval and pupal weight was significant among the different neem concentrations.

Table 1: Effect of different Neem Concentrations on Larval and pupal Weights (milligram) of P. xylostella (L.)

<table>
<thead>
<tr>
<th>Concentrations (%)</th>
<th>Larval wt ±SE (mg)</th>
<th>Larval wt decrease over control (%)</th>
<th>Pupal wt ±SE (mg)</th>
<th>Pupal wt decrease over control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.91±0.04 a</td>
<td>---</td>
<td>6.30±0.09 a</td>
<td>---</td>
</tr>
<tr>
<td>0.31</td>
<td>1.70±0.14 b</td>
<td>56.43</td>
<td>4.76±0.13 b</td>
<td>24.47</td>
</tr>
<tr>
<td>0.5</td>
<td>1.44±0.02 c</td>
<td>63.28</td>
<td>4.11±0.13 c</td>
<td>29.99</td>
</tr>
<tr>
<td>0.6</td>
<td>1.40±0.07 c</td>
<td>64.24</td>
<td>4.18±0.02 c</td>
<td>33.76</td>
</tr>
<tr>
<td>1</td>
<td>1.24±0.04 c</td>
<td>68.20</td>
<td>3.36±0.09 d</td>
<td>46.77</td>
</tr>
<tr>
<td>LSD value (0.05 )</td>
<td>0.23</td>
<td>---</td>
<td>0.31</td>
<td>---</td>
</tr>
</tbody>
</table>

For each larval and pupal weight means with sd (standard deviation) with the same letter are not significantly different (Least Significant Difference test, p<0.05)

Effects of various neem extracts on P. xylostella larvae and pupal weight have been well documented [27]. Generally, azadirachtin extracts or azadirachtin-based insecticides are effective against P. xylostella with significant lethal and antifeedant effects accompanied by significant reduction in food consumption and ultimately on their weight. These azadirachtin concentrations were toxic to all larval instars and pupae. A different economic threshold for the major lepidopterous pests may be needed when azadirachtin-based or other bioinsecticides are the primary components in the management programs. The effects on different developmental stages of P. xylostella were not always significantly different, although their azadirachtin contents vary greatly: 0.15% in Agroneem, 0.25% in Neemix, and 3% in Ecozin. Nevertheless, it has been proved that biological activity of azadirachtin-based insecticides cannot be solely judged by the azadirachtin content and azadirachtin extracts can contain numerous other chemicals, i.e. aflatoxins, salamin, nimbandiol, etc. that also have insecticidal activities and cause a great reduction in larval and pupal weight [28]. In addition, differences in extraction process, formulation solvent and adjuvant can dramatically influence toxicity and biological activity [28,29].

Effect of oviposition and hatching

Increasing concentrations of neem oil decreased egg production and subsequent larval emergence as adults which developed from treated larvae. At all concentrations tested, mean egg laying was reduced significantly from 53.75 to 200.25 when compared to the control and mean egg hatch was significantly reduced from 271.75 in the controls to 18.75 in treated samples (Table-2).

There are few published results available on the reproduction-inhib-
iting effects of L. petersonii oil on P. xylostella. The chemical components responsible for this activity have not been investigated further. A group of chemicals likely to be involved in this activity include neral, which was reported as being an oviposition deterrent effect on the leaf-hopper, Amrasca devastans (Distant.) (Hemiptera: Cicadellidae) [30]. The neem oil had potential insecticidal action since it was observed in this study that at 1%, this oil caused more than 50% mortality of P. xylostella. It produced strong antifeedant effects and insect growth inhibition in P. xylostella. In addition, the adult survivors produced less egg than the control insects.

Other researchers also concluded that compounds in the neem extracts other than azadirachtin might be responsible for oviposition deterrent effects [28, 31, 32]. Suggested that non-volatile neem components contain primarily azadirachtin, appeared to be associated with azadirachtin concentration.

Neem extracts and Neem-based insecticides appear to significantly deter oviposition many lepidopterous pests [33, 34, 35, 36] found that neem extracts reduced P. xylostella oviposition [37] found that the azadirachtin extract Margosan-O exerted a significant inhibitory effect on the oviposition of P. xylostella. Nonetheless, [38] reported that cabbage plants treated with neem seed kernel water extracts were more attractive for egg-laying by P. xylostella both in the laboratory and in the field. The possible explanation may be that P. xylostella moths were attracted to cabbage leaves by their stronger volatile that overshadows the odor of neem and deposited more eggs.

**Antifeedant index choice and no choice**

Maximum Anti Feeding Index (AFI) was recorded for 1% concentration whilst the lower was observed for 0.31% for 3rd instar larvae in the choice experiment and same situation were noticed for 4th instar larvae (Table-3).

In the no choice experiment, maximum area of the untreated leaf was consumed by the 3rd instar larvae followed by leaves treated with neem concentrations 0.31, 0.5, 0.6 and 1% which were 0.12, 0.08, 0.01 and 0.00 cm respectively (Table 4). High AFI were recorded for 1 and 0.6% concentration whilst the lowest was for 0.31% in the experiment. Likewise, 4th instar larvae also behave in the same way like 3rd instar larvae in the no choice experiment after 24 hours exposure to the leaves treated with different neem concentrations. Maximum areas of the leaves were consumed of untreated leaves whilst lowest areas of the leaves were consumed treated with 0.31% concentration. AFI for the 4th instar larvae showed that no significant of different neem concentrations effect has been noticed on 4th instar larvae.

| Table 2: Effect of different Neem Concentrations on oviposition and hatching of P. xylostella (L). |
|-----------------|-----------------|-----------------|
| Concentrations (%) | Mean # Eggs | Mean eggs hatched |
| 0  | 272.50 a | 271.75 a |
| 0.31 | 200.25 b | 153.25 b |
| 0.5 | 160.25 c | 105.50 c |
| 0.6 | 97.50 d | 70.75 d e |
| 1  | 53.75 e | 18.75 d |
| LSD value (0.05 ) | 22.87 | 19.24 |

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

| Table 3: Antifeedant Index (AFI) for 3rd and 4th instar larvae of P. xylostella after 24 hours exposure to different neem concentrations treated leaves (Choice Method). |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Concentrations (%) | Area consumed by 3rd instar larvae (T) (cm) | Area consumed in (C) (cm) | AFI for 3rd instar | Area consumed by 4th instar larvae (T) (cm) | Area consumed in (C) (cm) | AFI for 4th instar |
| 0.31 | 0.42 | 1.95 | 63.39 b | 0.28 | 2.87 | 82.34 b |
| 0.5 | 0.12 | 2.73 | 91.73 a | 0.30 | 2.78 | 80.52 b |
| 0.6 | 0.03 | 2.78 | 97.86 a | 0.15 | 2.84 | 90.29 ab |
| 1 | 0.00 | 2.55 | 99.80 a | 0.00 | 2.95 | 99.66 a |
| LSD value (0.05 ) | --- | --- | 18.47 | --- | --- | 16.97 |

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

| Table 4: Antifeedant Index (AFI) for 3rd and 4th instar larvae of P. xylostella after 24 hours exposure to different neem concentrations treated leaves (No Choice Method). |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Concentrations (%) | Area consumed by 3rd instar larvae (T) (cm) | AFI for 3rd instar larvae | Area consumed by 4th instar larvae (T) (cm) | AFI for 4th instar larvae |
| 0.31 | 0.12 | 96.01 b | 0.1 | 96.42 a |
| 0.5 | 0.08 | 97.40 ab | 0.15 | 94.94 a |
| 0.6 | 0.01 | 99.74 a | 0.23 | 92.06 a |
| 1 | 0 | 99.91 a | 0 | 100.00 a |
| Control | 2.88 | --- | 2.87 | --- |
| LSD value (0.05 ) | --- | 2.73 | --- | 9.19 |

Means in columns followed by different letters are significantly different at α = 0.05 level of significance by using Fischer’s Protected LSD test.
An antifeedant effect on larvae of the diamondback moth, *P. xylostella* was observed on Agroneem, Ecozin and Neemix treated cabbage, Brascica oleracea leaves and larvae fed on neem-based insecticide-treated leaves were smaller than those fed on non-treated controls [20, 39] reported that aqueous plant extract against *P. brassicae*, at different concentrations caused 3.2 to 81.8 percent protection to the foliage over control [39]. Extract of *A. indica* resulted in maximum protection to foliage against *P. brassicae*. However, [41] reported feeding inhibition up to 90 percent against the third instar larva of Leptinotarsa decemlineata with crude leaf extract of *A. indica*. Also reported feeding deterrent activity of aqueous extract of *M. azedarach* and *A. indica* against the larvae of *P. xylostella*. The strategy that can include antifeedants is the stimulodeteter variantion strategy (SDDS), sometimes called the “push-pull” strategy [43]. Further work is however required to fully understand its mode of action. It is now accepted that neem have a wide margin of safety for both user and consumer. Antifeedant effects of neem products have been reported to persist after replacement of treated leaves with untreated ones' and similar effects were noted in the present work. These observations, together with evidence from topical application and injection studies, suggest that neem products may possess anorectic activity. 

A reduced rate of feeding in the presence of neem extracts may also explain the time taken (48-120 h) to accumulate a dose lethal to the majority of the population. Some studies have reported habitation as a reason to feed but have not been able to distinguish insects that accept treated food or the ability of insects to reduce the chemical responsible for their aversive behavior more readily because they were hungry [44] based on this reason, it suggests thathabitation might occur during this stage. Habitation or the ability of insect to reduce their averesive behavioral response to unpleasan or toxic compounds in a plant can be recorded if the insect is repeatedly exposed to toxic plant over a period of time [45].

It is concluded that, these concentrations should enhance the management of lepidopterous 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. Neem extracts seem to be suitable for combination with biological control, as these extracts have a profound effect on larval pupal weight, oviposition and hatching and also on antifeedant effect.

Conclusions

These various concentration of neem were have a profound effect on larval and pupal weights (milligram) reduction of *P. xylostella*, oviposition and hatching, antifeedant index for 3rd and 4th instar larvae of *P. xylostella* after 24 hours exposure (choice & no choice method). This study indicates the potentialities of neem plant extracts for the management of *P. xylostella*. Neem plants are locally available in Australia, having plenty of leaves and as such preparing extract of leaves will be economical and sustainable for the protection of agricultural crop. These research works can be of great importance for the farming community in many areas of the developing world. Nevertheless, further research is required to isolate the active components of foliage extract for their effective use in the IPM of this insect pest.

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