In Vitro Management of Diamondback Moth (*Plutella xylostella* L.) Using Different Concentrations of Parthenium and Neem Extracts

B. Ahmad1, N. Mehmood1*, K. Sohail2, A. U. R. Saljoqi1, A. Khan1, A. Rab3, H. Zada1, and S. Hussain1

ABSTRACT

The study investigated the toxicity of Parthenium extracts in comparison with Neem extracts against 3rd and 4th instar larvae of the diamondback moth, *Plutella xylostella* L.. The side effect of Parthenium extracts was also examined against larval parasitoid, *Cotesia plutellae*. Extracts obtained from their respective plants were dissolved independently to get stock solutions, which were further diluted to different concentrations (1%, 2%, and 3%) with distilled water. The experiment was laid out in completely randomized design with four treatments, including the control, and replicated six times in vitro. Results showed that Parthenium crude extracts was very effective in comparison with the control against *P. xylostella*, whilst the highest mortality was recorded at 3% concentration of Neem in comparison with Parthenium extracts against both stages of *P. xylostella*. Overall, the highest mortality was recorded at 3% concentration of Neem extracts followed by 2% Neem extracts, 3% Parthenium extracts, 1% neem, 2% and 1% Parthenium extracts. The lowest mortality was noted in the control (i.e. 70%, 58.33%, 51.67%, 35%, 33.33%, 18.33% and 16.67% in case of 3rd instar mortality whilst 61.67%, 50%, 41.67%, 30%, 16.67% and 15% in case of 4th instar mortality, respectively). Less harmful effect was observed for larval parasitoid, *C. plutellae*. Finally, it was concluded that all the treatments had the ability to control *P. xylostella* to some extent, but Parthenium crude extract was less efficient in comparison with Neem extract, as 3% crude extract of Neem had a sufficiently toxic effect on the *P. xylostella*.

**Keywords:** Botanical insecticide, Cabbage, *Cotesia plutellae*, Larval parasitoid, Toxicity

INTRODUCTION

Cabbage, *Brassica oleracea* var. capitata L. is one of several vegetables belongs to the family Brassicaceae (also called Cruciferace). It is important cash crop of Pakistan and grown for edible purpose. It is grown as a winter crop as well as summer vegetable (Qamar et al., 2014). During vegetative growth, this crop is attacked by several numbers of insect pests including whitefly, armyworm, cabbage looper, cabbage butterfly and the most serious pest is the *P. xylostella* (Shuaib et al., 2007). The *P. xylostella*, (Lepidoptera: Plutellidae) is the most destructive insect pest of crucifer crops such as cabbage, cauliflower, collard, radish, mustard and turnip worldwide (Furlong et al., 2013).

*P. xylostella* has worldwide distribution, including Southeast Asia, North America, New Zealand, and Europe. In Southern Sindh (Hyderabad and Karachi region) of Pakistan, it is very destructive pest where...
cruciferous crops are grown all over the year (Abro et al., 1994). It causes damage throughout the year excluding the rainy period (Talekar and Lee, 1985). The first instars larvae generally mine the spongy mesophyll tissues (Capinera, 2000). Second, third, and fourth instars are surface feeders and they devour leaves, flowers, buds, and siliques. The third and fourth instars are big feeders and cause the cruelest damage commonly creating window-pane damage on leaves. Globally, *P. xylostella* annual management costs, in addition to the crop losses, requires a predictable US$1.0 billion (Talekar and Shelton, 1993).

Numerous predators and parasitoids attack on different stages of *P. xylostella*. *Trichogramma chilonis* is an important egg parasitoid that is reported to be mostly effective against *P. xylostella* (Miura and Kobayashi, 1998). The two main genera *Cotesia* and *Diadegma* are the most effective larval parasitoids of *P. xylostella* (Liu et al., 2000). *Chrysoperla carnea* and *C. plutellae* have been used in an IPM program to control diamondback moth in cabbage crop (Reddy and Guerrero, 2000).

The continuous use of synthetic pesticides causes development of resistance in insect pests, disturbance in natural biological control systems, adverse effects on non-target organisms, and reappearance of target insect species (Papachristos and Milonas, 2008). Sarfraz and Keddie (2005) also stated that *P. xylostella* had developed resistance to different insecticides. Therefore, a new way to manage *P. xylostella* is botanical insecticides. Several plants have insecticidal active ingredients. Dev and Koul (1997) stated that 60 families with 2000 species of plants have active ingredients that are chemical insecticides. The secondary metabolites of plants that act as insecticides are called botanical insecticides (Martin and Gopalakrishnan, 2005). For the control of destructive insect pests, Azadirachtin, Pyrethrum, Nicotine, and Rotenone are the successful plant products that have been examined (Saxena, 1998). Due to the multiactive principles, insects have less chances of resistance to botanical insecticides (Govindarajan et al., 2011).

*Parthenium hysterophorus* L. (Asteraceae) is commonly known as white head, white top, carrot grass, or congress grass. It is a serious weed of wastelands, grazing land and agricultural field in the world (Aneja et al., 1991). It has exposed several well-known biological activities. The sesquiterpene lactone parthenin is the key secondary metabolite of *P. hysterophorus* and has insecticidal, phytotoxic, and antifeedant properties. The latest reports recommend that Parthenin can be used in pathogen and pest control (Fazal et al., 2011). The extracts of *P. hysterophorus* have the capability to act as antifeedent, which reduce the damage caused by destructive insect pests (Datta and Saxena, 2001).

Neem tree, *Azadirachta indica* is usually known as Neem or Dharak found in Indo-Pak subcontinent. In southern areas (Sindh and Punjab) of Pakistan, Neem tree is found. They have more than 100 chemical compounds, of which the more effective bioactive compound is *Azadirachtin*. *Azadirachtin* is a triterpenoid extracted from Neem seed and its oil is used as a botanical insecticide with repellent, antifeedant, and normal growth disrupting activities. Neem products also affect the fecundity potential of insect pests (Schmutterer, 1990).

Due to pesticidal properties of Parthenium and Neem, both can be an alternative to synthetic pesticides, therefore, the aim of the present study was to assess the efficacy of Parthenium and Neem extracts against *P. xylostella* under standard laboratory conditions.

**MATERIALS AND METHODS**

The present investigation was carried out in the laboratory of Plant Protection, The University of Agriculture Peshawar and in the National Agricultural Research Center (NARC), Islamabad, during 2015.
Development and Maintenance of Laboratory Culture of P. xylostella

Culture of P. xylostella was maintained on host plant cabbage based on the method described by Golizadeh et al. (2009) with some modifications. Initially, cabbage was planted in pots in a greenhouse and seeds were sown in suitable soil of compost mixtures in seedling flats. After five weeks (plants with six to eight leaves), each seedling was transplanted in 20 cm diameter plastic pot. Population of P. xylostella larvae was collected from field. The stock culture of P. xylostella was initiated on potted host plant and was maintained at 25±1 °C and 65±5% relative humidity (RH) with photoperiod of 12:12 (L: D) hours in growth chamber. Leaves of host plants were put inside an oviposition cage containing both sexes of the moths reared on the corresponding host plant. The oviposition cage was a clear cubic Plexiglass container (35×35×35 cm), the top and sides of which were cut off and replaced with fine mesh gauze coverings. One side of the cage was modified so that it could be opened for either insertion or removal of plant leaves, moths, as well as feed. Ten percent honey solution was placed in the oviposition chamber for adults feeding.

Botanical Extracts

Collection and Preparation of Parthenium and Neem Extracts

Parthenium and Neem extracts were prepared by the procedure described by Khan et al. (2014) and Badshah et al. (2015) with some modifications. Extraction was carried out in the laboratory. About 50 g of ground sample of each plant was mixed with 300 mL of distilled water in electric blender and stirred for an hour continuously and then each extract was allowed to stand for at least another hour. After this resting period, each extract was mixed vigorously and then filtered by Whatman 42 filter paper. Afterwards, each filtrate was mixed again with 100 mL of distilled water, shaked for 30 minutes and then filtered. All the filtrates were then mixed. The water contents from this pooled filtered solution were evaporated in a water bath at 65 °C. After complete evaporation of the final crude extracts, 20 g in the form of a paste were mixed with 100 mL of distilled water and mixed 2 mL of 1% detergent (Arial) solution as emulsifier (Amaugo and Emosairue, 2003). This solution corresponded to 20% of water extract and was stored in the refrigerator as stock solution. Further concentrations of 1%, 2%, and 3% (V/V) of the stock solution were prepared by dilution.

Percent Corrected Mortality

Mortality observations were made after 24, 48, 72 and 96 hr by counting the number of dead insects. For this experiment, procedure of Akbar et al. (2014) was followed with some modifications. Percent corrected mortality was calculated in comparison with the control by using Abbott’s formula (Abbott, 1925).

Experimental Procedure

Bioassay of 3rd and 4th Larval Instars Mortality of P. xylostella with Parthenium and Neem Extracts

The leaf-disc immersion method was used to determine larval toxicity. The experiment was conducted in completely randomized design (CRD) under laboratory conditions. Leaf discs (5 cm in diameter) were treated with their respective doses by dip method for 10 seconds and allowed to dry on blotting paper at room temperature for 10 minutes (Park et al., 2002). The fresh sound leaf discs (1/petri dish) were placed in Petri dishes. Moistened filter paper was placed beneath the leaf disc to avoid desiccation of leaves in the Petri dishes. From the
laboratory maintained culture, ten 3rd and 4th instar *P. xylostella* larvae/petri dish were carefully distributed on the leaf discs evenly using camel hair brush to avoid mechanical injury, and were allowed to forage and settle down properly for at least 24 hr. The experiment was replicated 6 times and was carried out at 25±1 °C with a photoperiod of 12:12 (L: D) and relative humidity of 65±5%.

Mortality was assessed after 24, 48, 72, and 96 hr by counting the number of dead larvae.

### Bioassay of Adult Mortality of *Cotesia plutellae* with Parthenium Extracts

Ten adults of *C. plutellae*, 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 activities. Three different concentrations of Parthenium extracts i.e. 1%, 2%, and 3% were assayed with six replications. A cut filter paper (10 cm in length; 1 cm in width) was dipped in the corresponding concentration suspensions and in the surfactant solution alone (control) for 30 seconds. They were left to dry for 1 hour and then inserted into a glass vial. Ten adults of *C. plutellae* were introduced into each 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 (Purwatiningsih et al., 2012).

### Statistical Analysis

All the data regarding the *P. xylostella* mortality in each treatment were subjected to statistical analysis through analysis of variance (ANOVA) under CRD by using Statistix 8.1 version. All the means were separated by applying the least significant difference (LSD) test at 5% level of probability (Steel and Torrie, 1980).

### RESULTS

The current studies were carried out to determine the effectiveness of Parthenium extracts in comparison with Neem extracts against *P. xylostella*. Results of the findings are described below:

#### Third Larval Instar Mortality

Table 1 disclosed the percent mortality of *P. xylostella* after 24, 48, 72, and 96 hr to various concentrations of Parthenium and Neem seed extracts. The data obtained after 24 hr showed that maximum larval mortality (26.67±2.10%) of *P. xylostella* 3rd instar larvae was recorded in 3% concentration of Neem extracts, followed by 2% Neem concentration (23.33±2.10%), but both of these concentration were statistically at par with each other. Mortality of *P. xylostella* 3rd instar larvae/petri dish was carefully distributed on the leaf discs evenly using camel hair brush to avoid mechanical injury, and were allowed to forage and settle down properly for at least 24 hr. The experiment was replicated 6 times and was carried out at 25±1 °C with a photoperiod of 12:12 (L: D) and relative humidity of 65±5%. Mortality was assessed after 24, 48, 72, and 96 hr by counting the number of dead larvae.

### Table 1. Percentage of mortality of 3rd instar larvae of *P. xylostella* at different time intervals during 2015 (% mortality, mean±SE, n=6).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Concentration (%)</th>
<th>24hrs</th>
<th>48hrs</th>
<th>72hrs</th>
<th>96hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parthenium (crude)</td>
<td>1</td>
<td>8.33±1.66 cd</td>
<td>11.67±2.10 de</td>
<td>16.67±1.66 d</td>
<td>18.33±1.66 d</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>13.33±3.33 c</td>
<td>25.00±3.41 c</td>
<td>33.33±3.33 c</td>
<td>35.00±2.23 c</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>20.00±0.00 b</td>
<td>35.00±2.23 b</td>
<td>48.33±1.66 b</td>
<td>51.67±3.07 b</td>
</tr>
<tr>
<td>Neem (crude)</td>
<td>1</td>
<td>11.67±3.07 c</td>
<td>20.00±3.65 cd</td>
<td>30.00±4.47 c</td>
<td>33.33±4.21 c</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>23.33±2.10 ab</td>
<td>40.00±3.65 ab</td>
<td>53.33±3.33 b</td>
<td>58.33±1.66 b</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>26.67±2.10 a</td>
<td>46.67±2.10 a</td>
<td>63.33±3.33 a</td>
<td>70.00±3.65 a</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>3.33±3.33 d</td>
<td>10.00±3.65 e</td>
<td>15.00±3.41 d</td>
<td>16.67±3.33 d</td>
</tr>
<tr>
<td>LSD at α 0.05</td>
<td></td>
<td>6.570</td>
<td>8.673</td>
<td>9.221</td>
<td>8.559</td>
</tr>
</tbody>
</table>

* Means followed by the same letters are not significantly different from each other at α = 0.05 level by using LSD test.
instar larvae calculated in 3% Parthenium extracts were (20.00±0.00%) followed by 1% Neem, 2%, and 1% Parthenium extracts, respectively. Lowest mortality (3.33±3.33%) of 3rd instar larvae was recorded in the control and was at par to 1% concentration of Parthenium. After 48 hr, maximum mortality was proofed in 3% Neem extract, which was statistically similar to 2% concentration of the same extracts followed by 3% (35.00±2.23%) and 2% Parthenium extracts, 1% Neem extracts, and 1% concentration of the Parthenium, respectively. Nevertheless, minimum mortality was recorded in untreated check (10.00%±3.65) which was at par to Parthenium 1% concentration.

Likewise, on day-3, greater larval mortality was recorded in Neem seed extract 3% concentration, followed by 2% Neem extract, 3% and 2% Parthenium extract, Neem 1%, and 1% concentration of Parthenium water extracts, respectively. While lower mortality was observed in the untreated control. After 96 hours of exposure, significantly highest mortality (70.00±3.65%) was obtained in 3% Neem extract, followed by 2% Neem extract, 3% Parthenium extract, 1% Neem, 2% and 1% concentration of Parthenium extracts, respectively. The lowest mortality was found in the control (16.67±3.33%), which was statistically similar to 1% Parthenium extract. Overall mortality was found as 70% and 51.67% by Neem extract and Parthenium extracts, respectively, for 3% concentration, while for 2% concentration mortality was 58.33% and 35%, and for 1% concentration, it was recorded as 33.33% and 18.33%, respectively. Overall results showed that the Neem extracts caused highest mortalities that were significantly different from Parthenium extracts and the efficacy of those extracts increased when their concentrations increased, because significantly higher mortality was recorded in 3% concentration of both extracts. Moreover, data recorded also showed mostly the highest percentage of mortality in both extracts in comparison with the control.

### Fourth Larval Instar Mortality

The fourth larval instar mortality after 24 hr of the application of various concentrations of Neem and Parthenium extracts is shown in Table 2. The data recorded showed that the highest larval mortality (23.33±2.10 %) was recorded in 3% Neem extracts followed by 2% concentration of the Neem, but both of these concentrations were statistically at par with each other as there was no significant difference recorded. A mortality of 11.67±1.66% was confirmed at 1% concentration of Neem extracts followed by

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Concentration (%)</th>
<th>24hrs</th>
<th>48hrs</th>
<th>72hrs</th>
<th>96hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parthenium (crude extracts)</td>
<td>1</td>
<td>8.33±1.66</td>
<td>11.67±1.66</td>
<td>15.00±2.23</td>
<td>16.67±2.10</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>11.67±1.66</td>
<td>20.00±2.58</td>
<td>26.67±2.10</td>
<td>30.00±3.65</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>13.33±2.10</td>
<td>26.67±2.10</td>
<td>38.33±1.66</td>
<td>41.67±3.07</td>
</tr>
<tr>
<td>Neem (crude extracts)</td>
<td>1</td>
<td>11.67±1.66</td>
<td>20.00±2.58</td>
<td>26.67±3.33</td>
<td>30.00±3.65</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>20.00±2.58</td>
<td>33.33±2.10</td>
<td>45.00±2.23</td>
<td>50.00±2.58</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>23.33±2.10</td>
<td>41.67±3.07</td>
<td>56.67±3.33</td>
<td>61.67±4.01</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>5.00±2.23</td>
<td>10.00±2.58</td>
<td>13.33±3.33</td>
<td>15.00±2.23</td>
</tr>
<tr>
<td>LSD at α 0.05</td>
<td></td>
<td>5.832</td>
<td>6.957</td>
<td>7.715</td>
<td>8.970</td>
</tr>
</tbody>
</table>

*Means followed by the same letters are not significantly different from each other at α = 0.05 level by using LSD test.
Table 3. Percentage of mortality of *C. plutellae* adult in different Parthenium concentrations at different time intervals.

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>24hrs</th>
<th>48hrs</th>
<th>72hrs</th>
<th>96hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.00±2.23ab</td>
<td>8.33±1.67ab</td>
<td>10.00±0.00b</td>
<td>11.67±1.67b</td>
</tr>
<tr>
<td>2</td>
<td>6.67±2.10ab</td>
<td>11.67±1.67ab</td>
<td>15.00±2.23ab</td>
<td>16.67±2.10ab</td>
</tr>
<tr>
<td>3</td>
<td>10.00±0.00a</td>
<td>15.00±2.23a</td>
<td>20.00±3.65a</td>
<td>23.33±3.33a</td>
</tr>
<tr>
<td>Control</td>
<td>3.33±2.10b</td>
<td>6.67±3.33b</td>
<td>8.33±3.07b</td>
<td>10.00±2.58b</td>
</tr>
<tr>
<td>LSD at α 0.05</td>
<td>5.497</td>
<td>6.865</td>
<td>7.773</td>
<td>7.375</td>
</tr>
</tbody>
</table>

*Means followed by the same letters are not significantly different from each other at α = 0.05 level by using LSD test.*
was substantiated as 23.33%, 16.67%, 11.67%, and 10% by Parthenium extracts. Likewise, data further revealed that 2% Parthenium extracts acted like 3%, whereas the control was at par to 1% concentration of Parthenium extracts. Data also disclosed that greater numbers of Cotesia adults were found after 24 hr, and then, mortality slowly decreased up to 96 hr.

**DISCUSSION**

Parthenin is the most important secondary metabolite in *Parthenium hysterophorus* having insecticidal, antifeedant, and phytotoxic properties. Fazal et al. (2011) reported that parthenin could be used for controlling insect pests and diseases. Datta and Saxena (2001) also reported that Parthenin have the ability to act as antifeedant against destructive insect pests. In Pakistan, Ahmad et al. (2011) confirmed that Parthenin have high toxicity against the larvae of *Anopheles* species. Sathish (2008) stated that *P. hysterophorus* showed larvicidal activity against larvae of *Culex quinquefasciatus*.

Azadiractin is the most effective bio-active compound in *Azadirachta indica*. It acts as an antifeedant and repellent, and prolongs the insect’s growth. It is very effective against destructive insect pests and affects the fecundity potential of insect pests (Schmutterer, 1990). Satti et al. (2010) and Sadre et al. (1983) reported that it has also harmful effects on the physiology and behavior of homopterous and hemipterous pests. In this study, Parthenium and Neem seed extracts were used in water to find out its efficacy against 3rd and 4th instar of diamondback moth, and their compatibility with its natural enemies was also checked.

The toxic effects of Parthenium and Neem extracts against 3rd instar larvae of *P. xylostella* showed that the highest mortality was recorded after four days exposure to 3% concentration of Neem seed extract in water, followed by 2% of the same extract and 3% and 2% Parthenium extracts, respectively. These results are in contrast with Khan et al. (2014) who compared Parthenium with *Stevia, Chrysanthemum* and Neem extract in oil against the 3rd and 4th instars larvae of *Aedes albopictus* and showed that toxicity of Parthenium was superior to Neem and other botanical extracts. Similar results were also recorded by Tatiana et al. (2005) against *Leishmania amazonensis*. As per our results, mortality was increased by increasing concentration of the respective botanical extracts as found by Badshah et al. (2015) who reported that the highest mortality was recorded in the highest dose of Neem extract. It was recorded that mortality was high after one day exposure and then gradually decreased up to day 4. These results are in close concordance with findings of Khan et al. (2002).

The toxicity of Parthenium in comparison with Neem extracts was also tested against 4th instar larvae of *P. xylostella*. From our results, it was found that after 96 hr exposure, 3% Neem crude extract showed the highest mortality. Similar results were recorded by Sharma et al. (2014) who reported that 3% concentration of Neem seed methanol extract have high mortality against *P. xylostella* larvae. Similarly, Web et al. (1983) have found that aqueous solution of Neem seed extract have shown high toxicity against the larvae of leaf miners. These results further showed that the efficacy of both extracts was time dependent as highest mortality was found on the first day and then decreased with time. Similar results were found by Khan et al. (2002) in which they found that the efficacy of Neem extract decreases with time against target pest. Khattak et al. (2006) have also reported that because of the deterrent and antifeedant effect of Neem products against sucking pest, its efficacy decreases with time as pest leave the target place.

To examine the compatibility of Parthenium extracts with natural enemies, its effect was checked against adult of parasitoid wasp, *C. plutellae*. Our results revealed less toxicity of *P. hysterophorus* against *C. plutellae*. The same results were
also found by Hoelmer et al. (1990) who reported that A. indica extracts have less effect on coccinellid larvae. Purwatiningsih et al. (2012) also stated that A. calamus extracts have significant effects against adult Trichogramma pretiosum. They also reported that mortality of adult was concentration dependent and it increased with increasing concentrations.

**CONCLUSIONS**

On the basis of our findings, it was concluded that Parthenium extracts were more effective against P. xylostella than the control. It was further confirmed that all the treatments had the ability to control P. xylostella to some degree, but Parthenium extract was less effective in comparison with Neem crude extract, as 3% Neem crude extract had a sufficiently toxic effect on the target pest. All these treatments were affected by time and dose. Also, the experiments have shown that the effect of these treatments on the larval parasitoid is much less. It is, therefore, suggested that further concentration of Parthenium extract should be tested to find out its efficacy against P. xylostella. Neem crude extract 3% concentration can be used in the IPM program in combination with biological control agent. Further investigation is needed to evaluate its efficacy in field conditions.

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قرار داشت. کمترین مرگ و میر در تیمار شاهد بود (که مقادیر آن در سه سوم به Parthenium ترتیب 20/7و 18/33/77/57/16/41/20/50/1% بود و برای سه چهارم 67/15/50/1% بود). اثرات صدماتی در مورد لارو پارازیت Cotesia plutellae کمتر بود. بالاخره، چنین نتیجه گرفته شد که همه تیمارها تا حدودی قادر به کنترل P. xylostella بودند، ولی عصاره خام Neem در مقایسه با عصاره از کارآیی کمتری برخوردار بود Parthenium نشان داد. P. xylostella اثر سمی کافی روزی Neem/3% جناگه عصاره با غلظت 3%