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TOXICITY OF DIFFERENT PLANT EXTRACTS AND GREEN SILVER NANOPARTICLES AGAINST *PLUTELLA XYLOSTELLA* (LEPIDOPTERA: PLUTELLIDAE)

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ABSTRACT

Diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) is one of the most notorious and cosmopolitan insect pests of brassica crops around the world. *P. xylostella* may cause 90% yield losses in brassica crops. Various control measures have been adopted to manage this pest; however, the most effective control method is the use of synthetic chemical insecticides. Overuse of insecticides have many adverse effects including insecticide resistance, hazardous to environment, long persistency, interference with food chain. There is an urgent need for alternative control measures which should be effective, environmental friendly and economically safer. Bio-pesticides include plant extracts and green synthesized nano-based insecticides are among the feasible alternative measures which can be useful for the management of *P. xylostella*. Less work has been carried out on the use of green synthesized plant products against *P. xylostella*. Therefore, the current study was planned to evaluate the toxicity of plant extracts and green synthesized nano-based plant products against *P. xylostella*. Eight plants extracts (neem, bakain, bitter gourd, clove, eucalyptus, dathura, garlic and ginger) and their nano-based products (green synthesis silver nanoparticles) were applied in different concentrations against 3rd larval instars of *P. xylostella*. Mortality were recorded after 24, 48 and 72 hours of application of treatments. Corrected mortality was calculated using Abbot's formula and LC_{50} values were calculated. All the plants extract had great potential to kill the maximum population of diamondback moth at high concentration. All these plants extract gave more than 80% mortality which was at the rate of 23, 24, 20 and 30 mg/ml respectively after 72 hour interval. The present study indicated that the botanical insecticides have good toxic effect against the 3rd instar larvae of diamondback moth.

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INTRODUCTION

Diamondback moth, *Plutella xylostella* (Lepidoptera; Plutellidae) is a notorious pest of brassica crops and is distributed around the globe. *P. xylostella* is a major pest of cauliflower and other brassica crops and damage the crops throughout the year due to availability of many

host plants (Li et al., 2016). This pest has attained the status of most extensively distributed insect pest among all the Lepidoptera insect pests (Grzywacz et al., 2010). The diamond back moth has this exceptional pest status due to the diversity and abundance of its host plants (Li et al., 2016). Another important factor is its high reproductive potential and studies have shown more than 20 generations per year for *P*. *xylostella* (Shelton and Nault, 2004). This pest has the potential to cause 90% yield losses and the annual damage may reach approximately $ 4–5 billion per year. It is estimated that the global annual management cost for this pest is over US $1.0 billion (Furlong et al., 2013).

Various control measures have been adopted to manage *P*. *xylostella* however, the most effective control method is the use of synthetic chemical insecticides (Li et al., 2016). The intensive application of synthetic insecticides against *P*. *xylostella* resulted in insecticide resistance to all groups of synthetic insecticides (Sayyed et al., 2004). The overuse of insecticides have many adverse effects including insecticide resistance, hazardousness to environment, long persistency, interference with food chain and negative impact on non-target organisms (Li et al., 2016).

To overcome above mentioned problems, use of biopesticides derived from plants with potential insecticidal activity is one of the available options. Biopesticides are considered valid alternative control measures to conventional synthetic insecticides against *P. xylostella.* Biopesticides include plant extracts and green synthesized nano-based insecticides are among these measures which can be useful for the management of *P. xylostella* (Patil et al., 2017). The use of natural insecticides extracted from plants have shown considerable success against *P. xylostella* and these plant extracts are environmentally suitable and development of resistance against them has not yet been reported.

Various plant extracts have been used in many countries throughout the world for the management of insect pests including *P. xylostella* (Patil et al., 2017). These plant extracts have many bioactive compounds which act as insecticides in terms of anti-feedants, repellents, fecundity reduction and respiration inhibiting agent (El-Bokl, 2016). For example, *Azadirachta indica*, *Zingiber officinale, Momordica charantia*, *Melia azedarach*, *Allium sativum* and over 2000 plant species have insecticidal properties (Ahmad et al., 2012; Ahmed et al., 1984; Bullangpoti et al., 2012; Isman, 1999; Jacobson, 1989; Li et al., 2001; Loc et al., 2014; Rembold, 1989; Sarker et al., 2007; Schmutterer, 1990).

Green synthesized nanoparticles are one of the most effective and environmental friendly biopesticides (Benelli, 2016). Recent study has shown that green synthesized silver nanoparticles are most reliable biopesticides (Benelli, 2016) which can be used as alternative control measure against *P*. *xylostella*. As less work has been carried out on the use of green synthesized plant products against *P. xylostella*, therefore, current study has been planned to evaluate the toxicity of eight plant extracts and green synthesized silver nanoparticles of these plant products against *P. xylostella*.

MATERIAL AND METHODS

In the current study, different parts of eight plants were used to prepare plant extracts. The details of these plant parts are given in Table 1. Silver nitrate (AgNO3) and distilled water was used to prepare the silver nanoparticles and ethanol and above plants materials were used to prepare the extracts of plants.

Preparation of plant extracts: The above plant material was washed to remove dust and impurities. All these plant materials were dried under the shade for about three weeks. The dried plant materials were crushed into fine powder with the help of electric grinder. The powder of each plant extract was sieved through 20 mesh size sieve to obtain a favorable size range.

The botanical extracts were prepared by mixing 100 g powder with 500 ml ethanol of each plant. The solution was stirred with a magnetic stirrer. The suspension was filtered through Whatman No. 1 filter paper and the flask was covered with aluminum paper. The plant extract was shaken manually twice a day. The ethanol solvent was removed by rotary evaporator and dry crude extract of plant material was collected. Same procedure was used for each plant material. There were eight treatments.

Synthesis of silver nanoparticles: Ten grams extract powder of each plant material were used for the preparation of green synthesis silver nanoparticles by adding in 250 ml double distill water in flask. The mixture was boiled on hot plate for 5 minutes. The extract was filtered using Whatman filter paper No.1. The filtrate was mixed with 1mM of silver nitrate (AgNO3) solution and the solution was placed on hot plate for 10 minutes until its colour became brown

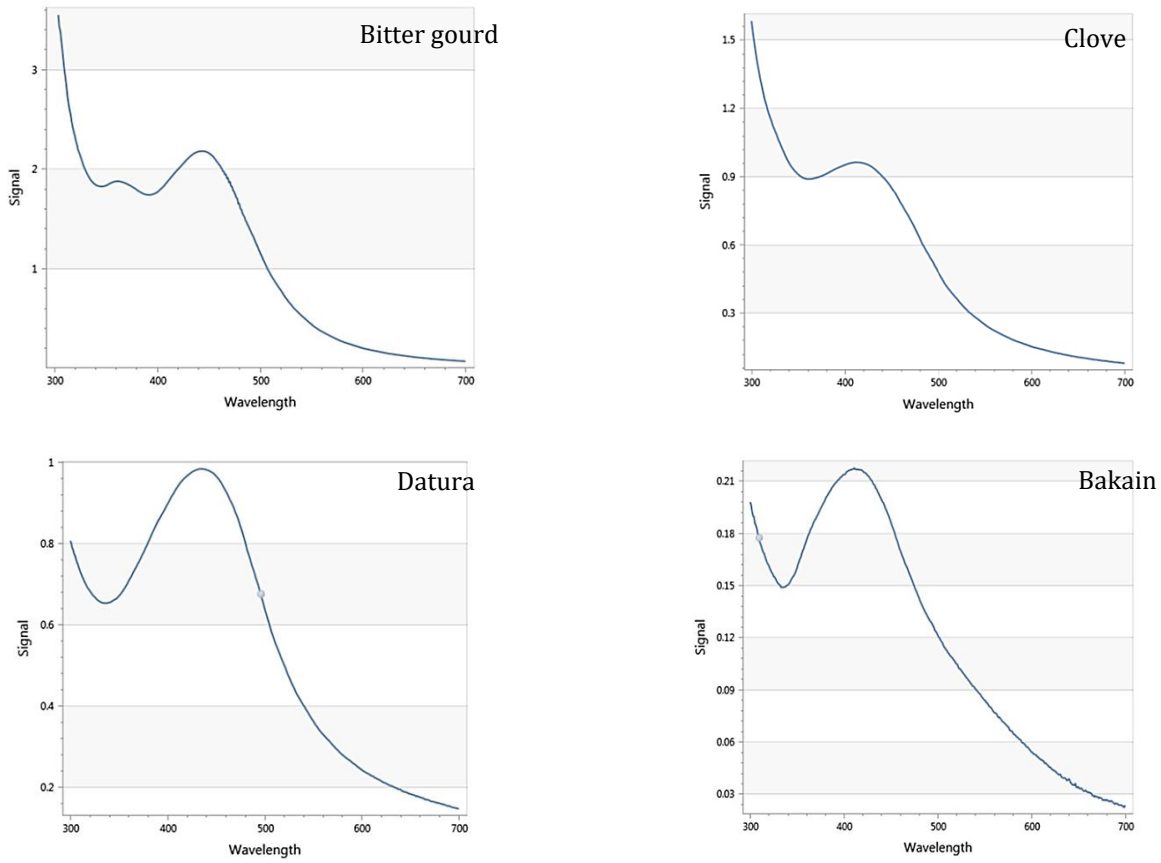
which confirmed the formation of AgNPs (Parashar et al., 2009).

Rearing of *Plutella xylostella* larvae: The larvae of *P. xylostella* were collected from the cultivated field of cauliflower. They were kept in plastic jars at 25 ± 2°C with 70 ± 5% relative humidity and a photoperiod of 12L:12D in the Insect Molecular Laboratory, Department of Entomology, University Arid Agriculture Rawalpindi. The mouths of cages were covered by muslin cloth for providing food and management. Fresh cauliflower leaves were provided as food for the larvae and were replaced daily. The adults were fed by 10% sugar solution soaked in cotton wick as a food. A fresh cauliflower leaf was kept in each jar for oviposition. The insects were reared under these conditions and used for bioassay.

Characterization of green silver nanoparticles: Green silver nanoparticles were checked for the corresponding and absorbance wavelengths by using UV-Vis spectrophotometer at the Alpha Genomics Laboratory, Islamabad (Figure 1).

Table 1. Detail of plant parts of different plants used in the study.

Sr. No.	Plant	Scientific Name	Part used
1	Eucalyptus	*Eucalyptus camaldulensis*	Leaves
2	Neem	*Azadirachta indica*	Leaves
3	Bakain	*Melia azedarach*	Leaves
4	Datura	*Datura stramonium*	Fruits
5	Dried clove	*Syzygium aromaticum*	Fruits
6	Bitter gourd	*Momordica charantia*	Fruits
7	Garlic	*Allium sativum*	Fruits
8	Ginger	*Zingiber officinale*	underground stem fruit



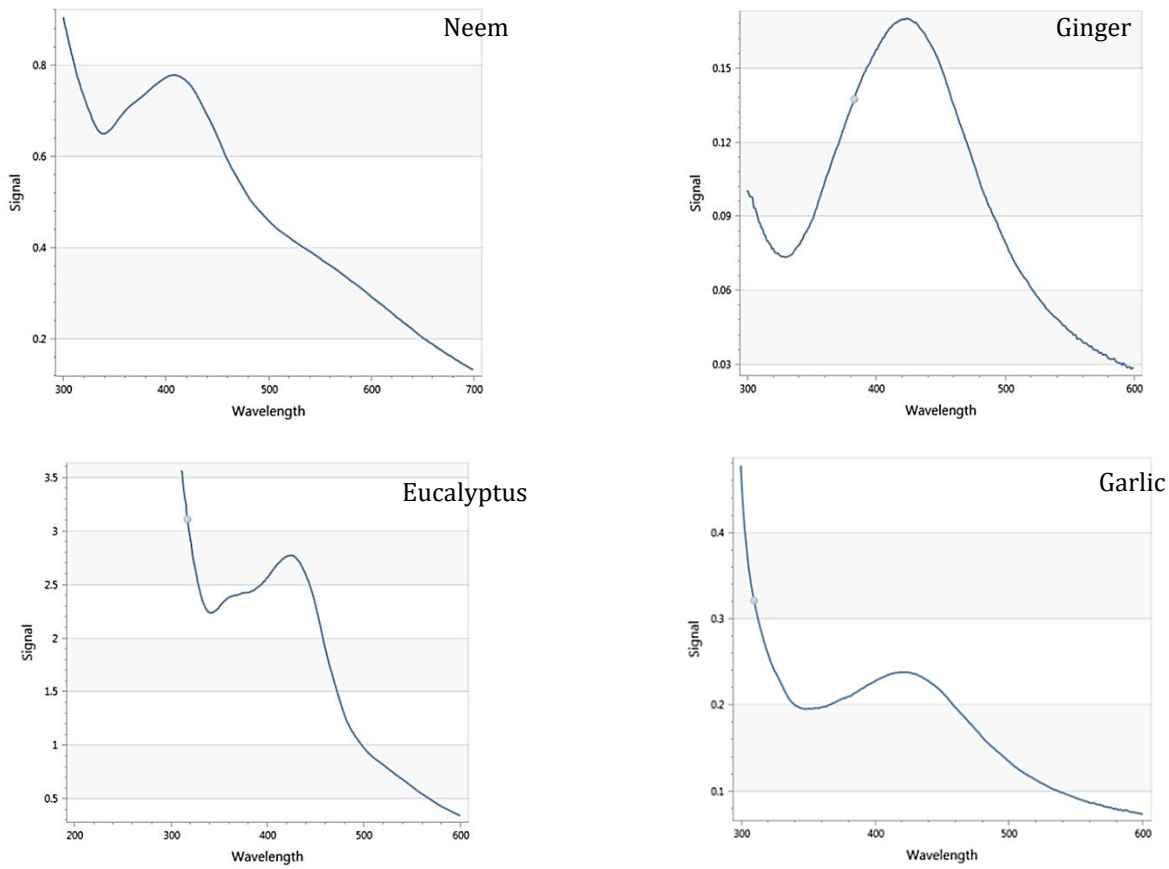


Figure1. UV characterization of silver nanoparticles of different plants.

Experimental bioassay

Larvicidal toxicity of plant extracts: The toxicity of eight treatments was tested against 3rd larval instar of *P. xylostella.* Twenty five larvae of *P. xylostella* were used in single concentration. Five concentrations for each treatment with five replications were used in each bioassay. All bioassays were conducted in Insect Molecular Laboratory, Department of Entomology, University of Arid Agriculture Rawalpindi under control condition. Mortality data were recorded after 24, 48 and 72 hours after application of each treatments.

Larvicidal toxicity of green synthesis Ag nanoparticles: The above mentioned green synthesized silver nanoparticles of eight plant extracts were used against 3rd larval instar of *P. xylostella* to observe the toxicity of green silver nanoparticles as mentioned above.

Statistical analysis: Mortality data for each treatment were recorded and corrected mortalities were calculated using Abbot's formula. LC_{50} values were calculated from probit analysis software. Statistical Package for Social Sciences version 16 was used for the standard error mean analysis.

RESULTS

Larvicidal potential of ginger (*Zingiber officinale*) against 3rd instar larvae of *Plutella xylostella:* The ethanol plant extract of *Z. officiale* showed 72% mortality at the highest concentration of 23 mg/ml against the 3rd instar larvae of *P. xylostella*. Ethanol extracts at the lowest concentration of 1.4375 mg/ml showed only 20% mortality when exposed for 24 hours. For 48 hours, 76% mortality was observed whereas the percentage mortality of the lowest concentration only increased up to 24%. The final values at the 72 hours interval for the highest and lowest concentrations were 84% and 32% respectively. The results were obtained based on the Probit Analysis between the concentrations of plant extract against the 3rd instar larvae of *P. xylostella* for the entire experiment. For 24, 48, and 72 hours, the LC_{50} values obtained were 12.701, 8.898, and 5.704 mg/ml respectively (Table 2).

The ginger enhanced silver nanoparticles showed 76% mortality at the highest concentration of 2.3 mg/ ml

against the 3rd instar of *P. xylostella*. Silver nanoparticles at the lowest concentration of 0.014375 mg/ml showed only 24% mortality when exposed for 24 hours. For 48 hours, 84% mortality was observed whereas the percentage mortality of the lowest concentration only increased up to 28%. The final values at the 72 hours interval for the highest and lowest concentrations were 88% and 36% respectively. For 24, 48, and 72 hours, the LC_{50} values obtained were 1.024, 0.716, and 0.382 (mg/ml) respectively (Table 3).

Table 2. LC_{50} values and fiducial limit of different plant extracts for different time intervals against 3rd instar larvae of *P. xylostella.*

Plant extract	LC_{50} (mg/ml)with fiducial limit		
	24 hours	48 hours	72 hours
Ginger	12.701 (8.899-18.604)	9.898 (4.652-13.429)	5.704 (-0.622-9.340)
Neem	9.368 (4.120-14.975)	7.248 (1.136-12.002)	4.331 (-2.461-8.234)
Clove	14.123 (9.222-21.488)	9.496 (4.278-14.372)	6.567 (0.315-10.901)
Datura	9.432 (4.919-16.170)	6.697 (1.561-11.142)	2.990 (-5.698-6.882)
Bakain	17.654 (9.856-27.599)	11.857 (-0.046-20.557)	6.746 (-6.528-13.715)
Bitter Gourd	15.095 (9.031-24.781)	11.715 (4.971-18.935)	8.683 (1.877-13.998)
Eucalyptus	16.126 (9.442-29.041)	11.172 (3.746-18.559)	6.542 (-8.175-12.758)
Garlic	12.135 (7.977-18.822)	9.237 (5.250-13.682)	6.689 (1.632-10.684)

Table 3. LC_{50} values and fiducial limit of different plants silver nanoparticles for different time intervals against 3rd instar larvae of *P. xylostella.*

Plant silver nanoparticles	LC_{50} (mg/ml)with fiducial limit		
	24 hours	48 hours	72 hours
Ginger	1.024 (0.656-1.484)	0.716 (0.331-1.062)	0.382 (-0.185-0.7180)
Neem	0.948 (0.481-1.446)	0.660 (0.116-1.070)	0.611 (-0.203-0.628)
Clove	1.358 (0.869-2.062)	0.856 (0.286-1.339)	0.517 (-0.224-0.955)
Datura	0.851 (0.438-1.354)	0.609 (0.155-0.978)	0.337 (-0.326-0.686)
Bakain	1.565 (0.583-2.624)	1.029 (-0.347-1.877)	0.532 (-0.753-1.182)
Bitter Gourd	1.324 (0.739-2.070)	0.951 (0.163-1.590)	0.639 (-0.129-1.125)
Eucalyptus	1.367 (0.620-2.451)	0.951 (0.163-1.590)	0.514 (-1.454-0.971)
Garlic	1.156 (0.720-1.805)	0.902 (0.481-1.354)	0.729 (0.205-1.162)

Larvicidal potential of neem (*Azadirachta indica*) against 3rd instar larvae of *Plutella xylostella:* At 24 hours, the ethanol extract of neem plant showed 72% and 20% mortalities at higher and lower concentrations of 24 mg/ml and 1.5 mg/ml respectively. For 48 hours, the ethanolic plant extract of neem showed 76% mortality at higher limit and 32% at lower limit of plant extract. The final data obtained at 72 hours shoed the 84% mortality at higher concentrations and 36% at lower concentrations. The LC_{50} values calculated from probit and logit analysis software which are different for different time intervals 24, 48, 72 hours were 9.368, 7.248 and 4.33 mg/ml respectively (Table 2).

The green silver nanoparticles of neem plant gave 76% mortality at the highest concentration of 2.4 mg/ml against the 3rd instar larvae of *P. xylostella*. Silver nanoparticles at lowest concentration of 0.15 mg/ml showed only 28% mortality when exposed for 24 hours. For 48 hours, 80% mortality was observed whereas the percentage mortality of the lowest limit of concentration increased only up to 32%. The final values were recorded at the 72 hours interval for higher and lower limits of concentration were 92% and 36% respectively. Based on the probit analysis between the concentrations of plant extract against the 3rd instar larvae of *P. xylostella* for the entire experiment for 24, 48, and 72 hours, the LC_{50} values obtained were 0.948, 0.660 and 0.611 mg/ml respectively (Table 3).

Larvicidal potential of clove (*Syzygium aromaticum*) against 3rd instar larvae of *Plutella xylostella*: The application of clove plant extract of 28 mg/ml concentration showed the highest mortality of 72% while 1.75 mg/ml concentration showed the lowest mortality 20% after 24 hours. The treated values observed after 48 hours gave the maximum of 80% mortality at higher concentration and minimum 24% mortality at lower concentration. After 72 hours, the final mortality values increased up to 84% and 28%. The LC_{50} values observed after 24, 48 and 72 hours were 14.123, 9.496 and 6.567 respectively which were calculated from the probit and logit analysis software (Table 3).

The silver nanoparticles of clove were applied on the 3rd larval instar of *P. xylostella* gave 76% mortality and 24% mortality at 2.8mg/ml and 0.175mg/ml after 24 hours. For 48 hours, the mortality values were 84% and 32% respectively. The final values which obtained after 72 hours were 88% and 36% at higher and lower limit of clove silver nanoparticles against 3rd instar larvae of *P. xylostella.* The final values of LC_{50} for different time intervale were 1.358, 0.856 and 0.517 (in mg/ml) respectively (Table 3).

Larvicidal potential of datura (*Datura stramonium*) against 3rd instar larvae of *Plutella xylostella*: In 20 mg/ml concentration after 24 hours, the larvae of *P. xylostella* treated with datura plant extract was recorded the highest mortality of 68% at 20 mg/ml and 1.25 mg/ml at lower value of concentration gave the minimum mortality of 24%. The maximum and minimum mortalities of 76% and 28% were observed after 48 hours' time interval and larvae of *P. xylostella* were treated with datura plant extract and the mortality data was recorded after 72 hours gave the highest mortality value of 80% and 36%. For 24, 48 and 72 hours, the LC_{50} values of 9.432, 6.697 and 2.990 were obtained from the probit and logit analysis software (Table 2).

The larvae of *P. xylostella* treated with the green silver nanoparticles of datura at higher concentration of 2 mg/ml and lowest concentration of 0.125 mg/ml gave the highest mortality of 72% and the lowest mortality of 28% after 24 hours. The values increased up to 80% and 32% after 48 hours. For 72 hours, the final values recorded were 84% and 40%. The LC_{50} values from probit and logit analysis were 0.851, 0.609 and 0.337 for 24,48 and 72 hours (Table 3).

Larvicidal potential of bakain (*Melia azedarach*) against 3rd instar larvae of *Plutella xylostella*: The toxicity of bakain plant extract against 3rd instar larvae of *P. xylostella* exhibited that bakain plant extract was effective against 3rd instar larvae of *P. xylostella* at different time intervals showing the highest mortality of 68% with 40 mg/ml and the lowest mortality of 24% after 24 hours. For 48 hours, the bakain plant extract showed 76% and 28% mortalities. The mortality values were increased up to 84% and 40% after 72 hours. The LC_{50} values for different time intervals of 24, 48 and 72 hours were 17.654, 11.857 and 6.746 respectively (Table 2).

The toxicity of bakain enhanced silver nanoparticles against 3rd instar larvae of *P. xylostella*. The application of bakain silver nanoparticles against 3rd instar larvae of *P. xylostella* after 24 hours showed the highest mortality of 72% at the concentration limit of 4 mg/ml and the lowest 28% mortality at the concentration of 0.25 mg/ml. For 48 hours and 72 hours, the values of the

highest and lowest mortalities were 80%, 32% and 88%, 44% respectively. The LC_{50} values for 24, 48 and 72 hours were 1.565, 1.029 and 0.532 (in mg/ml) respectively (Table 3).

Larvicidal potential of bitter gourd (*Momordica charantia*) against 3rd instar larvae of *Plutella xylostella*: The results of present studies have shown that the plant extract of bitter gourd was found to be toxic against 3rd instar larvae of *P. xylostella* at different time intervals. The 68% and 24% mortality values recorded after 24 hours at the concentration limits of 30 mg/ml and 1.875 mg/ml. The per cent mortalities were 72 at high concentration and 28 at lower limit of concentration after 48 hours. The final values obtained after 72 hours were 80% and 32% respectively. The major variations in LC_{50} values recorded after 24, 48 and 72 hours were 15.095, 11.715 and 8.683 respectively (Table 2).

The toxicity of bitter gourd nanoparticles was the most effective when applied to the 3rd larval instar of *P. xylostella*. For 24 hours, the highest concentration 3 mg/ml showed 72% mortality and lowest concentration of 0.1875 mg/ml showed 24% mortality. The final values at the maximum and minimum concentrations of bitter gourd silver nanoparticles were 75%, 32% and 84%, 36% after 24 hours and 72 hours respectively. The LC_{50} values were 1.324, 0.951 and 0.639 (in mg/ml) respectively for different time of intervals (Table 3).

Larvicidal potential of eucalyptus (*Eucalyptus camaldulensis*) against 3rd instar larvae of *Plutella xylostella*: Results of eucalyptus plant extract against 3rd instar larvae of *P. xylostella* showed promising effects with exposure to different concentrations at 24 hours with highest per cent mortality up to 64%. The bar for the lowest concentration was set at 24%. At the 48 hour interval only a 4% increase in mortality was observed for the lowest concentration whereas the increase was double (8%) for the highest concentration of 30 mg/ml. The reverse was observed at the last 72 hours observation where the increase in per cent mortality was 4% for 1.875 mg/ml concentration and 8% for 30 mg/ml concentration. The LC_{50} values observed were 16.126, 11.172, and 6.542 mg/ml for 24, 48, and 72 hours observations respectively (Table 2).

For the Ag-nanoparticles of eucalyptus, 4% increase in mortality was recorded for both 0.1875 mg/ml and 3 mg/ml concentrations as compared to the ethanol-based plant extracts and the values were 28% and 68% respectively after 24 hours. For 48 hours interval, 32% mortality was the lowest with 3 mg/ml. Minimum concentration of 0.1875 mg/ml gave 44% mortality for after 72 hour observation and 3 mg/ml concentration was recorded much higher at 80% killing 20 out of 25 *P. xylostella* larvae in the petri dish. The LC_{50} values for this experiment were 1.367, 0.951, and 0.514 mg/ml for 24, 48, and 72 hours' observations respectively (Table 3).

Larvicidal potential of garlic (*Allium sativum*) against 3rd instar larvae of *Plutella xylostella*: Regarding the results of *Allium sativum*, the per cent mortality observed at the 24 hours was 20% at the concentration limit of 1.4375 mg/ml and 68% at 23 mg/ml. There was no death in the control populations and therefore no reason to calculate the corrected mortality values. The per cent mortality increased to 24% and 76% at the 48 hours interval respectively. Final observed values noted were 32% and 80% for the highest and lowest concentrations at the 72 hours mark. The lethal concentration values for 50% of the population were calculated via probit analysis as 12.135, 9.237, and 6.689 mg/ml for the 24, 48, and 72 hours marks respectively (Table 2).

Regarding the silver-nanoparticles of garlic, the observed results for per cent mortality were 24% and 72% for lowest and highest concentrations at the 24 hours interval. For the 48 hours mark, an increase of 8% was observed for highest concentration of 23 mg/ml, making its mortality equal to 80%. The lowest concentration only saw an increase of 4% i.e. at 28%. For the final 72 hours mark, the observed per cent mortality values for lowest concentration and highest concentration were 36% and 84% respectively. The LC_{50} values calculated via probit analysis were 1.156, 0.902, and 0.729 mg/ml for the 24, 48, and 72 hours marks respectively (Table 3).

DISCUSSION

The present investigation suggests the potential of plant species in controlling diamondback moth. Garlic ethanolic extract gave good control against diamondback moth. Garlic has well been reported to contain pesticidal properties. Zehnder and Griggs (1996) described that garlic gave effective control of the caterpillars on lettuce and cabbage. The authors applied garlic oil as sprays which killed aphids, cabbage loopers, earwigs, june bugs, leafhoppers, squash bugs and white flies. Similar results have also been reported by Reuben et al. (2006) who used garlic extract to control larval instar of

diamondback moth.

The present study revealed that *Zingiber officinale* ethanolic extracts gave the lowest LC_{50} value of 12.701 mg/ml after 24 h and after increase of time the percent mortality increased and the LC_{50} values decreased. This show the toxicity of ginger extract as the value of LC_{50} decreased down to 5.704 mg/ml after 72 h. The results had some resemblance with those of Babu et al. (2018) because in the present study we used the ethanol for the extraction instead of aceton.

Similarly, the extracts of datura affected the activity of larval instars of *P. xylostella* throughout the experiment conducted in the laboratory. The results are in line with those reported by Mari (2012). The researcher proved that the datura plant extract are cheap and effective for the control of insect pests. Likewise, neem extract was also found effective against the pest. Neem extract slightly increased the death of larvae of *P. xylostella.* Similar results were also reported by Mari (2012) when neem extract was applied on 2nd and 3rd instar larvae of *P. xylostella.*

The present study indicated that the botanical insecticides have good toxic effect against the 3rd instar larvae of diamondback moth. The different plants extract i.e. ginger, neem, clove and bitter gourd had great potential to kill the maximum population of diamondback moth at high concentration. All these plants extract gave more than 80% mortality which was highest percentage mortality at the rate of 23, 24, 20 and 30 mg/ml respectively after 72 hour interval. These results are mostly similar with the those of Abbasipour et al. (2010) who reported that extract of *Peganum harmala* were toxic against the larvae of diamondback moth.

The silver nanoparticles studied here were synthesized using a botanical byproduct. Notably, neem cake-synthesized silver nanoparticles were extremely effective against *P. xylostella*. Neem cake-synthesized AgNP were extremely toxic and the LC_{50} value was 0.611 mg/ml which was lowest value and killed 92 percent population of 3rd larval instar of diamondback moth. The results are in line with the finding of Chandramohan et al. (2016) against larvae and pupae of the dengue vector *Aedes aegypti*.

Synthesized silver nanoparticles blended with datura extracts applied on 3rd instar larvae of diamondback moth showed 84 percent mortality at the rate of 2 mg/ml. These results are in agreement with those of Murugan et al. (2015) against malaria mosquitoes.

It was practical that plant extracts can be successfully used as an excellent substitute to synthetic insecticides. Further studies are essential to discover the required lower application rates and extraction methods that will provide a more effective control of diamondback moth to economic threshold levels.

Authors' contribution: FA, MT and FAS conceived the idea, FA conducted the research trials; ZRM and TZ helped in the preparation of nanoparticles; MT performed the statistical analysis; FA wrote the manuscript; All the authors reviewed and edited the manuscript.

Conflict of interest: The authors declare no conflict of interest.

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