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Evaluation of Neem (*Azadirachta indica*) Derivatives against Jassids (*Emrasca devastans*) and Cotton Mealybug (*Phenacoccus solenopsis*), and Side Effects on the Feeding Potential of Green Lacewing (*Chrysoperla carnea*) on Cotton Aphid (*Aphis gossypii*)

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ABSTRACT

Laboratory studies were conducted to investigate the toxic, deterrent and anti-feedent effects of neem oil and neem seed water extract at 1, 2, and 3% concentrations against jassids, (*Emrasca devastans* Dist.) cotton mealybug (*Phenacoccus solenopsis* Tinsley) and their side effects on the feeding potential of *Chrysoperla carnea*on cotton aphid (*Aphis gossypii* Kalten) at 27±2°C and 65% R.H. in Khyber Pakhtunkhwa Province, Pakistan. The results revealed that neem oil at 2 and 3% concentrations and neem seed water extract at 3% concentration was effective against jassids compared to control. Neem oil and neem seed water extract caused more mortality of cotton mealybugs at 3% concentration than the control. When cotton aphids were offered for feeding to *C. carnea*, statistically fewer aphids were consumed by *C. carnea*at higher concentrations of both neem derivatives as compared to that in the control. However; both neem derivatives at lowest concentration had no effect on the feeding ability of *C. carnea*. It can be concluded from the present findings that *C. carnea* is sensitive to neem derivatives and can be used at appropriate concentrations in combination with *C. carnea* in swapping to synthetic insecticides for a safer control of the test insects.

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Authors' Contribution

MMR, SJ and QK conceived and designed the study. PK, AL, KW and SN executed the experimental work. OS and MMH statistically analyzed the data and wrote the article.

Key words

Cotton, Jassids, Mealybug, Chrysoperla carnea, Neem derivatives

INTRODUCTION

Cotton, *Gossypium hirsutum* L., is a major crop and is a source of foreign exchange for Pakistan. It was grown over an area of 2.80 million hectares with an annual production of over 12.76 million bales (170 kg each) (Anonymous 2015*).* Still, it is low as compared to other cotton growing countries because of several reasons. The most important factor is the attack of a variety of insect species. The most economically important of these insect pests are whitefly (*Bemisia tabaci* Genn.), jassid (*Emrasca devastans* Dist.), thrips (*Thrips tabaci* Lind), a recently introduced pest, cotton mealybug (*Phenacoccus solenopsis* Tinsley) and bollworm complex. The cotton mealybug is distributed worldwide and is polyphagous species (Ben-Dov, 2005).

These insect pests do not only reduce the quantity but also the quality and spread diseases in the crops. The attack of insect pest complex causes 15-20% loss in cotton yield (Zahidullah, 1992). About 15-20% crop is damaged every year by insect pests and 10% by diseases (Ahmad, 1999). Sucking insect pests alone cause 4-6% damage, while bollworms complex cause 19-21% loss in the yield of cottonseed (Satpute *et al.*, 1998*).* These insect pests are mainly controlled by synthetic insecticides which are hazardous to humanity and other living organisms. Therefore, it is necessary to find some alternative methods for the management of these insect pests to avoid the harmful effects of conventional insecticides on non-target organisms.

Botanical insecticides could be the best option as they are being used in village pharmacology as well as for the control of insect pests since the advent of synthetic organic chemicals. Among these plants, neem (*Azadirachta indica*) has great potential for commercial exploitation. Neem derivatives act as repellent, deterrent, anti-ovipositional and growth inhibitors against insect pests (Khattak *et al.*, 2001; Mamoon-ur-Rashid *et al.*, 2012). Neem products affect insect vigor, longevity and fecundity (Arora and Dhaliwal, 1994). Unlike synthetic insecticides, neem derivatives have no or less toxicity to human being, other animals and useful insects (Schmutterer, 1985). The triterpenoids, meliontriol, salannin and azadirachtin occurring mainly in the seed kernels of neem act as antifeedant, ovipositional

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deterrence and disturb insect growth and development (Prakash and Rao, 1997). Azadirachtin has no side effects on non-target organisms such as birds, parasitoids, predators and pollinators Neem derivatives do not leave any toxic effects to contaminate environment and insects do not develop resistance against neem derivatives (Lowery and Isman, 1995; Naumann and Isman, 1996; Charleston *et al.*, 2005; Zehnder and Warthen, 1988).

Green lacewing, *Chrysoperla carnea,* is a generalist predator widely used in various situations due to its wide geographical distribution, broad habitats, good searching ability and easy rearing under laboratory conditions (Tolstova, 1986). Lacewing larvae voraciously feed on different insect pests. In general, they attack the eggs and the immature stages of most soft-bodied pests such as aphids, thrips, spider mites, whiteflies, mealybugs, leafhoppers and the eggs and caterpillars of most pest moths (Geethalakshmi *et al*., 2000; Sattar *et al*., 2007; Joshi *et al*., 2010). *Chrysoperla carnea* is the most important naturally occurring predator and used extensively for the management of insect pests of many crops in Pakistan (Iqbal *et al*., 2008; Zia *et al*., 2008; Usman *et al*., 2012)

Looking into the importance of cotton crop, the losses caused by the insect pests and the safe nature of the neem derivatives, these studies were carried out to determine (i) the toxic, growth-inhibiting and antifeedant/deterrent effects of neem derivatives against jassids and cotton mealybug, and (ii) to study the effect of neem derivatives on the feeding potential of *C. carnea* on cotton aphid.

MATERIALS AND METHODS

Preparation of neem oil concentrations

Neem seeds were collected from the local farmers during autumn, dried under shade conditions for 30 days, the neem seeds were dehulled and oil was extracted with the help of experimental oil extractor (10.5 kg/cm). Different concentrations (1, 2 and 3%) of neem oil were prepared in water to be used in the trials.

Preparation of neem seed water extract concentrations

Two kilogram dried neem seeds collected from the local farmers was grinded. The dried neem seed were grinded by using Mortar and pestle. The grinded seeds were tied in a cotton cloth in the form of a bag and dipped in ten liters of water at 80ºC for 16 h. In this way concentrated solution of 20% was obtained and diluted into 1, 2 and 3%, respectively, by adding calculated amount of distilled water using the method described by Musabyimana *et al.* (2001).

Effect of neem derivatives on cotton jassids and mealybugs

An experiment was conducted in the laboratory of Entomology Department, Faculty of Agriculture, Gomal University, D.I. Khan to investigate the toxic effect of neem derivatives against cotton jassids and mealybugs. The experiments were conducted at 27±3ºC and 65% R.H. and a photoperiod of 12:12 h (L:D). The design of experiment was completely randomized. Neem oil and neem seed water extract used in this experiment were each diluted at 1, 2 and 3%. There were seven treatments including control/untreated cotton leaves. Fresh cotton leaves were dipped in each aqueous solution of neem oil and neem seed water extracts. After complete drying in the open air, the leaves of each concentration were placed in separate transparent cages. The arena was made of transparent cages of 30.5 cm×30.5 cm × 30.5 cm. The untreated fresh cotton leaves were also maintained in a separate cage for comparing with the treated ones. Each of these treatments was replicated three times. Ten unsexed, uniform size adults of jassids and mealybugs collected from the cotton field were released in each treatment. After 24 h of exposure to aqueous solution of neem oil and neem seed water extracts, mortality of jassids and mealybugs was recorded and changed to percent mortality.

Deterrent and anti-feedent effects

The deterrent and anti-feedant effect of neem derivatives was evaluated under choice experimental condition. Different concentrations (1, 2 and 3%) of neem oil in water and neem seed water extract was applied to fresh leaves of cotton. The jassids and mealy bugs were presented with a choice of dispersing on to either treated or untreated leaves in the arena. The arena was made of transparent cages of 30.5 cm × 30.5 cm × 30.5 cm. Six treated cotton leaves and six untreated leaves were placed on an alternating design and at equal distance from each other in the arena. Ten adults of each jassids and mealybug were released in the middle of the arena and the arena was then closed to settled the escape of insects. After 24 h, the number of insects on treated and untreated leaves was recorded. Each treatment was replicated three times. The data for percent jassids and mealy bug distribution were analysed using T-test. Those insects were excluded from the data which did not respond *i.e.* they settled neither on treated leaves nor on untreated ones.

Rearing of C. carnea

The *C. carnea* adults were collected from berseem (*Trifolium alexandrinum*) crop and were reared in the laboratory at 27±2ºC and 65% R.H and a photoperiod of

12:12 h (L:D) in transparent plastic cages of (37 × 37 ×12 cm) on artificial food (20 gm yeast + 98.9 gm sugar + 19.89 gm water + 10% honey) and a black sheet on the top. Limited numbers of available aphids were also added in the cages during the predator's rearing. Eggs of *C. carnea* were collected from black sheet with razor blade and kept for hatching in petri-dishes. Three days after hatching larvae of the predator were shifted to other petri-dishes.

Effect of neem derivatives on the feeding ability of C. carnea *on cotton aphid*

A Completely Randomized Design (CRD) experiment was conducted in the laboratory to see the effect of neem derivatives on the percent consumption of *C. carnea* on cotton aphid. There were seven treatments including control and each treatment was replicated three times. Neem derivatives were applied to cotton leaves at different concentrations (1, 2 and 3%) with hand atomizer sprayer (barber sprayer) with 500 ml capacity. The sprayer was thoroughly washed and rinsed after each treatment. For each concentration, leaves were placed in separate petri-dishes. White filter paper was placed in the bottom of each petri-dish to facilitate free movement of the *C. carnea* larvae. Counted number of cotton aphids (16, 32, 48, 64 and 80) were released in each treatment. A soaked cotton swab was placed to avoid desiccation of the larvae. A *C. carnea* larva was released in each treatment. After 24 h, the numbers of aphids consumed by the *C. carnea* larvae were recorded. The data were then converted into percent consumption with the following formula.

$$\text{Percent consumption} = \frac{\text{Number of aphids consumed}}{\text{Total number of aphids}} \times 100$$

All the data collected were analyzed with Analysis of Variance (ANOVA) and means were separated with LSD at $\alpha = 0.05$ using computer software (SPSS ver. 13).

RESULTS

Toxic effect of neem derivatives (no choice test)

When cotton jassids were forced to feed on cotton leaves treated with neem derivatives at different levels of concentrations, neem oil at higher concentrations killed more jassids with 26.67 % and 56.67 % mortality at 2 and 3% concentrations, respectively, which were significantly ($P<0.05$) more than the 3.33 % mortality in the control. However, neem oil at 1% showed no significant toxic effect to the test insect (Table I). Similarly neem seed water extract at lower concentrations (1 and 2%) used in the experiment was statistically similar in its toxicity to that in the control but 3 % concentration killed significantly more (43.33 %) jassids as compared to control (3.33 %). Overall results showed that increase in the concentrations of neem derivatives resulted in more reduction of the jassids.

Table I.- Effect of neem oil and neem seed water extract on the mortality of mealybugs and jassids on cotton.

Treatment	Mortality (%)	
	Jassids	Cotton mealybugs
Neem oil concentration		
1%	16.66±0.25 c	11.33±1.21 d
2%	23.33±0.53 bc	26.67±2.08 c
3%	36.67±1.72 a	56.67±2.10 a
Neem seed water extract		
1%	13.33±1.05 c	9.33±1.60 d
2%	16.66±0.30 bc	14.33±2.60 d
3%	26.67±1.42 b	43.33±3.41 b
Control	6.66 ±3.72 cd	3.33±4.01 d
LSD	8.386	11.09

Each value is a mean of 3 replications. Means followed by the same letters are not significantly different at $\alpha = 0.05$

Neem oil and neem seed water extract at low levels of concentrations used in these trials were found non-toxic to cotton mealybugs. Both neem derivatives used in this experiment showed their maximum toxic effect against cotton mealybugs at 3% concentration. Neem oil and neem seed water extract at 3% concentrations caused 36.67 and 26.67% mortality of the mealybugs which were significantly more than the 6.66% mortality in the control. The data also showed that neem oil at 3% was more toxic to the mealybugs than the neem seed water extract at 3% concentration.

Deterrent and anti-feedant effect on cotton jassids and mealybugs

When jassids were given a chance of distribution on untreated cotton leaves and treated with neem oil and neem seed water extract in a closed arena, they showed different response to the neem product at different levels of concentrations. Results showed that neem oil at 1% concentration did not show any deterrent effect against the test insect; however, kept more insects away from the leaves treated with 2% and 3% neem oil. Number of jassids (39.16 and 15.15%) in these concentrations was significantly fewer than the test insect settled in their respective controls (Table II).

Table II.- Effect of neem oil and neem seed water extract on the distribution of mealybugs and jassids on cotton leaves.

Treatment	Distribution (%)	
	Jassids	Cotton mealybugs
Neem oil		
Control vs 1 %	48.14±0.31 a vs 51.85±0.6 a 0	48.15±0.21 a vs 51.85±0.1 a 6
T value	-1.00	-1.00
Control vs 2 %	39.16±0.57 a vs 60.83±0.53 b	41.48±0.30 a vs 58.52±0.25 b
T value	-13.00	-5.74
Control vs 3 %	15.13±0.63a vs 84.86±0.67 b	36.66±0.20 a vs 63.33±0.38 b
T value	-13.41	-4.00
Neem seed water extract		
Control vs 1 %	48.14±0.31 a vs 51.85±0.60 a	50.00±0.21 a vs 50.00±0.1 a 6
T value	-1.00	0.00
Control vs 2 %	42.50±0.57 a vs 57.50±0.53b	45.80±0.30 a vs 54.23±0.25 b
T value	-1.96	-1.95
Control vs 3 %	27.38±0.63 a vs 72.61±0.67 b	43.33±0.20 a vs 56.67±0.38 b
T value	-18.97	-2.00

Paired values within column followed by the same letters are not significantly different at $\alpha = 0.05$.
Each paired value represents the choice offered in an arena.

Similarly neem seed water extract was not effective against jassids at 1% concentration but deterred more insects from the leaves treated with higher concentrations. The number of jassids (42.50 and 27.38%) settled on the cotton leaves treated with 2 and 3% neem seed water extract, respectively were statistically lower than the number of jassids (57.50 and 72.61%) settled on their respective control. It is also evident from the results that the increase in the concentrations of neem products showed more negative effect on the test insect.

When cotton mealybugs were given a choice of distribution onto cotton leaves treated with neem derivatives at different concentrations and untreated cotton leaves in a closed arena, lower number of mealybugs were observed on leaves treated with 2 and 3% neem oil and 2 and 3% neem seed water extract (Table II)**.** Both neem derivatives at 1% did not deter more mealybugs than their respective controls, as the presence of 48.15 and 50.00% mealybugs were statistically similar to the 51.85 and 50.00% mealybugs present in their respective controls. Neem oil showed some higher deterrent effect at 3% which differed non significantly from the 3% neem seed water extract.

Effect of neem oil and neem seed water extract on the feeding ability of C. carnea *on cotton aphid*

Neem oil and neem seed water extract at highest concentrations affected the prey consumption by the *C. carnea* larvae when 16 aphids/treatment were offered; as 64.58 and 79.17% consumption of aphids in 3% neem oil and neem seed water extract concentrations respectively was statistically lower than the 97.91% consumption of the prey in the control. When 32 aphids/day were offered, neem oil and neem seed water extracts at 3% concentrations significantly affected the feeding ability of the *C. carnea* larvae (Table III). It is also evident from the results that neem derivatives used in these trials at 3% concentrations significantly affected the feeding ability of *C. carnea* larvae when 48, 64 and 80 aphids per treatment/day were offered. Statistically fewer aphids were consumed in all these treatments as compared to the control. Moreover, there was also statistical difference in the feeding ability of the predator among the treatments; however, the higher the neem oil or neem seed water extract concentration, the fewer the number of aphids consumed by *Chrysoperla* larvae. It is very much apparent from the results that *C. carnea* is very much sensitive to the repellent and anti-feedant/deterrent activities of the neem derivatives at higher concentrations.

DISCUSSION

Botanical insecticides offer a better and much safer alternative for IPM systems compared to chemical insecticides (Copping and Menn, 2000). Neem products have been reported benign to parasitoids and predators and are compatible with integrated management programs (Lowery and Isman, 1995; Naumann and Isman, 1996). In the present investigations, neem oil and neem seed water extract at 1% did not affect the distribution of jassids on treated and untreated cotton leaves but they deterred significantly more jassids from the cotton leaves treated with 2% and 3% concentrations as compared to their respective controls. The same concentrations of the neem oil and neem seed water extract killed significantly more jassids than the control while neem derivatives were statistically similar as regards to the percent mortality of the jassids in the control.

Other research workers in their studies obtained similar results with regards to the effect of neem derivatives on different insect pests of cotton. Jat and Jeyakumar (2006) found that neem oil was more effective

Table III.- Effect of neem derivatives on the percent feeding ability of *Chrysoperla carnea* on cotton aphid, *Aphis gossypii.*

	No. of Aphids offered per day				
	16	**32**	**48**	**64**	**80**
Neem oil					
1%	89.50 a	91.50 a	91.41 a	95.93 a	95.00 a
2%	81.83 ab	88.21 ab	81.08 b	72.39 c	71.67 c
3%	64.58 d	67.71 d	65.28 d	65.10 d	64.17 e
Neem seed water extract					
1%	89.58 a	93.75 a	91.19 a	94.58 a	94.92 a
2%	87.25 ab	88.94 a	82.64 b	78.12 b	75.83 b
3%	79.17 bc	79.16 c	75.69 c	72.39 c	69.17 d
Control	97.91 a	94.79 a	94.44 a	97.40 a	97.12 a
LSD	10.48	6.261	4.148	4.016	2.344

Each value is a mean of 3 replications. Means followed by the same letters are not significantly different at $\alpha = 0.05$

against jassids than neem seed kernel extract. The jassids population decreased by 20.4, 34.4 and 42.5% with 1, 2 and 3% neem oil concentrations, respectively. In laboratory as well as in field studies, Mansoor *et al.* (1996) found that the neem samples gave significant control of jassids, thrips and aphids. The nymphal duration was prolonged in all tested insects. Saxena *et al.* (1981) reported that neem oil was good anti-feedant for the control of rice brown hopper. Hoppers generally avoided the rice plants treated with 3, 6, and 12% crude emulsifiable neem oil. Similarly, in spray schedule where neem was used alternately with *Bacillus thuringiensis* and synthetic pyrethroid successfully managed the bollworms and *B. tabaci.* Neem derivatives killed small bodied insects and immature stages of several insect species (Gupta and Sharma, 1997). Neem oil extract at 0.04% caused 100% mortality of the 1st and last larval instar of mosquitoes in 24 h (Attri and Prasad, 1980). Hellpap (1984) found that 5 and 10ppm of methanol extract of neem seed kernel when mixed with rearing diet caused complete mortality of 4-10 day old fall armyworm larvae. Feeding on diet containing 250 and 500ppm of the extract caused death of armyworm larvae in 24 h. Neem derivatives caused 25% mortality of *Plutella xylostella* larvae when these insects were fed on foliage treated with neem leaf extract or neem oil (Sharma *et al.*, 2014).

In this study, neem derivatives were highly toxic to cotton mealybugs at 3% concentration. Similarly, significantly few cotton mealybugs were found on the cotton leaves treated with 3% neem oil and neem seed water extract as compared to their respective controls. Neem oil and neem seed water extract at highest concentration (3%) negatively affected the prey consumption by *C. carnea* larvae when 16 and 32 aphids/day were offered. However, when the number of aphids/day was increased, both the neem derivatives at higher concentrations (2 and 3%) significantly affected the prey consumption by *C. carnea* larvae.

CONCLUSION

In conclusion, the findings of the present studies suggest that neem oil at appropriate concentrations can be used in combination with *C. carnea* in swapping to synthetic insecticides for a safer control of the test insects.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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