Journal of Entomology and Zoology Studies
Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(5): 916-919

Received: 10-07-2019
Accepted: 12-08-2019

Prabina Regmi
Institute of Agriculture and Animal Science Paklihawa Campus, Rupandehi, Nepal

Shrinu Kumari Jha
Institute of Agriculture and Animal Science Paklihawa Campus, Rupandehi, Nepal

Pragya Kiju
Institute of Agriculture and Animal Science Paklihawa Campus, Rupandehi, Nepal

Hira Poudel
Institute of Agriculture and Animal Science Paklihawa Campus, Rupandehi, Nepal

Shiva Shankar Bhattarai
Assistant Professor, Institute of Agriculture and Animal Science Paklihawa Campus, Rupandehi, Nepal

Corresponding Author:
Prabina Regmi
Institute of Agriculture and Animal Science Paklihawa Campus, Rupandehi, Nepal

Effect of neem treatment on seven spotted ladybugs (*Coccinella septempunctata*) in a laboratory condition in Nepal

Prabina Regmi, Shrinu Kumari Jha, Pragya Kiju, Hira Poudel and Shiva Shankar Bhattarai

Abstract
Effects of neem on fecundity and mortality of seven spotted ladybirds (*Coccinella septempunctata*) were evaluated under laboratory conditions in the Entomology laboratory at Paklihawa Agriculture campus of Rupandehi, Nepal from 13 March 2018 to 12 April 2018. The experiment was laid out according to a Complete Randomized Design (CRD) with four treatments and four replications each. Ladybirds were treated with the control, 2%, 3%, and 4% of neem solution. We found the effects of neem treatments on egg-laying, hatchability, and mortality to be non-significantly different. However, effects on male mortality and female mortality were significantly different. The impact of treatments on hatchability, mortality, and egg-laying were 31.04, 8.65, and 42.3 percent respectively. These results indicate that under the lab condition, neem treatment does not pose a negative effect on the insect.

Keywords: *Coccinella septempuctata*, complete randomized design, laboratory

Introduction

A major challenge of agriculture is to increase the production of crops to meet the food requirements of a growing population without harming the environment. There is no doubt that the modern agricultural system has been successful in increasing the production of crops, but at the cost of environmental pollution due to the excessive use of agrochemicals. Due to the high consumption of chemical pesticides and fertilizers, concern over the sustainability of the existing farming system has been growing, motivating the use of bio-insecticides commercially.

Bio-insecticide has eco-toxicological advantages over chemical insecticide due to low human toxicity, rapid degradation, and reduced ecological effects, making them suitable for organic farming (Bourget *et al*., 2000) [2]. Bio-insecticides are found to have secondary metabolites like amides, chalcones, lignans, neolignans, flavones, or phenols (Zanuncio *et al*., 2016) [22]. The presence of such metabolites facilitate the plant-insect interactions and make bio-insecticides suitable in the Integrated Pest Management, henceforth IPM.

Farmers have been using varieties of plants for preparation of bioinsecticides. Some of the commonly used plants for the purpose are neem, calotropis, lemongrass, lemon leaves, lantana, and moringa. Among all the bio-insecticides used, neem is considered to be the least toxic to human beings but considered promising to control the targeted pest. Neem, *Azadirachta indica is* a member of Meliaceae and originates from the Indian subcontinent. Neem is a small to medium-sized plant with wide branches. The plant can tolerate high temperatures as well as degraded soils. The young leaves are purple, which turns to green with maturity, consisting of the petiole, lamina, and stipules (Forim *et al*. 2014) [6].

It is found that the neem consists of at least 100 biologically active ingredients (Campos *et al*., 2016) [3]. Among them, the significant elements are triterpenes, the most important being the Azadirachtin. Azadirachtin has been found to contribute ninety percent of all the neem effects in insects. Apart from Azadirachtin, other essential compounds present in the plant include meliantriol, nimbin, nimbidin, nimbinin, nimbolides, fatty acids, and salanine. Neem extract is found to have a strong insecticidal effect against pests belonging to Coleoptera, Diptera, Hemiptera, and Lepidoptera orders (Ventura and Ito, 2000; Souza and Vendramin, 2005; Tedeschi, Alma, and Tavella, 2001; Viana and Prates, 2003) [20, 17, 19, 21].

Ladybugs (*Coccinella septempunctata)*, often called a ladybird beetle is a predatory insect.

The insect is a natural enemy of many pests, especially aphids and other sap feeders. It has been reported that a lady beetle can eat as many as five thousand aphids in its lifetime (Bessin, n.a.) [1]. Given its hunger towards harmful insects like aphids, ladybugs provide an active control agent in the context of IPM. The potential of *Coccinella septempunctata* as a biological control agent has been reported for sweet potato whitefly, mealybugs, adults and larvae of aphids, and scale insects in varieties of crops (Sanchez-Arroyo, 2014; Frank and McCoy, 1990; Michaud, 2001) [15, 5, 13].

Because the neem products are spread directly onto the leaves, the bio-insecticide affects non-targeted insects like ladybugs (Kumar *et al*. 2005) [10]. Although previous studies report the suitability of neem products in the IPM practices, its application is limited for specific species of insects. Therefore, additional studies on the effect of neem on the natural enemy are still necessary before we can recommend the product for the IPM program nationally.

The objective of our study was to evaluate the effect of different doses of neem on survivability and egg hatchability of the beneficial insect, *C. septempunctata,* in Nepal under laboratory conditions.

Materials and Methods

Study Area, Experimental Design, and Neem Solution Preparation

To assess the effects of neem-based insecticides on seven spotted ladybugs, a laboratory experiment was conducted in the entomology laboratory of Paklihawa Agriculture campus from 13 March 2018-12 April 2018. The experiment was set up in a complete randomized design (CRD) with four replications and four treatments.

Adults of the insects were collected from the agronomy field of the campus. The insects were raised in a bowl and petri dish. Each individual Coccinellid insect was fed with thirty aphids/day to maintain its daily nutrient requirements.

In the research, four treatments of neem with varying concentrations were prepared, i.e. control, 2ml/1L, 3ml/1L, and 4ml/1L. Neemraj super 3000ppm, a natural pesticide consisting of Azadirachtin, Nimbin, Nimbidin, Salanin, etc., was mixed with water to prepare the neem-based treatments. Plastic syringes were used to spray the different concentrations of the bio-insecticide on the insects kept on the Petri dishes.

Ladybugs Counting and Statistical Analysis

Observation on the impact of neem treatment on the insect was done every day around 1 pm and after. Data were collected for the following variables: a) mortality of ladybird beetles and b) fecundity. Hatching observations were made twice a day. Any dead insects, if observed during the monitoring, would be removed, and would be considered for analysis.

Standard statistical procedures were followed according to Gomez and Gomez (1984) [7]. One way ANOVA and mean comparison tests were employed for assessing the bio-insecticide effects on insects.

Results

Effect on Egg Laying, Egg Hatchability, and Mortality

Results on the effect of neem treatments on the ladybugs are shown in table 1. Egg-laying behavior of Coccinellids varied at different concentrations compared to the control. Egg-laying decreased with an increase in concentrations of neem (control>2>3>4). Egg-laying decreased from 193.75 to 25.5 when treated with the control and 4% neem concentrations, respectively. However, such variations were not significant at the five percent significance level (P=0.05).

Neem extract solution had negative effects on egg hatchability. However, the relationship between the treatment and egg hatchability was not significant at the 5% level, but significant at the 10% level i.e., P-value= 0.09. In general, egg hatchability decreased with an increase in neem concentration while treating.

The fourth column in table 1 shows neem treatment on mortality of ladybugs. The column shows that a nonsignificant relationship between various concentrations of neem and mortality existed (P=0.296). The mean mortality of the insects in control was 17.5. The variable was 35, 22.5, and 35 when treated with 2%, 3%, and 4% neem concentrations.

Table 1: Effect of Neem Treatment on Ladybugs in Laboratory Condition

Neem Concentrations (%)	Egg Laying	Egg Hatchability	Mortality
Control	193.75 ± 32.74	86.27 ±1.74	17.5 ± 8.54
2	96.25 ± 62.39	34.94 ± 20.17	35 ±8.66
3	49.5 ± 28.93	36.42 ± 21.03	22.5 ± 6.29
4	25.5 ± 25.5	20.1 ± 20.1	35 ± 6.45
F Statistics	3.4	2.65	1.392
P-value	0.05	0.09	0.29

Note: Table 1 shows the effect of Neem treatment on Ladybugs (Mean ± SE).

Mortality of Ladybugs According to Sexes

When comparing the mortality of male and female ladybugs when subjected to different concentrations of neem, it was found that female insects performed better. Males had an average mortality of 72.5 in control, while that for females was around 28. When they were subjected to 2%, 3%, and 4% neem solution, average male mortality decreased to 65, 78, and 65, respectively. In contrast to male mortality, female mortality remained almost constant. Average female mortality was found to be 35, 21.1, and 35, when they were treated respectively with 2%, 3%, and 4% concentrations of the bio-insecticide. Mortality of the insect according to sexes is shown in figure 1 below.

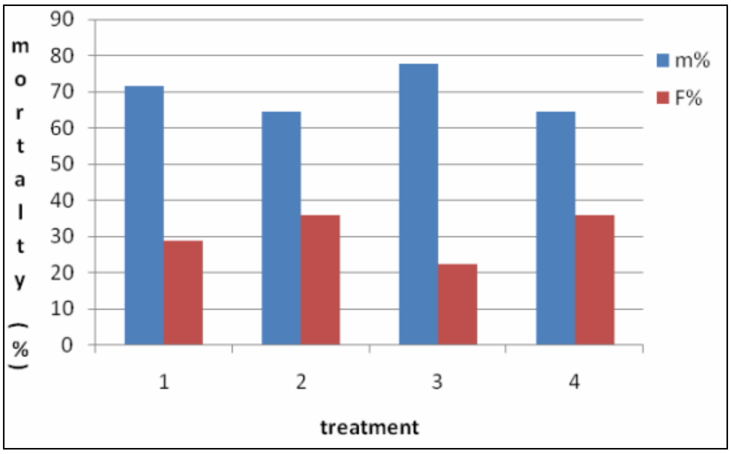

Fig 1: Mortality of Ladybugs according to Sexes when Treated with Different Concentrations of Neem.

Discussions

Pest control programs require high selectivity for application in the integrated pest management program (IPM), for example, broad-spectrum insecticides are not preferred. Selection of proper pest control programs can be achieved through environmental procedures that minimize the effect on the predators. Additionally, the use of insecticides that are more harmful to insects than to the predators, can also help in selection of suitable insecticides.

Here, the word selectivity refers to the tendency of neem treatments on the predator, seven spotted ladybugs, *Coccinella septempunctata*. Results of the research indicated that neem treatment at different concentration (i.e., control, 2%, 3%, and 4%) affected egg laying and egg hatchability of the insect. However, such results were not statistically significant at 5% probability. Egg laying was higher when treated with the control than when treated with 4% neem solution i.e., 193.75 vs 25.5. Egg hatching when treated with neem also decreased with an increase in the concentration of the treatment. Such results are in line with the activity reported on the effect of neem on the predator of Hemiptera order (Schumutterer, 1990; Matsuda *et al*. 2001; Ishaaya, 2007) [16, 12, 8].

The mortality of ladybugs after treatment with different concentrations of neem suggests the tolerance of the insect to the bio-insecticide. In the research, it was found that mortality of the insect increased when treated with neem solution. Again, the relationship remains insignificant. The result is in contrast with the previous findings that treating predator such as *Bemisia argentifolli*, *Clavigralla scutellaris*, and *Macrolophus caliginosus* with neem oil of different concentrations from 0.1 to 10 percent, increased mortality significantly (Masood *et al*. 2006; Mitchell, 2004; Tedeschi *et al*. 2001) [11, 14, 19].

Conceptually, any insecticides should be toxic to targeted insects with less or almost zero impact on beneficial insects like ladybugs. In this study, applications of neem solution of different concentrations showed negative but statistically non-significant toxicity for the ladybugs. These results suggest that neem treatment is safe for application.

Conclusions

Biological analysis of the neem-based treatment of different concentrations revealed that neem treatment did not have statistically significant negative effects on the ladybugs. Treating the insect with the control, 2%, 3%, and 4% neem-based insecticide showed that egg-laying, hatchability, and mortality of ladybird beetles were not significantly different at five percent probability. Based on the lab research, it is recommended that neem is a safe alternative to chemical insecticides. The conclusions are not without constraints. The research was conducted for a period of a month in strict lab conditions with a limited sample of insects. In order to better understand the neem effects on Coccinellids, field research with large insect sample over a longer period of time is recommended, which is the immediate future work of the authors.

Acknowledgment

We would like to extend our appreciation to Mr. Subodh Khanal, Dr. Keshav Prasad Adhikari, Dr. Kannhaiya Prasad Singh, and Mr. Dhan Bahadur Rana for providing valuable guidance throughout the execution of this research, which is our undergraduate thesis project. We would also like to convey deep thanks to our friends Sanjay Yadav, Pratima Subedi, Anita Paneru, Shovana GC, Dibya Bhatta, and Sapana Thapa for supporting us during data collection and analysis.

References

1. Bessin R. Ladybugs. Cooperative Extension Service, University of Kentucky, ENTFACT105. Available at: https://entomology.ca.uky.edu/files/efpdf1/ef105.pdf. [Accessed on 17 August 2019].
2. Bourguet D, Genissel A, Raymond A. Insecticide resistance and dominance levels. Journal of Economic Entomology. 2000; 93(6):1588-1595.
3. Campos EVR, de Oliveira JL, Pascoli M, de Lima R, Fraceto LF. Neem Oil and Crop Protection: From Now to the Future. Frontiers in Plant Science. 2016; 7:1494.
4. Choudhary R, Chandrakar G, Bhardwaj GR, Khan HH. Effects of neem based insecticides on Coccinellids and Staphylinids population in rice fields. Journal of Entomology and Zoology Studies. 2017; 5(5):1688-1692.
5. Frank JH, McCoy ED. Endemics and Epidemics of Shibboleths and other things causing chaos. Florida Entomologist. 1990; 73:1-8.
6. Forim MR, Fernandes DSMF, Fernandes JB, Vieira PC. Processo de Obtencao de Nanoparticulas Biopolimericas Contendo Oleo e Extratos de *Azadirachtha indica* a Juss (neem), Nanoparticulas Biopolimericas e Microparticular em po. Available at: http://www.google.co.ve/patents/WO2014113860A1 [accessed August 12, 2019].
7. Gomez KA, Gomez AA. Statistical Procedures for

Agricultural Research- Handbook. John Wiley & Sons, New York, 1984.

8. Ishaaya I, Barazani A, Kontsedalov S, Horowitz AR. Insecticides with novel modes of action: Mechanism, selectivity and cross-resistance. Entomol Research. 2007; 37:148-152.
9. Kraiss H, Cullen EM. Insect growth regulator effects of azadirachtin and neem oil on survivorship, development, and fecundity of *Aphis glycines* (Hemiptera: Aphididae) and its predator, *Harmona axyridis* (Coleoptera: Coccinellidae). Pest Management Science. Doi: 10.1002/ps. 2008.
10. Kumar P, Poehling HM, Borgemeister C. Effects of different application methods of azadiracthin against sweetpotato whitefly *Bemisia tabaci* Gennadius (Hom., Aleyrodidae) on tomato plants. Journal of Applied Entomology. 2005; 129:489-497.
11. Masood KK, ur-Rashid M, Syed A, Hussain S, Islam T. Comparative effect of neem (*Azadirachta indica* A. Juss) oil, neem seed water extract and baythroid against whitefly, jassids and thrips on cotton. Pakistan Entomologist. 2006; 28:31-37.
12. Matsuda K, Buckingham SD, Kleir D, Rauh JJ, Grauso MM, Sattelle DB. Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. Trends Pharmacological Sciences. 2001; 22:573-580.
13. Michaud JP. Numerical responses of *Olla v-nigrum* (Coleoptera: Coccinellidae) to infestations of Asian citrus psyllid (Hemiptera: Psyllidae) in Florida. Florida Entomologist. 2001; 18:608-612.
14. Mitcheli PL, Gupta R, Singh AK, Kumar P. Behavioural and development effects of neem extracts on *Clavigralla scutellaris* (Hemiptera: Heteroptera: Coreidae) and its egg parasitoid, *Gryon fulviventre* (Hymenoptera: Scelionidae). Journal of Economic Entomology. 2004; 97:916-923.
15. Sanchez- Arroyo H. Mexican bean beetle, *Epilachna varivestis* Mulsant. UF/IFAS Featured Creatures, 2014.
16. Schmutterer H. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Annual Review of Entomology. 1990; 35:271-297.
17. Souza AP, Vendramim HD. Efeito translaminar, sistêmico e de contato de extrato aquoso de sementes de nim sobre *Bemisia tabaci* (Genn.) biótipo B em tomateiro. Neotropical Entomolgy. 2005; 34:83-87.
18. Swaminathan R, Jat M, Hussain T. Side effects of a few botanicals on the aphidophagous coccinellids. Journal of Biopesticides. 2010; 3(1):81-84.
19. Tedeschi R, Alma A, Tavella L. Side-effects of three neem (*Azadirachta indica* A. Juss) products on the predator *Macrolophus caliginosus* Wagner (Het., Miridae). Journal of Applied Entomolgy. 2001; 125:397-402.
20. Ventura MU, Ito M. Antifeedant activity of *Melia azedarach* (L) extracts to *Diabrotica speciosa* (Genn.) (Coleoptera: Chrysomelidae) beetles. Brazilian Archives of Biology and Technolgy. 2000; 43:215-219.
21. Viana PA, Prates HT. Desenvolvimento e mortalidade larval de *Spodoptera frugiperda* em folhas de milho tratadas com extrato aquoso de folhas de *Azadirachta indica*. Bragantia. 2003; 62:69-74.
22. Zanuncio JC, Mourao SA, Martinez LC, Wilcken CF, Ramalho FS, Reuda AP *et al*. Toxic effects of the neem oil (*Azadirachta indica*) formulation on the stink bug predator, *Podisus nigrispus* (Heteroptera: Pentatomidae). Scientific. 2016; 6:30261.