International Journal of Entomology Research
ISSN: 2455-4758
Impact Factor: RJIF 5.24
www.entomologyjournals.com
Volume 3; Issue 2; March 2018; Page No. 159-163

Evaluation of toxicity of biopesticides against okra moth, *Earias vittella* (Fabricius) (Noctuidae: Lepidoptera)

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Abstract
The toxicity of garlic bulb aqueous extract, neem leaf aqueous extract and NeemGold (neem oil) (Azadiractin 0.03% EC, 300 ppm) was evaluated against okra moth, *Earias vittella* (F.) (Noctuidae: Lepidoptera) under laboratory conditions. The LC_{50} values were determined at different doses (20, 30, 50 and 70 mg) were used for the aqueous extract of garlic bulb and neem leaf, and NeemGold (neemoil) for 24 h to 96 h exposure periods. The significant time and dose dependent toxic effect of all biopesticides were observed against *E. vittella.* The result demonstrated that *E. vittella* is very sensitive to NeemGold (LC_{50} = 39.31 mg and 22.45 mg for 24 h and 96 h treatment, respectively) followed by neem leaf aqueous extract (LC_{50} = 42.03 mg and 28.02 mg for 24 h and 96 h treatment, respectively) and garlic bulb aqueous extract (LC_{50} = 61.85 mg and 39.42 mg for 24 h and 96 h treatment, respectively). It evinced that the NeemGold is more effective than neem leaf aqueous extract and garlic bulb aqueous extract for reducing the number of *E. vittella* larvae. Thus, the botanical tools are also valuable for managing the population of agricultural pests and being ecofriendly it gives better results for the management of *Earias vittella.*

Keywords: *Abelmoschus esculentus*, *Earias vittella*, Biopesticides, LC_{50}

1. Introduction

Okra (*Abelmoschus esculentus* (L.) Moench) is the only vegetable crop of significance in the Malvaceae family and is very popular in the Indo-Pak subcontinent [1]. It is not only the important source of vitamins and minerals but also proteins and carbohydrates. According to The okra pods can be consumed in different forms as well as important as fresh fruits [2]. It has also medicinal value and provide relief from ulcers and hemorrhoids [3]. Like other food crops, okra crop is also attacked by several insect pests among which spiny bollworm, *Earias vittella* (F.) (Noctuidae: Lepidoptera) is most injurious [4, 5]. *E. vittella* cause direct damage to tender fruits. The larvae attack and damage growing points of the vegetative parts, feed mostly inside squares, flowers and fruits. *E. vittella* causes 8.4-73.2% infestation on fruits of okra depending on the season [6]. Since, the synthetic insecticides cause health problems in humans, animals and also create environmental hazards, their field use should be minimum [7, 8]. In recent decades, effective uses of plant extracts against several insect pests have been reported [9, 10, 11, 12, 13]. Neem (*Azadirachta indica* A. Juss., Meliaceae) and garlic (*Allium sativum* L. var. *sativum*, Amaryllidaceae) based biopesticides have already shown their potentiality [14, 15]. Neem based biopesticides such as has systemic activity and it is even active at low concentrations but degrades rapidly in the environment [16]. Among the isolated Neem constituents, limonoids (azadirachtin) have multiple functions, such as insect growth regulation, affects the reproductive and digestive process of insects in such a manner that the insect can no longer feed, breed or undergo metamorphosis [17]. More specifically, it disrupts moulting by inhibiting biosynthesis or metabolism of ecdysone, the juvenile moulting hormone [18]. Garlic consists of varieties of volatile sulphur-based compounds which are effective as insect repellent as well as insecticides. Its one component, diallyl disulfide has a strong odour and acts as a powerful insecticide [5]. Commercial preparations of garlic are certified as insecticides against different pests infesting a variety of crops [19, 20, 21]. Aqueous garlic extracts have been shown to inhibit egg hatchability of mosquitoes [22], to have toxic and antifeedant effects on stored product pests [23] and Lepidoptera [24]. Repellent and toxic effects upon Hemiptera have also been observed [25, 24]. In India, *E. vittella* is one of the very common and notorious pest of okra. Therefore, the present study was undertaken to find out the LC_{50s} of aqueous extract of garlic bulb and neem leaf, and NeemGold against *E. vittella* larvae.

2. Materials and Methods

The *E. vittella* were reared following the method of Bhardwaj [26] in the laboratory. Under laboratory condition, the stock of *E. vittella* was maintained by procuring infested okra fruits from the local fields. As per requirement, different lots of such infested okra fruits were kept in rectangular insectaries (15x15x15 cm) made of card board, the top and four sides were fitted with soft fine mesh plastic net for ventilation. Inside the infested fruits, the larvae steadily grew till pupation by consuming the developing seeds. The full grown larva (approx. 2.0 cm in size) after completing development inside the okra fruit makes its exit and selects a suitable spot viz., the wall of the insectary, or even the outer surface of the fruit itself to pupate in a tough silken cocoon. Under optimal conditions, the pupal period lasts for 5 to 9 days at the end of which new generation of adult moths emerges after sunset.

Preparation of biopesticides

a. Aqueous extract of garlic bulbs (AEG): Aqueous extract of garlic bulb was prepared by grinding 1 kg of garlic bulbs with 1 l of distilled water. The extract was squeezed through fine meshed rayon cloth and finally filtered through Whatman filter paper. The filterate (w/v) was used for foliar application as test biopesticide on experimental plant.

b. Aqueous extract of neem leaves (AEN): Leaves of neem were locally collected. Its aqueous extract was prepared by grinding 1 kg of fresh leaves with 1 l of distilled water. The extract was squeezed through fine meshed rayon cloth and finally filtered through Whatman filter paper. The filterate (w/v) was used as test biopesticide for foliar application on experimental plant.

c. NeemGold preparation (NG): NeemGold was purchased from local market. It contains Azadiractin A 0.03%, Neem oil 90.57%, Hydroxy EL 5.00%, Epichorohydrin 0.50% and Aromex 3.90%, and is manufactured by Foliage Crop Solution Private Limited, Chennai, 600006, India. For preparation of its test solution, 1 ml of NeemGold was dissolved in 100 ml of distilled water.

Experimental set up

Above mentioned 3 biopesticides were used for their toxicity test, viz. AEG, AEN and NG. For each set, 20 third instar larvae of *E. vittella* were kept in an insectary and were kept in the laboratory at around 25°C. For every treatment of biopesticides, doses used were 20, 30, 50 and 70 mg/cage/20 larvae. After 24, 48, 72 and 96 h of exposure periods, the larvae were counted to record mortality. The lethal dose (LC_{50}), slope value, t-ratio and heterogeneity were calculated by the POLO computer programme following Robertson *et al.*, (2007). In the control set up, instead of biopesticides, only distilled water was sprayed over the larvae. The experiment was repeated 6 times for each biopesticide.

3. Results

The results are demonstrated in Table 1-3. The time and dose dependent toxic effect were observed in all the treatment of AEG, AEN and NeemGold in all the exposure periods (Fig. 1). Table 1 displays that the LC_{50} values of AEG was 61.85 mg after 24 h which decreased upto 39.42 mg after 96 h of exposure period. Table 2 shows that the LC_{50} values of AEN was 42.03 mg after 24 h of exposure period which decreased upto 28.02 mg after 96 h. Similarly, there was higher decrease in LC_{50} value from 39.31 mg (24 h of exposure period) to 22.45 mg (96 h of exposure period) of NeemGold (Table 3). Regression coefficient showed a significant ($P < 0.05$) negative regression between exposure periods and different values of LC_{50}. The values of heterogeneity were very low and t-ratios are significant that demonstrate that the decrease in LC_{50} value due to longer exposure period are significant, i.e., the toxicity of these biopesticides is time dependent. Enhanced exposure period increases the larval mortality in *E.* vitella. The data also demonstrated that the toxicity of NeemGold was much higher followed by AEN and AEG.

Table 1: Toxicity of garlic bulb aqueous extract againts *Earias vittella.*

Treated period (hrs)	Effective dose (LC_{50} mg)	Confident limits (mg)		Slope value	t- ratio	Heterogeneity
		LCL	UCL			
24	61.85	48.25	106.18	2.44±0.77	3.16	0.076
48	59.07	46.66	94.83	2.52±0.77	3.26	0.099
72	50.98	39.72	79.73	2.23±0.73	3.05	0.192
96	39.42	30.25	52.36	2.34±0.72	3.25	0.135

Table 2: Toxicity of neem leaf aqueous extract againts *Earias vittella.*

Treated period (hrs)	Effective dose (LC_{50} mg)	Confident limits (mg)		Slope value	t- ratio	Heterogeneity
		LCL	UCL			
24	42.03	33.41	55.14	2.56±0.73	3.509	0.035
48	36.92	28.55	46.98	2.54±0.72	3.50	0.024
72	31.29	22.61	39.24	2.50±0.73	3.423	0.028
96	28.02	20.11	34.51	2.76±0.75	3.664	0.283

Table 3: Toxicity of neem gold againts *Earias vittella.*

Treated period (hrs)	Effective dose (LC_{50} mg)	Confident limits (mg)		Slope value	t- ratio	Heterogeneity
		LCL	UCL			
24	39.31	31.82	48.97	2.90±0.74	3.895	0.068
48	33.34	24.65	42.15	2.46±0.72	3.391	0.029
72	29.29	20.67	36.59	2.54±0.73	3.438	0.061
96	22.45	14.46	28.06	2.89±0.816	3.553	0.244

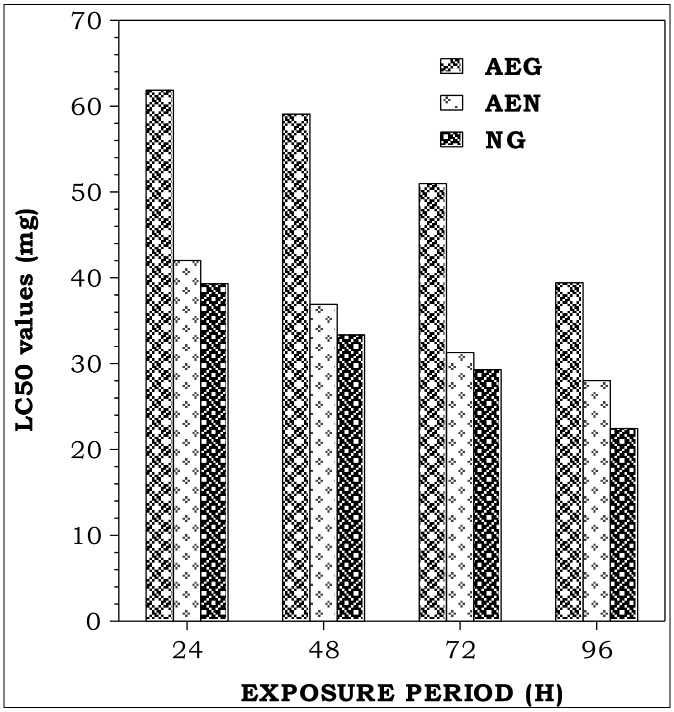

Fig 1: Relationship between LC_{50} values of biopesticides tested and exposure periods.

4. Discussion

Biopesticides are certain types of pesticides derived from biological materials such as animals, plants, bacteria, etc. They are a set of tools and applications that help our farmers' transition away from highly toxic conventional chemical pesticides into an era of truly sustainable agriculture. India's rich biodiversity is an ace factor, always providing a wide source of biopesticides which can be effectively used in agriculture at a large scale. Also increasing health consciousness of Indian citizens has created a demand of organic food. This indicates huge scope for growth of biopesticides sector. Pesticidal property of garlic has earlier been reported against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) [27]. A 225.8 µl/l air of the garlic extract caused 83.3% larval mortality after 48 h. Even its very low dose (2.13 µl/cm^2) caused more than 95% repellency. Two compounds extracted from garlic, (methyl allyl disulfide and diallyl trisulfide) have been observed toxic to other store grain beetles by reducing the egg hatchability and larval death [28, 29]. Bachrouch [11] also observed a dose dependent rate of egg mortality, higher dose of garlic extract increased the egg mortality. Garlic oil (2 %) protected the okra fruits from infestation of *E. vittela* when sprayed on the crop [30]. In other study of garlic essential oil was demonstrated to possess insecticidal activity against *Blattella germanica* (L.) (Blattodea: Blatellidae) [31]. Recently, Sohail [32] found reduced reproduction rate and high larval mortality of another okra moth *E. insulana* in Pakistan. Garlic oil caused substantial mortality and repellency in larva, pupa, and adult stages. The best results were obtained with concentrations of 16 and 32% in *Tenebrio molitor* (Coleoptera: Tenebrionidae) as reported for other stored grain pests according to the concentration of these products. [33, 34, 35]

A number of neem-based products have been observed to induce mortality of different kinds of insect pests on several vegetable and cereal crops [36, 37, 38, 12] and have been used in their management [39]. Its active ingradient, azadirachtin has been reported to reduce the fertility and fecundity of adults of green peach aphid *Myzus persicae* (Sulzer), lettuce aphid *Nasonovia ribisnigri* (Mosley) and strawberry aphid *Chaetosiphon fragaefolii* (Cockerell) in a linear concentration-dependent manner [40]. Neem-based insecticides have been found to deter feeding and thus influencing growth rate of beet armyworm (*Spodoptera exigua*) [41], *Trogoderma granarium* (Everts) (Coleoptera: Bostrichoidea) [42], *Cryptolestes pusillus* (Schon.) (Coleoptera: Polyphaga) [43]. Insecticidal influence of neem seed acetone extract was observed on the third instar larvae of *Corcyra cephalonica* (Staint.) (Lepidoptera: Pyralidae) and caused a depressive effect on the developmental stages of this moth. A dose level of 0.16% (a.i) v/w of this extract caused 100% larval mortality, which may be considered as extremely toxic to the pest [13]. Third instars larvae of the cabbage moth, *Mamestra brassicae* L. revealed that Neem EC inhibited larval development, greatly increased mortality, had antifeedant/deterrent effects and acted as a growth regulator for *Mamestra brassicae* (Lepidoptera: Noctuidae) larvae and pupae [44]. Experiments by Luik & Viidalepp [45]showed that when caterpillars of *Phytometra brassicae* (Lepidoptera: Noctuidae) were fed with cabbage treated with NeemAzal T/S, all caterpillars perished within 7 days. Active ingredient of neem i.e. azadirachtin inhibits the release of prothoracicotropic and allatotropic hormones and hence affect the metamorphosis [46] and the growth [47]. Direct toxicity effect of neem based biopesticides (Neemazal, Neemarine, Bioneem, Neemgold, Nimbicidine and Achook) on *E. vitella* and *D. koenigii* was done by Kumar [48]. The result of toxicity effectiveness on the basis of the mortality ranged in descending order: Neemazal > Neemarine > Bioneem > Neemgold > Nimbicidine and Achook. Present study reveals that the biopesticides based on neem derivatives is more effective for the insect *E. vittella* than to garlic bulb extract.

5. Conclusion

It is concluded from the present experiments that the NeemGold (Azadiractin 0.03% EC) is more potential in comparison to neem leaf extract and garlic bulb extract against *E. vittella*. Biopesticides are easily biodegradable, most effective and non hazardous to human and environment, leaves no residue and it is soft to insect predators and pollinators. Biopesticides may also offer solutions to concerns such as pest resistance to traditional chemical pesticides, public concern about side effects of pesticides on the surrounding environment and ultimately, on human health.

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