



Relative toxicity of natural products and biopesticides against brinjal shoot and fruit borer, *Leucinodes orbonalis* (Guenee)

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Abstract

The investigation was conducted to study the intrinsic toxicity of *Agniastra*, *Brahmastra*, azadirachtin, *Bacillus thuringiensis*, emamectin benzoate and spinosad against neonate larvae of *Leucinodes orbonalis* by residue film method of bioassay under laboratory conditions. The results revealed that LC₅₀ value of the emamectin benzoate was found to be minimum (0.0043 ppm), followed by spinosad (0.0303 ppm), Bt (3.989 ppm), azadirachtin (8.847 ppm), *Brahmastra* (24203.194 ppm) and *Agniastra* (29795.975 ppm). Almost similar trend in LC₉₀ values was observed.

Key words: Toxicity, *Leucinodes orbonalis*, insecticides, biopesticides and natural products

Eggplant (*Solanum melongena* L.) is one of the most popular and important commercial vegetable crops grown throughout the world. In production and productivity, India stands second in the world after China. It is a native of India and is grown throughout the country except in higher altitudes (Jena, 2006). In India, brinjal is cultivated in 7.28 lakh ha area with an annual production of 12.66 million tons (NHB, 2020). It is grown in the states of Orissa, Bihar, Karnataka, West Bengal, Andhra Pradesh, Maharashtra and Uttar Pradesh in India. In Himachal Pradesh, brinjal is grown over an area of 1012.20 ha with a production of 20918.00 MT (Anonymous, 2020). It is an important vegetable due to its nutritive value consisting of minerals like iron, phosphorus, calcium and vitamins like A, B and C.

Brinjal is attacked by many insect pests viz., *Leucinodes orbonalis* (Guenee), *Henosepilachna vigintioctopunctata* Fabricius, *Euzophera perticella* Ragonot, *Urentius sentis* Distant, *Eublemma olivacea* Walker, *Amrasca biguttula biguttula* Ishida, *Bemisia tabaci* (Gennadius), *Trialeurodes vaporariorum* (Westwood), *Aphis gossypii* Glover, *Myzus persicae* (Sulzer) and mite, *Tetranychus urticae* Koch. starting from seedling stage to senescence (Chandel *et al.* 2016; Ghongade *et al.* 2021). The most important of which is the shoot and fruit borer which causes significant losses of 60-70 per cent (Patial *et al.* 2007).

The pest is very active during the rainy and summer season and cause 85 to 90 per cent damage (Jagginavar *et al.* 2009; Thapa, 2010). The larva is a internal feeder, it immediately bores into the nearest tender shoot or flower or fruit just after hatching. Larval feeding inside shoots, result in wilting of young shoots. The minute entrance hole in fruit is closed by the excreta of feeding larvae (Alam *et al.* 2006). A number of insecticides have been recommended for the management of this pest but their indiscriminate use has posed a resistance in pest species to insecticides along with many other side effects (Pal, 2004). Therefore, the present investigation was planned to study the intrinsic toxicity of natural product, biopesticides and insecticides against *L. orbonalis*.

Materials and Methods

The experiment was conducted during 2020-21 in the Toxicology laboratory, Department of Entomology, CSK HPKV, Palampur. The toxicity tests of natural products, *Agniastra*, *Brahmastra*, biopesticides, *Azadirachtin*, *Bacillus thuringiensis*, and insecticides, emamectin benzoate and spinosad were carried out against neonate larvae of *L. orbonalis*.

Test insect population

L. orbonalis infested shoots and fruits were collected from field and placed inside the perforated

aluminium cage (15 cm x 15 cm x 15 cm). The larvae were segregated and reared on healthy and untreated fruits of brinjal till pupation. The pupae were collected and placed inside the glass jars until emergence. The emerged moths were transferred to clean circular glass jar (20 cm x 15 cm) for pairing, covered with black muslin cloth and secured tightly with rubber band (Sharma, 2018). On hatching, neonate larvae were transferred to fresh brinjal fruits with the help of fine camel hair brush and fruits were placed in aluminium cage provided with filter paper at the bottom. The neonate larvae were preconditioned for 2 hours without food. After starvation, the healthy and active larvae were selected.

Bioassay

For preparing the working concentration of each treatment, stock solutions of the natural products, biopesticides and insecticides were prepared in distilled water. The concentrations were freshly prepared every time before the conduct of an experiment. In preliminary experiment, the concentrations giving a mortality of 20 to 90 per cent were ascertained. Healthy brinjal fruits were cut into small discs and treated with different concentrations with the help of hand atomizer. The fruit discs were treated with Bavistin to check fungal infection. Treated discs were then shade dried for 15 to 30 minutes. In each treatment, 10 neonate larvae were released on a small disc which was replicated thrice. The treated disc was kept in a petri dish on a filter paper. In case of *Agniastra*, *Brahmastra*, *Azadirachtin* and *Bacillus*

thuringiensis, the mortality data were recorded after 24, 48 and 72 hours of treatment, whereas the data were recorded after 24 hours of treatment in case of emamectin benzoate and spinosad. The moribund larvae were treated as dead. The corrected per cent mortality was calculated as per Abbott's (1925) formula. The data so obtained were subjected to Probit analysis (Finney, 1971) to work out LC_{50} and LC_{90} values of natural products, biopesticides and insecticides for neonate larvae of *L. orbonalis*.

Results and Discussion

The results on intrinsic toxicity of natural products, biopesticides and insecticides against neonate larvae of *L. orbonalis* are presented in table 1 to 6:

1. Natural Products

Agniastra when tested against neonate larvae of *L. orbonalis* at concentration ranging from 6250 to 100000 ppm caused mortality in the range of 13.3 to 80.0 per cent after 72 hours of exposure. There was no mortality in control treatment (Table 1). LC_{50} value of 29795.975 ppm was obtained with the fiducial limits of 21812.114 and 41918.579 ppm. The LC_{90} value was estimated to be 177629.326 ppm with the fiducial limits of 102949.074 and 492621.844 ppm. No significant heterogeneity was recorded in the data as the calculated chi-square ($\chi^2 = 0.108$) was less than the tabulated value ($\chi^2 = 7.82$). *Brahmastra* formulation tested at concentration ranging from 6250 to 100000 ppm against neonate larvae of *L. orbonalis* resulted in mortality of 20.00 to 80.00 per cent, whereas in control

Table 1. Concentration-mortality response of *Agniastra* against neonate larvae of *L. orbonalis*

Conc.(ppm)	Log Conc $\times 10^{-3}$ X	No. of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical Probit	Expected Probit
6250.00	0.80	30	13.3	13.3	3.8877	3.9
12500.00	1.10	30	26.7	26.7	4.3781	4.4
25000.00	1.40	30	43.3	43.3	4.8313	4.9
50000.00	1.70	30	66.7	66.7	5.4316	5.4
100000.00	2.00	30	80.0	80.0	5.8416	5.9
Control	-	30	0.0	-	-	-

Regression equation, $Y = 2.551 + 1.665X$

$LC_{50} = 29795.975$ ppm

Fiducial limits= 21812.114 and 41918.579 ppm

$LC_{90} = 177629.326$ ppm

Fiducial limits= 102949.074 and 492621.844 ppm

$\chi^2_{cal} (p=0.05) = 0.108$

$\chi^2_{tab} (p=0.05) = 7.814$

the mortality was 3.33 per cent (Table 2). Probit analysis of the data revealed that the LC_{50} and LC_{90} values were 24203.194 and 208439.897 ppm, respectively. Chi-square test showed that the data were homogeneous ($\chi^2 = 0.077$) at 5 per cent level of significance and 3 degree of freedom.

2. Biopesticides

Brinjal fruit discs treated with Azadirachtin at concentration of 2.34, 4.69, 9.38, 18.75 and 37.5 ppm

when given to neonate larvae of *L. orbonalis*, showed mortality of 16.67 to 86.67 per cent and in control the mortality was 3.33 per cent (Table 3). Probit analysis of the data revealed the LC_{50} value as 8.847 ppm and LC_{90} as 46.891 ppm. The fiducial limits of LC_{50} were 6.504 and 11.943 ppm whereas; fiducial limits of LC_{90} were 29.350 and 107.943 ppm. Bt formulation tested at concentration of 1.03, 2.06, 4.13, 8.25 and 16.5 ppm against neonate larvae of *L. orbonalis* resulted in mortality of 20.00, 33.33, 46.67, 70.00 and 83.33 per

Table 2. Concentration-mortality response of *Brahmastra* against neonate larvae of *L. orbonalis*

Conc.(ppm)	Log Conc. $\times 10^{-3}$ X	No. of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical Probit	Expected Probit
6250.00	0.80	30	20.00	17.24	4.0537	4.10
12500.00	1.10	30	36.67	34.48	4.6011	4.53
25000.00	1.40	30	50.00	48.28	4.9574	4.90
50000.00	1.70	30	66.67	65.52	5.3989	5.40
100000.00	2.00	30	80.00	79.31	5.8169	5.83
Control	-	30	3.33	-	-	-

Regression equation, $Y = 2.939 + 1.246X$

$LC_{50} = 24203.194$ ppm Fiducial limits= 16356.343 ppm and 35497.732 ppm

$LC_{90} = 208439.897$ ppm Fiducial limits= 107292.640 ppm and 839028.164 ppm

$\chi^2_{cal} (p=0.05) = 0.077$ $\chi^2_{tab} (p=0.05) = 7.814$

Table 3. Concentration-mortality response of Azadirachtin against neonate larvae of *L. orbonalis*

Conc.(ppm)	Log Conc. X	No. of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical Probit	Expected Probit
2.34	0.37	30	16.67	13.79	3.9107	3.89
4.69	0.67	30	30.00	27.59	4.4052	4.44
9.38	0.97	30	50.00	48.28	4.9574	4.99
18.75	1.27	30	73.33	72.41	5.5948	5.55
37.50	1.57	30	86.67	86.21	6.0893	6.10
Control	-	30	3.33	-	-	-

Regression equation, $Y = 3.218 + 1.822X$

$LC_{50} = 8.847$ ppm Fiducial limits= 6.504 ppm and 11.943 ppm

$LC_{90} = 46.891$ ppm Fiducial limits= 29.350 ppm and 107.943 ppm

$\chi^2_{cal} (p=0.05) = 0.134$ $\chi^2_{tab} (p=0.05) = 7.814$

cent, respectively, whereas in control the mortality was 10.00 per cent (Table 4). Probit analysis of the data revealed that the LC_{50} and LC_{90} values as 3.989 and 27.783 ppm, respectively. Chi-square test showed that the data were homogeneous ($\chi^2=0.300$).

3. Insecticides

Neonate larvae of *L. orbonalis* when fed on the brinjal fruit discs treated with emamectin benzoate at concentrations ranging from 0.00098 to 0.01563 ppm

resulted in mortality of 16.67 to 86.67 per cent after 24 hours of exposure (Table 5). In control, no mortality was recorded. After subjecting the mortality data to probit analysis, LC_{50} and LC_{90} values of 0.0043 and 0.0228 ppm, respectively were obtained. The data were homogenous ($\chi^2 = 0.236$) at 5 per cent level of significance and 3 degree of freedom. Spinosad when tested at concentrations ranging from 0.0156 to 0.25 ppm against neonate larvae of *L. orbonalis* resulted in

Table 4. Concentration-mortality response of Bt against neonate larvae of *L. orbonalis*

Conc. (ppm)	Log Conc. X	No. of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical Probit	Expected Probit
1.03	0.01	30	20.00	11.11	3.7788	3.78
2.06	0.31	30	33.33	25.93	4.3536	4.31
4.13	0.62	30	46.67	40.74	4.7647	4.84
8.25	0.92	30	70.00	66.67	5.4316	5.38
16.50	1.22	30	83.33	81.48	5.8965	6.0
Control	-	30	10.00	-	-	-

Regression equation, $Y = 3.756 + 1.779 X$

$LC_{50} = 3.989$ ppm Fiducial limits= 2.805 ppm and 5.642 ppm

$LC_{90} = 27.783$ ppm Fiducial limits= 15.614 ppm and 85.325 ppm

$\chi^2_{cal} (p=0.05) = 0.300$ $\chi^2_{tab} (p=0.05) = 7.814$

Table 5. Concentration-mortality response of emamectin benzoate against neonate larvae of *L. orbonalis*

Conc.(ppm)	Log Conc. $\times 10^4$ X	No. of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical Probit	Expected Probit
0.00098	0.99	30	16.67	13.79	3.9107	3.87
0.00195	1.29	30	30.00	27.59	4.4052	4.41
0.00391	1.59	30	46.67	44.83	4.8693	4.94
0.00782	1.89	30	66.67	65.52	5.3989	5.47
0.01563	2.19	30	86.67	86.21	6.0893	6.00
Control	-	30	00.00	-	-	-

Regression equation, $Y = 2.133 + 1.763 X$

$LC_{50} = 0.0043$ ppm Fiducial limits= 0.0015 ppm and 0.0058 ppm

$LC_{90} = 0.0228$ ppm Fiducial limits= 0.0073 ppm and 0.0301 ppm

$\chi^2_{cal} (p=0.05) = 0.236$ $\chi^2_{tab} (p=0.05) = 7.814$

mortality of 16.67 to 83.33 per cent (Table 6) while, 6.67 per cent mortality was observed in control. The LC_{50} value was worked out as 0.0303 ppm with upper and lower fiducial limits of 0.0183 and 0.0486 ppm, respectively. The LC_{90} value was 0.4104 ppm with fiducial limits of 0.1960 and 0.6064 ppm. The chi-square test revealed no heterogeneity of the data as the calculated value of χ^2 was less (0.402) than its tabulated value (7.82).

In present studies the lowest LC_{50} value was obtained for emamectin benzoate (0.0043 ppm) and highest for *Agniastra* (29795.975 ppm) against neonate larvae of *L. orbonalis*. The order of relative toxicity of natural products, biopesticides and insecticides against *L. orbonalis* was found to be emamectin benzoate (0.0043 ppm) > spinosad (0.0303 ppm) > Bt (3.989 ppm) > azadirachtin (8.847 ppm) > *Brahmastra* (24203.194 ppm) > *Agniastra* (29795.975 ppm), whereas, based on LC_{90} the trend remained similar (Table 7). When compared to *Agniastra*, emamectin benzoate was 6.92×10^6 times

more toxic to *L. orbonalis* and spinosad was 9.83×10^5 times more toxic. The LC_{50} values obtained for emamectin benzoate (0.0043 ppm) and spinosad (0.0303 ppm) in the present studies are different from those of Munje *et al.* (2015) who found the LC_{50} value of emamectin benzoate as 0.277-0.351 ppm and spinosad as 3.094-4.940 ppm against third instar of *L. orbonalis*. The reason for higher values of LC_{50} could be the third instar larvae used for testing, which have eventually developed greater surviving potential than the neonate larvae and hence require greater doses to achieve mortality. Sharmila *et al.* (2015) evaluated different plant extracts against *Pieris brassicae* under laboratory and found that azadirachtin showed highest antifeedant effect (100%) after 24 hours of treatment. Badiyala and Sharma (2013) and Thakur and Sood (2019) also reported azadirachtin, the toxic principle of neem to be highly effective against *Helicoverpa armigera*, *Agrotis ipsilon* and *T. urticae* with LC_{50} value of 0.0045 per cent and 0.0051 per cent, respectively. Similar results were found by Shashni

Table 6. Concentration-mortality response of spinosad against neonate larvae of *L. orbonalis*

Conc.(ppm)	Log Conc. $\times 10^2$ X	No. of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical Probit	Expected Probit
0.01562	0.19	30	16.67	10.71	3.7574	3.76
0.03125	0.49	30	30.00	25.00	4.3255	4.29
0.06250	0.80	30	46.67	42.86	4.8211	4.82
0.12500	1.10	30	63.33	60.71	5.2715	5.34
0.25000	1.40	30	83.33	82.14	5.9192	5.87
Control	-	30	6.67	-	-	-

Regression equation, $Y = 4.314 + 1.217X$

$LC_{50} = 0.0303$ ppm Fiducial limits = 0.0183 ppm and 0.0486 ppm

$LC_{90} = 0.4104$ ppm Fiducial limits = 0.1960 ppm and 0.6064 ppm

$\chi^2_{cal} (p=0.05) = 0.402$ $\chi^2_{tab} (p=0.05) = 7.814$

Table 7. Relative toxicity of different treatments to neonate larvae of *L. orbonalis*

Treatment	LC_{50} (ppm)	Relative toxicity	LC_{90} (ppm)	Relative toxicity
<i>Agniastra</i>	29795.975	1	177629.326	1.17
Azadirachtin	8.847	3.40×10^3	46.891	4.45×10^3
<i>B.t.</i>	3.989	7.50×10^3	27.783	7.50×10^3
<i>Brahmastra</i>	24203.194	1.23	208439.897	1
Spinosad	0.0303	9.83×10^5	0.4104	5.08×10^5
Emamectin benzoate	0.0043	6.92×10^6	0.0228	9.14×10^6

(2020) who has reported spinosad (0.034 ppm) most toxic to neonate larvae of *P. brassicae* followed by diafenthiuron (0.059 ppm), and *Agniastra* (3508.232 ppm) and *Brahmastra* (3720.866 ppm) were found least toxic. The higher LC₅₀ values of *Agniastra* and *Brahmastra* indicated that least effect on insect mortality but have their effect on biology of the pest. Hewady *et al.* (2002) found that natural product and biopesticides show deformation and reduction in longevity of *Pectinophora gossypiella* moths.

Conclusion

Among the biopesticides, Bt proved to be superior

in terms of larval mortality and *Brahmastra* proved better than *Agniastra*, but these were toxic at very high concentrations. While comparing biopesticides to natural products, the toxicity of biopesticides was higher to neonate larvae of *L. orbonalis*. Intrinsic toxicity studies revealed that emamectin benzoate was found most toxic to neonate larvae of *L. orbonalis* followed by spinosad.

Conflict of interest: The authors declare no competing interests.

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