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RELATIVE AND PERSISTENCE TOXICITY OF DIFFERENT INSECTICIDES AGAINST BRINJAL SHOOT AND FRUIT BORER (*LEUCINODESORBONALIS* GUENEE)

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ABSTRACT

An *in vitro* experiment was conducted at the Organic Farming Research Centre (OFRC), KSNUAHS, Shivamogga, to investigate the toxicity of six different insecticides, *viz.*, spinosad 45% SC, fenpropathrin 30% EC, cypermethrin 25% EC, chlorantraniliprole 18.5% SC, malathion 50% EC and flubendiamide 39.35% SC, against third-instar larvae of Brinjal shoot and fruit borer, *Leucinodesorbonalis* (Guenee), via the fruit dip bioassay method. The results indicated that chlorantraniliprole was 1.59, 3.72, 7.19, 10.16 and 11.98 times more toxic than Spinosad, flubendiamide, cypermethrin, malathion and fenpropathrin, respectively. Chlorantraniliprole 18.5% SC proved to be highly persistent among the tested insecticides where it effectively controlled *L. orbonalis* larvae, reducing their population for up to 11 days post-treatment, with a recorded PT_{50} value of 7 days. Next best persistent insecticides were spinosad and flubendiamide with PT_{50} values of 6 days and 4.5 days, respectively. Compared to conventional insecticides, diamide and spinosyn insecticides were more toxic to *L. orbonalis*. Hence, compared with other insecticides, chlorantraniliprole 18.5% SC with novel mode of action was highly toxic to larvae of *L. orbonalis*, with the lowest LC_{50} values and high persistence.

***Key words*: *Leucinodesorbonalis*, Insecticides, Relative toxicity, Persistent toxicity**

Introduction

Vegetables have been part of the human diet since time. They provide essential minerals, proteins and minerals as a part of a healthy human diet (Duguma, 2020). Vegetable cultivation is important from an economic point of view as it provides income to the farmers (Kumar *et al*., 2022). Brinjal, *Solanum melongena* L. is one of the prominent vegetable crop grown across the globe, especially in South East Asian countries. It is an important vegetable that is grown in all seasons. In India, brinjal occupies an area of 743 thousand hectares, with a production of 12,774 thousand metric tonnes (MTs) and an average productivity of 17.17 t/ha (Anonymous, 2022). The cultivation of brinjal is becoming a menace to farmers because of insect pest infestations that starts right from nursery to harvest in major brinjal growing areas. Shoot and fruit borer, *Leucinodesorbonalis* Guenee is a major threat among the insect-pests as it damages most important and economic parts of the crop such as shoots and fruits. Its infestation starts a few weeks after transplantation where the larva bores into the shoots causing the damaged shoots to droop, wither, and dry. Later the larvae enter into fruits and hampers its market value (Shanmugan *et al*., 2015). Thus, shoot and fruit boreris considered as limiting factor in brinjal cultivation and result in approximately 70 to 92% loss in yield (Rahman *et al*., 2019) and 93% loss in yield of brinjal (Kodandaram *et al*., 2017).

Insecticidal control is first line defence to combat *L. orbonalis*, however many insecticides do not effectively combat this pest. Insecticides are recognized as important inputs for increasing agricultural production, and their consumption has increased markedly. Large-scale farmers are completely dependent on chemical methods to protect crops (Raza *et al*., 2018). Farmers take up nearly 22 sprays of insecticides to manage this pest in South Indian states which is leading to residual toxicity on marketable fruits (Kariyanna *et al*., 2020). These insecticides are highly toxic compounds that must be used at most levels for the eco-safety and proper management of this pest. It is also necessary to manage these pests economically to obtain the desired amount of profit. Pest populations on a crop are highly dependent on insecticide persistence and residual toxicity following foliar sprays.

The results of this investigation will aid in choosing the appropriate insecticide for incorporation into the pest management module. Additionally, they will help in selecting an eco-friendly and safer insecticide formulation to effectively manage major pests affecting vegetable crops like brinjal.

Materials and Methods

Insect culture

Field visits were done in major vegetable growing areas of Shivamogga (14° 1' 41.103" N, 75° 35' 45.72" E) to collect infested fruits. Fruits bearing boreholes with fresh excreta were considered as *L. orbonalis* infested fruits. The larvae within the fruits were collected and further mass reared as per the methodology suggested by Munje *et al*., (2015) under proper laboratory conditions, *i.e.*, a temperature of 25±2°C and a relative humidity (RH) of 75±2%.

Insecticides used

Six commercial insecticides were purchased from local market belonging to different groups as per Insecticide Resistance Action Committee (IRAC). Spinosad 45% SC, malathion 50% EC, fenpropathrin 30% EC, chlorantraniliprole 18.5% SC, cypermethrin 25% EC and flubendiamide 39.35% SC were selected for bioassay studies (Table 1).

Relative toxicity studies

In order to determine the relative toxicity of different insecticides fruit-dip method of bioassay was adopted as per previous investigation by Kodandaram *et al*., (2015) with slight modifications. The third-instar larvae of the F_1 generation of *L. orbonalis* that were collected from field and reared in laboratory were used for the bioassay. The larvae were starved for 2 hours before being released into the treated fruit discs. Preliminary tests were conducted to establish the range of concentrations likely to cause mortality between 10 and 90% (bracketing) through serial dilution from stock solutions of insecticides and utilized in the bioassays. On the basis of those tests, at least five different concentrations in parts per million (ppm) were determined for each insecticide to estimate

Table 1: Insecticides used for bioassay and persistency studies on *L.orbonalis*.

S. No.	Chemical class	Common name	MoA[1]	Formulation	Trade name	IRAC MoA[1] class	Manufacturer	Dose (ml/l) for persistency studies
1	Pyrethroid	Fenpropathrin	Sodium channel modulator	30% EC[5]	Meothrin	IRAC 3A	Sumitomo chemical India Ltd.	0.34
2	Pyrethroid	Cypermethrin	Sodium channel modulator	25% EC[5]	Superkiller	IRAC 3A	Dhanuka Agritech Ltd.	0.40
3	Diamide	Chlorantraniliprole	RyR[2] modulator	18.5% SC[4]	Coragen	IRAC 28	Dupont Private India Ltd., Mumbai, Maharashtra	0.40
4	Spinosyn	Spinosad	NAChR[3] allosteric modulator	45% SC[4]	Delegate	IRAC 5	Corteva Agriscience	0.38
5	Organophosphate	Malathion	Acetylcholine esterase (AChE) inhibitors	50% EC[5]	Cythion	IRAC 1B	Coromandel International	2.00
6	Diamide	Flubendiamide	RyR[2] modulator	39.35% SC[4]	Fame	IRAC 28	Bayer crop science	0.25

[1]MoA: mode of action; [2]RyR: ryanodine receptor; [3]nAChR: nicotinic acetylcholine receptor; [4]SC: suspension concentrate; [5]EC: emulsifiable concentrate; IRAC: Insecticide Resistance Action Committee.

the concentration response curves. Healthy, uninfested and pesticide free brinjal fruits were thoroughly washed in distilled water, air dried and cut into small discs of 25-30 mm thickness. Fruit discs were dipped for 30 s with gentle agitation in each insecticidal concentrations and air dried on filter paper at room temperature. The treated fruit discs were transferred to each plastic breeding dish, and ten early third-instar larvae of *L. orbonalis* were released into each dish. The control fruits were dipped into distilled water. The mortality assessment was recorded at 24 and 48 hours and up to 72 hours after the larvae were transferred to the treated fruits. The probit analysis was recorded for mortality data taken at 72 hours after treatment. Larvae showing no sign of movement when pressed gently with hair brush were scored as morbid/dead.

Persistent toxicity studies

The plots were appropriately labelled for each insecticide and sprayed with insecticide (Table 1) at the recommended doses. Upon the appearance of young tender fruits on brinjal plants (45 days after transplanting), insecticides were applied for the first time. We also maintained a control plot with simple water sprays. Spray operations were performed via a battery-operated power sprayer. Care was taken to achieve uniform application to all the plants in plots. A thorough cleaning of the sprayer was performed at every interval with water and detergent in order to ensure that residual traces of previously used insecticide could not be detected. Fresh, tender brinjal fruits were collected from each insecticide-treated plot at 1, 3, 5, 7, 9 and 11 days post-application. These fruits, including their peduncles, were then transported to the laboratory. There, they were placed in plastic boxes (5.5 cm in height × 14.5 cm in diameter), with their peduncles wrapped in moistened cotton cloth. We released ten larvae of *L. orbonalis* that were maintained in the laboratory on three insecticide-treated fruits after washing them and allowing them to feed at ambient temperature and relative humidity. As a control, ten *Leucinodes* larvae were released on three brinjal fruits from the untreated plot. A 48-hour observation period was used to measure mortality of treated and untreated fruits. The larvae that did not react to a camel hair brush were deemed dead and removed from the rearing container.

Data analysis

The data on relative toxicity and persistency were analysed using IBM-SPSS software version 16. The results obtained for mortality at each concentration, from which the corrected percentage mortalities were determined (Abbott, 1925), were used for probit analysis (Finney, 1971) to determine the LC_{50} values. These LC_{50} values were then used to determine the relative toxicities of different insecticides using the following formula:

$$\text{Relative Toxicity (RT)} = \frac{LC_{50}\text{ of least toxic insecticide}}{LC_{50}\text{ of most toxic insecticide}}$$

Corrected mortalities were then utilized to compute persistent toxicity values. The corrected percentage mortalities at various time intervals were summed and then divided by the number of observations to yield the average percent mortality (T). The product of this average percent mortality (T) and the duration (P) of toxicity in days was used to calculate persistent toxicity index (PTi). The persistent toxicity of each insecticide was assessed based on the criteria established by Pradhan and Venkatraman (1962), with PT (persistent toxicity) serving as the index, *i.e.*,

$$PT_i = P \times T$$

Where, PT_i= persistent toxicity index; P = time in days up to which some mortality was observed; T = mean corrected percent mortality of the period P

Results and Discussion

The results of the current bioassay experiment involving the fruit dip method are presented in Table 2. Based on the results, chlorantraniliprole 18.50% SC exhibited a lower LC_{50} (5.78 ppm) than other insecticides tested, indicating that it is toxic to larvae of the third instar of *L. orbonalis.* In all six insecticide bioassays, slopes of the probit test ranged from 1.35-1.84, suggesting homogeneity among larval populations. These results, which were obtained from investigations of six different

Table 2: Relative toxicity (RT) of different insecticides to *L. orbonalis* by the fruit dip method at 72 HAT

S. No.	Insecticides	LC_{50} (ppm)*	Fiducial limits (Lower-Upper)	Slope	X^2 hetero-geneity (DF)	Equation	Relative toxicity (RT)	Order of Relative Toxicity(ORT)
1	Fenpropathrin	69.24	49.13-101.3	1.35 ± 0.21	1.24 (4)	Y= 1.35x - 2.49	11.98	6
2	Cypermethrin	41.59	29.71-57.87	1.45 ± 0.21	1.86 (4)	Y= 1.45x - 2.36	7.19	4
3	Chlorantraniliprole	5.78	3.35-7.57	1.84 ± 0.24	1.14 (4)	Y= 1.84x - 1.40	1.00	1
4	Spinosad	9.20	7.44-11.31	1.65 ± 0.16	0.98 (4)	Y= 1.65x - 1.59	1.59	2
5	Malathion	58.78	44.10-77.72	1.52 ± 0.22	1.42 (4)	Y= 1.52x - 2.73	10.16	5
6	Flubendiamide	21.51	17.59-26.78	1.70 ± 0.16	0.67 (4)	Y= 1.70x - 2.267	3.72	3

*ppm – parts per million; HAT - Hours after treatment

Table 3: Persistence of different insecticides applied as sprays to brinjal fruits against *L. orbonalis.*

S. No.	Insecticides	Dose (ml/L)	Corrected Mortality (%) DAS							P	T	PTi	ORP	PT_{50} (Days)*	RP
			1	3	5	7	9	11	13						
1	Fenpropathrin	0.34	66.67	33.33	30	11.11	0	0	0	7	35.28	246.96	6	1.82	1.00
2	Cypermethrin	0.40	77.78	55.56	50	22.22	0	0	0	7	51.39	359.73	5	3.00	1.46
3	Chlorantraniliprole	0.40	100	88.89	80	55.56	30	22.22	0	11	62.77	690.56	1	6.85	2.80
4	Spinosad	0.38	100	88.89	70	44.44	20	11.11	0	11	55.74	613.14	2	6.05	2.48
5	Malathion	2.00	77.78	66.67	40	11.11	10	0	0	9	41.11	369.99	4	2.94	1.50
6	Flubendiamide	0.25	88.89	77.78	50	44.44	20	11.11	0	11	48.70	535.70	3	4.53	2.17
7	Control	-	10	10	0	10	0	10	0	-	-	-	-	-	-

* PT_{50} values were determined via probit analysis via SPSS software version 16; DAS - Days after spraying; PT_{50} - Persistent toxicity; P - Period of observation in Days; T - Average percent mortality; PTi - Persistent toxicity index; ORP - Order of relative persistence; RP - Relative persistence.

insecticides, can be arranged on the basis of their relative toxicity in decreasing order of toxicity: chlorantraniliprole> spinosad> flubendiamide> cypermethrin > malathion >fenpropathrin. Hence, *L. orbonalis* was highly susceptible to chlorantraniliprole in third-instar larvae. Similar results were reported as supporting evidence with related literature.

Earlier investigation by Kodandaram *et al.*, (2013) indicated lower LC_{50} values for cyantraniliprole and chlorantraniliprole over flubendamide meaning those two insecticides were highly effective against *L. orbonalis.* Similarly, Kaur *et al.*, (2014) and Botre *et al.*, (2014) reported low LC_{50} values for chlorantraniliprole against different populations of *L. orbonalis* collected from Punjab and Vidharba region, respectively. These variations in lethal dose might be due to local variations, host plants and insecticide usage patterns (Karuppaiah and Srivastava, 2013). As a result of their novel mode of action, diamide insecticides such as chlorantranilprole cause paralysis of muscles and finally death in *L. orbonalis*, since they activate ryanodine receptors (RyRs) at a faster rate and release Ca^{+2} ions more frequently (Sattelle *et al.*, 2008). In addition, Spinosad's higher toxicity and efficacy may be due to its special mode of action on the nervous system of insects. It operates on nicotinic acetylcholine receptors and has additional effects on GABA and H-glutamate receptor sites, which results in continual motor neuron stimulation and causes the insect to stop feeding, tremble most of its muscles and eventually become paralyzed and die (Semiz *et al.*, 2006).

The results of investigation on persistence toxicity studies of six different insecticides sprayed at their respective field doses and mortality in third-instar larvae of *L. orbonalis* on Brinjal are presented in Table 3. Results showed that all tested insecticides resulted in the mortality of *L. orbonalis* larvae. The median persistence toxicity (PT_{50}) values of chlorantraniliprole 18.5% SC, spinosad 45% SC, flubendiamide 39.35% SC, malathion 50% EC, cypermethrin 25% EC and fenpropathrin 30% EC were 6.82 days, 6.05 days, 4.53 days, 2.94 days, 3.00 days and 1.82 days, respectively. The relative persistence of fenpropathrin was 2.80, 2.48, 2.17, 1.50 and 1.46 times lower than that of other insecticides, *viz.*, chlorantraniliprole, spinosad, flubendiamide, malathion and cypermethrin, respectively.

The duration of protection needed for a high-quality harvest and the interval between harvesting brinjal fruits for the market were taken into consideration when future research on the brinjal crop was designed. The results of the persistence toxicity studies suggested that the persistence of the insecticide lasted up to 11 days of monitoring. The highest PT_{50} values of chlorantraniliprole 18.5 SC showed that this specific insecticide has the capacity to kill 50% of the larval population up to 6-7 days after spraying, which is promising for protection of brinjal fruits. The superiority of the diamide insecticide schlorantraniliprole and flubendamide, which have novel modes of action, is supported by the findings of Sharma *et al.*, (2018), who reported that chlorantraniliprole 0.007% demonstrated the greatest persistent toxicity (PT) compared to other insecticides. Similarly, Visnupriya and Muthukrishnan (2017) revealed that the persistence of spinetoram 12% SC against larvae of *Helicoverpaarmigera* remained up to 10-15 days, with a high persistent toxicity index. Treatment with spinetoram recorded 100% mortality in larvae of *Spodoptera littoralis* within 24 hours and mortality was when treated mortality declined to 58.1% after seven days of treatment, indicating that spinetoram has a limited residual period (Elbarky *et al.*, 2008). This indicated that spinosad and spinetoram which belong to same insecticide group have relatively high persistency.

Persistent toxicity resulting from foliar spraying is a good indicator of how long an insecticide remains biologically active. Chlorantraniliprole 18.5 SC showed that this specific insecticide has the capacity to kill 50% of the larval population up to 6-7 days after spraying, and may remain up to 11 days. Hence, chlorantraniliprole must be applied for an interval of 14 days on the basis of the economic threshold level (ETL). This finding was supported by the results of Visnupriya and Muthukrishnan (2017).

Conclusion

The study concludes that chlorantraniliprole is highly toxic to 3rd-instar larvae of *Leucinodesorbonalis* and has a longer persistence compared to conventional insecticides. Given its effectiveness and persistence, chlorantraniliprole should be applied every 14 days based on the economic threshold level.

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Conflict of interest

The authors declare that there is no conflict of interest

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