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Laboratory Bioassays to Study the Efficacy of Diamides against *Leucinodes orbonalis* (Guenee) of Brinjal

G. Prashanth [a*], Sunitha N D [a], V. Chavan S.S [a] and Shiva Kumar V [b]

[a] Department of Agricultural Entomology, College of Agriculture, Vijayapur, India.
[b] Department of Agricultural Entomology, College of Agriculture, VNMKV, Parbhani, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study was undertaken with the objectives to investigate the toxicity of insecticides against larval stage of L. orbonalis in the laboratory. Management stratagies of the pest requires frequent spraying of chemical insecticides. Due to continuous spraying of insecticides L. orbonalis had developed a resistance to different insecticides that are urgently needed to test new molecules. In this bioassay, seven insecticides such as Cyantraniliprole 10OD, chlorantraniliprole 18.5SC, flubendiamide 480SC thiodicarb 75WP, methomyl 40SP, cartap hydrochloride and profenophos 50EC belonging to different insecticides groups were tested against L. orbonalis in the laboratory. In this bioassay larval mortality showed increasing trend with maximum percent mortality observed at 72HAT. All the treatments were found significantly superior over UTC. Among the seven

**Corresponding author: E-mail: gprashanthgoud50@gmail.com;*

insecticides tested against L. orbonalis, three diamides were found to be significantly superior. Cyantraniliprole 10OD recorded significantly highest percent larval mortality (97.26%), followed by chlorantraniliprole 18.5SC (94.14%) flubendiamide 480SC (88.45%), thiodicarb 75WP (77.53%), methomyl 40SP (71.50%), cartap hydrochloride 50SP (68.04%) and profenophos 50EC (65.88%). It is suggested that diamides may be used for the control of L. orbonalis.

Keywords: Leucinodes orbonalis; brinjal; chemical insecticides; chlorantraniliprole.

1. INTRODUCTION

Brinjal, also known as egg fruit (*Solanum melongena* L.) belongs to the Solanaceae family and is a popular non-tuberous vegetable globally. It originated in India. The Solanum genus includes flowering plants such as brinjal, which are economically significant food crops [1]. The monophagous pest, brinjal shoot and fruit borer (BSFB), *Leucinodes orbonalis* (Guenee) is the most damaging to this crop, causing yield losses of up to 60-70% and significant production losses [2].Concerns about toxic residues in food, effects on non-target organisms, and pesticide resistance have prompted the development of safer compounds [3]. Insecticides with different active compounds and modes of action are likely to be more effective than those have been so far in controlling *L. orbonalis* control [3; 4]. Flubendiamide a pthalic diamide having novel mode of action with strong insecticidal activity against lepidopterans including their resistant strains [5]. Morever, reports on field efficacy of cartap hydrochloride, Thiodicarb and methomyl against this pest are scanty, and their comparative evaluation with flubendiamide has not been carried out so far. Therefore, this study was undertaken with the objectives to investigate the toxicity of insecticides against larval stage of *L. orbonalis* in the laboratory.

2. MATERIALS AND METHODS

Collection: Brinjal crop was grown at AgricultureCollege, Vijaypura under natural conditions, where organic farming was practiced. Brinjal fruits infested with shoot and fruit borer were collected for conducting laboratory bioassay. Fruits were cut open and fourth instar larvae which are of uniform size were selected.

Procedure: For the percent mortality studies, insecticides chlorantraniliprole@ 0.3ml/lit, cyantraniliprole@ 0.3ml/lit, flubendiamide@ 0.3ml/lit, profenophos 50 EC@2ml/lit, methomyl 40 SP@ 0.6g/lit, thiodicarb 75 WP@ 0.6g/lit, cartap hydrochloride 50 SP@1g/lit and control were examined (Table 1). All insecticide solutions, including the control, were made using distilled water. The toxicity and laboratory efficacy of insecticides were assessed using the fruit dip bioassay method. Brinjal fruits that were healthy and unblemished were properly watered, air dried and sliced into small slices of 25 mm thickness. Fruit slices were immersed in each insecticide solution for 30 seconds before being air dried on filter paper at room temperature. Treated fruit slices were placed in each of the 95 mm × 35 mm plastic insect breeding dishes and 10 third instar larvae were released into each petri dish (Plate 1). All of the plastic dishes holding treated fruits and insects were kept at 25-30 degrees Celsius, 60-70 percent relative humidity and a 16:8 light: dark cycle. Mortality was measured at 24, 48 and72hours following treatment. Insects that showed no signs of movement were considered dead. Under untreated control fruit slices were cut open and treated with distilled water.Abott's formula (1925) was used to adjust mortality in all of the treatments [3].

Preparation of insecticide solution: 1000 ml beaker was filled with distilled water and accurate concentration of active ingredient of respective insecticide with the help of micropipette was released into the water to make the desired solution and solution was thoroughly mixed with the help of glass rod to make the insecticide solution uniform.

3. RESULTS AND DISCUSSION

3.1 *Leucinodes orbonalis* (Guenee)

Laboratory bioassay was conducted on 3rd larval instar of *L. orbonalis* by fruit dip method with 7 insecticides and observations on larval mortality were recorded at 24, 48 and 72 hr after treatment (Plate 1). Two trials of bioassays were conducted simultaneously to conclude the efficacy of diamides against shoot and fruit borer.

3.2 Percent Larval Mortality

Trial I

The data pertaining to larval mortality at different intervals is presented in Table 1.

Table 1. Test chemicals for laboratory bioassay against *Leucinodes orbonalis* (Guenee)

Treatment No.	Treatment details	Dose(ml/g/lit)
1	Chlorantraniliprole 18.5 SC	0.3
2	Cyantraniliprole 10 OD	0.3
3	Flubendiamide 480 SC	0.3
4	Profenophos 50 EC	2.0
5	Methomyl 40 SP	0.6
6	Thiodicarb 75 WP	0.6
7	Cartap hydrochloride 50 SP	1.0
8	UTC	-

24 HAT

Significant difference was found between the treatments with respect to percent larval mortality at 24 HAT. Cyantraniliprole 10OD was found to be significantly superior by recording 33.33 per cent mortality. Chlorantraniliprole 18.5SC was found next best and significantly superior to other treatments (30.00%) followed by flubendiamide 480SC (26.66%). Methomyl 40SP (23.33%) and thiodicarb 75WP (23.93%) were found at par and significantly superior to cartap hydrochloride 50SP (13.76%). Profenophos 50EC was found significantly inferior by recording lowest percent larval mortality of 10.09%.

48 HAT

At 48HAT, all the treatments differed significantly from each other and three diamides recorded their superiority over other treatments. The descending order of superiority of performance of different treatments was cyantraniliprole 10OD (83.33%), chlorantraniliprole 18.5SC (76.66%) flubendiamide 480SC (70.00%), thiodicarb 75WP (66.81%), methomyl 40SP (63.33%), cartap hydrochloride 50SP (56.34%) and profenophos 50EC (50.00%).

72 HAT

Similar trend was observed as that of mortality at 48HAT. Cyantraniliprole 10OD recorded significantly highest percent larval mortality (96.67%) followed by chlorantraniliprole 18.5SC (93.33%) flubendiamide 480SC (86.28%), thiodicarb 75WP (76.37%), methomyl 40SP (70.00%), cartap hydrochloride 50SP (66.66%) and profenophos 50EC (63.33%) (Table 2)

Larval mortality showed increasing trend with maximum percent mortality observed at 72 HAT. All the treatments were found significantly superior over UTC.

3.3 Percent Larval Mortality

Trial II

Similar trail were conducted to know the accuracy of the results.

24 HAT

Significant difference was found between the treatments with respect to percent larval mortality at 24 HAT. Cyantraniliprole 10OD was found to be significantly superior by recording 34.36 per cent mortality. Chlorantraniliprole 18.5SC was found next best and significantly superior to other treatments (32.00) followed by flubendiamide 480SC (28.66%). Methomyl 45SP (22.34%) and thiodicarb 75WP (23.95%) were found at par and significantly superior to cartap hydrochloride 50SP (14.76%). Profenophos 50EC was found significantly inferior by recording lowest percent mortality of 12.39 per cent.

48 HAT

At 48HAT, all the treatments differed significantly from each other and three diamides recorded their superiority over other treatments. The descending order of superiority of performance of different treatments was cyantraniliprole 10OD (85.32%), chlorantraniliprole 18.5SC (77.82%), flubendiamide 480SC (72.16%), thiodicarb 75WP (67.81%), methomyl 40SP (63.95%), cartap hydrochloride 50SP (57.28%) and profenophos 50EC (51.67)

72 HAT

Trend was similar to 48HAT. Cyantraniliprole 10OD recorded significantly highest percent larval mortality (97.80%) followed by chlorantraniliprole 18.5SC (94.95%), flubendiamide 480SC (90.23%), thiodicarb 75WP (78.39%), methomyl 40SP (73.00%), cartap hydrochloride 50SP (69.42%) and profenophos 50EC (64.82%)

Larval mortality showed increasing trend with maximum percent mortality observed at 72HAT. All the treatments were found significantly superior over UTC (Table 2).

3.4 Percent Larval Mortality (Mean of Two Trials)

24 HAT

Significant difference was found between the treatments with respect to percent larval mortality at 24 HAT. Cyantraniliprole 10OD was found to be significantly superior by recording 33.85 per cent mortality. Chlorantraniliprole 18.5SC was found next best and significantly superior to other treatments. (31.00%) followed by flubendiamide 480SC (27.66%). Methomyl 40SP (22.84%) and thiodicarb 75WP (23.64%) were found at par and significantly superior to cartap hydrochloride 50SP (14.05%). Profenophos 50EC was significantly inferior by recording lowest percent mortality of 11.20%. All the treatments were found significantly superior to UTC (Table 3).

48 HAT

At 48HAT, all the treatments differed significantly from each other and three diamides recorded their superiority over other treatments. The descending order of superiority of performance of different treatments was cyantraniliprole 10OD (84.33%), chlorantraniliprole 18.5SC (77.24%), flubendiamide 480SC (71.08%), thiodicarb 75WP (67.24%), methomyl 40SP (63.64%), cartap hydrochloride 50SP (56.97%) and profenophos 50EC (50.84).

72 HAT

Similar trend was observed at 72HAT. Cyantraniliprole 10OD recorded significantly highest percent larval mortality (97.26%)followed by chlorantraniliprole 18.5SC (94.14%), flubendiamide 480SC (88.45%), thiodicarb 75WP (77.53%), methomyl 40SP (71.50%), cartap hydrochloride 50SP (68.04%) and profenophos 50EC (65.88%)(Table 3).

Larval mortality showed increasing trend with maximum percent mortality observed at 72HAT. All the treatments were found significantly superior over UTC. Among the seven insecticides tested against *L. orbonalis*, three diamides were found to be significantly superior. Among three diamides cyantraniliprole 10OD was found significantly superior by recording maximum percent larval mortality. Other insecticides showed their moderate effect.

Diamides are insecticides that promote disfunctions in ryanodine receptors. In Brazil this mode of action is registered for controlling lepidopteran and coleopteran Pests [6]. This class of insecticide is the most recent, launched in 2006 by Bayer with the active ingredient flubendiamide followed by chlorantraniliprole (DuPont) and cyantraniliprole (Syngenta) [7]. The mode of action of such insecticides is based on the uncontrolled calcium release from the sarcoplasmic reticulum into muscle fibers due to the abnormal modulation of the ryanodine receptor. In the insect organism, such calcium release causes irreversible muscle contraction, leading to a rapid food uptake paralysis, lethargy, heart muscle failure and lately death [5].

High susceptibility of fourth instar larva to cyantraniliprole 10OD@0.3ml/l may be due to the novel mechanism by which this new chemical belonging to carboxiamide group in which chlorine atom in chlorantraniliprole has been been replaced by cyano group in cyantraniliprole which activates ryanodine receptors (RyRs) and results in muscular paralysis and impairs insect feeding, movement and reproduction significantly by causing unregulated release of Ca ions even death of a larva. It enters larvae primarily by ingestion, but also by contact. It shows ovo- larvicidal and adulticide efficacy depending upon the pest species. Its exposure results in rapid feeding cessation within a few hours of exposure due to direct contact of insecticide to larvae, however the time to death may take 3 to 6 days depending on the species and it is having quick rainfastness, high penetration and spreading ability compare to other diamides [8].

The lethal and sublethal effects of chlorantraniliprole against *Spodoptera exigua* (Hübner) were evaluated under laboratory conditions by oral exposure of neonate larvae to the compound which resulted in the reduced pupation rates in exposure groups. The sublethal effects of this chemical were indicated by prolongation of larval period, the increase of pupal weight and decrease in hatch rate of egg. Chlorantraniliprole at LC_{30} and LC_{50} rate significantly delayed larval development; the developmental duration of surviving larvae was extended for 22.50 and 28.60 per cent respectively [9].

Table 2. Bio efficacy of diamides against *Leucinodes orbonalis* (Guenee) under laboratory conditions

Treatment No.	Treatment details	Dose (ml/g/lit)	Percent mortality of larva (Trial - I)			Percent mortality of larva (Trial– II)		
			24 HAT (Mean ± SE)	48 HAT (Mean ± SE)	72 HAT (Mean ± SE)	24 HAT (Mean ± SE)	48 HAT (Mean ± SE)	72 HAT (Mean ± SE)
1	Chlorantraniliprole 18.5 SC	0.3	30.00 [b] ± 0.00	76.66[b] ± 0.58	93.33[b] ± 0.61	32.00 [b] ± 0.08	77.82[b] ± 0.67	94.95 [b] ± 0.72
2	Cyantraniliprole 10 OD	0.3	33.33[a] ± 0.58	83.33[a] ± 1.15	96.67[a] ± 0.36	34.36 [a] ± 0.61	85.32 [a] ± 1.20	97.80 [a] ± 0.34
3	Flubendiamide 480 SC	0.3	26.66[c] ± 0.56	70.00[c] ± 0.23	86.28[c] ± 0.54	28.66 [c]± 0.65	72.16 [c] ± 0.32	90.23 [c] ± 0.84
4	Profenophos 50 EC	2.0	10.09[f] ± 1.00	50.00[g] ± 0.32	63.33[g] ± 0.56	12.39 [f] ± 1.15	51.67 [g] ±0.14	64.82 [g] ± 0.57
5	Methomyl 40 SP	0.6	23.33[d] ± 0.58	63.33[e] ± 0.57	70.00[e] ± 1.00	22.34 [d] ± 0.77	63.95 [e] ±0.58	73.00 [e] ± 1.11
6	Thiodicarb 75 WP	0.6	23.93[d] ± 0.54	66.81[d] ± 0.58	76.37[d] ± 0.52	23.95 [d]± 0.58	67.81[d] ± 0.61	78.39 [d] ± 0.58
7	Cartap hydrochloride 50 SP	1.0	13.76[e] ± 1.15	56.34[f] ± 0.51	66.66[f] ± 0.57	14.76 [e]± 1.18	57.28 [f] ± 0.58	69.42[f] ± 0.66
8	UTC	-	0.00[g] ± 0.00	0.00[h] ± 0.00	0.00[h] ± 0.00	0.00 [g]± 0.00	0.00 [h] ± 0.00	0.00 [h]± 0.00
	SEm±		0.39	0.33	0.35	0.42	0.39	0.51
	CD (0.01%)		1.61	1.37	1.46	1.81	1.42	1.98

HAT- Hours after treatment
Means followed by the same letter are not significantly different by DMRT

Table 3. Bio efficacy of diamides against *Leucinodes orbonalis* (Guenee) under laboratory conditions (Mean of two trails)

Treatment No.	Treatment details	Dose(ml/g/lit)	Mean percent mortality of larva (Trial 1 and Trial 2)		
			24 HAT (Mean ± SE)	48HAT (Mean ± SE)	72HAT (Mean ± SE)
1	Chlorantraniliprole 18.5 SC	0.3	31.00 [b] ± 0.00	77.24[b] ± 0.58	94.14[b] ± 0.67
2	Cyantraniliprole 10 OD	0.3	33.85[a] ± 0.59	84.33[a] ± 1.14	97.26[a] ± 0.72
3	Flubendiamide 480 SC	0.3	27.66[c] ± 0.58	71.08[c] ± 0.46	88.45[c] ± 0.54
4	Profenophos 50 EC	2.0	11.20[f] ± 1.00	50.84[g] ± 0.38	65.88[g] ± 0.42
5	Methomyl 40 SP	0.6	22.84[d] ± 0.57	63.64[e] ± 0.57	71.50[e] ± 1.00
6	Thiodicarb 75 WP	0.6	23.64[d] ± 0.52	67.24[d] ± 0.58	77.53[d] ± 0.54
7	Cartap hydrochloride 50 SP	1.0	14.05[e] ± 1.13	56.97[f] ± 0.51	68.04[f] ± 0.39
8	UTC	-	0.00[g] ± 0.00	0.00[h] ± 0.00	0.00[h] ± 0.00
	SEm±		0.41	0.35	0.37
	CD (0.01)		1.64	1.39	1.49

HAT- Hours after treatment

Means followed by the same letter are not significantly different by DMRT

Plate 1. Experimental setup for laboratory bioassay studies against *Leucinodes orbonalis* (Guenee)

Flubendiamide activates ryanodine sensitive intracellular calcium release channels (ryanodine receptors, RyR) in insects. It has a novel biochemical action as it affects calcium ion balance irrespective of sodium or potassium ion balance, which causes contraction of insect skeletal muscle. Flubendiamide is stable to hydrolysis under laboratory conditions, but direct aqueous photolysis appears to be a main route of degradation. Flubendiamide degrades to des-iodo flubendiamide under laboratory. It has shown longer persistence and is also reported to form toxic metabolites and it has high dissipation rate [10].

The present findings on efficacy of diamides are in the line with the findings of Kodandaram et al. [3] who revealed that, differences in mortality across the three diamide insecticides (cyantraniliprole10 OD, chlorantraniliprole 18.5 SC and flubendiamide 480 SC) were statistically significant, the highest mortality rate was noted in cyantraniliprole 10 OD 90g a.i./ha at 24 and 48 hours after treatment with 91.66 and 99.00 per cent larval mortality and mortality was dosage dependant. The next best treatment is chlorantraniliprole 18.5 SC with 82.00 and 100 percent larval mortality followed by flubendiamide 480 SC. Latif et al. [11] observed that, after 24 and 48 hours of treatment flubendiamide demonstrated the maximum toxicity (100%) against fourth instar larvae of *L. orbonalis* under laboratory condition. After 48 h thiodicarb 45 SP had recorded 87.96 per cent which is slightly contrary with our findings.Rehman et al*.* [12] conducted fruit dip bioassay against 3rd instar larvae of brinjal shoot and fruit borer and revealed that, flubendiamide 480 SC was most effective insecticide among all the tested insecticide with 87% mortality after 72 h of application while imidacloprid was the least effective. Tonishi et al. [5] determined that, flubendiamide was found to be a highly effective against various lepidopterous insect pest larvae, including their resistant strains. Patel et al. [13] observed 100 percent mortality of Spodoptera litura to cyantraniliprole @ 105 and 90 g a.i/ha.

Yadav et al. [14] reported that in laboratory bioassay, cyantraniliprole 10 OD at doses of 0.5, 0.6 and 0.7 ml/l resulted in 100 per cent mortality against Spodoptera frugiperda after 72 hours. Effect of insecticides other than diamides is reported on L. orbonalis and also on other lepidopteran pests. Silva et al. (2018) reported greater than 80 percent mortality was observed with methomyl and cartap hydrochloride showing strong toxicity against the eggs and larvae of Neoleucinodes elegantalis (Guenée) on tomato farms.McPherson and jones [15] conducted leaf dip bioassay to know the efficacy of methomyl 45 SP against tobacco horn worm. Hornworms were moderately susceptible to methomyl and hornworm larval feeding was disrupted within 24hr of exposure to the higher concentrations of methomyl with cessation of feeding accompanied by larvae moving off the treated foliage.

4. CONCLUSION

Bioassay studies on *L. orbonalis* revealed significant difference between the treatments with respect to percent larval mortality. Cyantraniliprole 10OD recorded significantly highest percent larval mortality (97.26%) followed by chlorantraniliprole 18.5SC (94.14%), flubendiamide 480SC (88.45%), thiodicarb 75WP (77.53%), methomyl 40SP (71.50%), cartap hydrochloride 50SP (68.04%) and profenophos 50EC (65.88%).Larval mortality showed increasing trend with maximum percent mortality observed at 72HAT. All the treatments were found significantly superior over UTC. Among the seven insecticides tested against *L. orbonalis*, three diamides were found to be significantly superior. Among three diamides cyantraniliprole 10OD was found significantly superior by recording maximum percent larval mortality.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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