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Seasonal toxicity pattern of novel insecticides and botanicals combination against *Leucinodes orbonalis* in Bhubaneswar

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Abstract

This study examines the seasonal toxicity patterns of novel insecticides and botanical combinations against *Leucinodes orbonalis* in Bhubaneswar. Managing this pest is challenging due to its resistance to conventional insecticides, leading to significant brinjal yield losses. This research evaluates the efficacy of spinetoram, tetraniliprole and emamectin benzoate, individually and in combination with plant extracts and piperonyl butoxide (PBO). Spinetoram exhibited the highest toxicity, followed by tetraniliprole and emamectin benzoate. The study also revealed strong synergistic effects, particularly with PBO, enhancing insecticide efficacy. These findings highlight the importance of integrating botanical extracts with insecticides for effective pest management.

Keywords

brinjal shoot and fruit borer; insecticide resistance; integrated pest management; spinetoram; synergistic effects; tetraniliprole

Introduction

Leucinodes orbonalis, commonly known as the brinjal shoot and fruit borer (SFB), is a highly destructive pest that affects the production of brinjal (*Solanum melongena*), a vital crop in many parts of the world. The pest's feeding habits cause substantial yield losses, ranging from 20% to 90% (1), depending on the severity of the infestation. The pest's high reproductive rate, overlapping generations and ability to develop resistance to chemical insecticides make it difficult to control. Additionally, its widespread presence in major brinjal-growing regions, including South and Southeast Asia, further underscores its global significance. In addition to brinjal, *L. orbonalis* also infests other economically important crops from the Solanaceae family, such as tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*) and even lesser-known species like nightshade (*Solanum nigrum*) and turkey berry (*Solanum torvum*). This wide host range, combined with the pest's year-round activity and overlapping generations in temperate climates, poses significant challenges for pest control and management.

The key problem in managing *L. orbonalis* is its rapid development of resistance to conventional insecticides (2). Over the years, extensive use of chemical insecticides has led to physiological and behavioural adaptations in the pest, making it increasingly resilient. These adaptations are manifested through mechanisms such as enhanced detoxification enzyme activity, modified target sites and reduced cuticular penetration (3). Resistance development occurs not only due to the insect's natural ability to metabolize insecticides but also due to the frequent and indiscriminate use of chemical compounds in pest control programs. Insects, including *L. orbonalis*, have evolved various detoxification mechanisms in response to both synthetic insecticides and natural plant toxins (4, 5), complicating pest management further.

Climate change exacerbates the issue of insecticide resistance. Rising global temperatures influence both pest biology and the efficacy of insecticides, altering pest behaviour and metabolism. Warmer conditions accelerate pest metabolism, increasing the rate at which they process and resist chemical compounds (6). This phenomenon calls for revaluating current pest management strategies, as resistance can develop faster under such conditions. Additionally, the broader impacts of climate change on pest dynamics make it even more challenging to control *L. orbonalis* effectively (7).

To address these challenges, it is essential to explore innovative and sustainable pest management strategies that combine both chemical and botanical solutions. This study aims to evaluate the efficacy of novel insecticides, such as spinetoram, tetraniliprole and emamectin benzoate, alongside botanical extracts, in managing *L. orbonalis* in Bhubaneswar. The potential synergistic effects of plant extracts, including those from *Terminalia arjuna*, *Alstonia scholaris*, *Eupatorium odoratum*, *Nyctanthes arbor-tristis*, *Spathodea campanulata* and *Vitex negundo*, in combination with the chemical synergist piperonyl butoxide (PBO), are also explored.

By studying the toxicity patterns of insecticide and botanical combinations, this research seeks to provide insights into integrated pest management (IPM) strategies that can offer more sustainable solutions. Investigating synergistic interactions between botanicals and insecticides is particularly important, as these combinations may enhance pest control efficacy, delay resistance development and reduce the reliance on chemical pesticides, making them an essential component of modern resistance management in brinjal cultivation.

Materials and Methods

Materials

The study was conducted in the Toxicology Laboratory, Department of Entomology, OUAT, Bhubaneswar. Infested brinjal fruits were collected from fields around Bhubaneswar to obtain the larvae of *Leucinodes orbonalis*. Selection of larvae was based on specific criteria to ensure reproducibility. Only actively feeding larvae found inside damaged brinjal fruits were considered. Larvae were identified based on size and developmental stage, selecting only 3rd instar larvae as they represent the most suitable stage for bioassays. Any larvae showing signs of disease, malformation, or parasitisation was excluded. The collected larvae were carefully extracted by cutting the fruits open and were placed in plastic jars covered with muslin cloth to maintain a controlled environment until testing. Three insecticides, *viz.,* emamectin benzoate, spinetoram and tetraniliprole, were selected for testing. Stock solutions of the insecticides were prepared by serially diluting commercial-grade formulations in acetone to deliver 1 µg/larva, with additional dilutions prepared at concentrations of $1x10^{-3}$, $5x10^{-4}$, $1x10^{-4}$, $5x10^{-5}$, $1x10^{-5}$ and $1x10^{-6}$ µg/larva.

Fresh leaves of *Alstonia scholaris*, *Spathodea campanulata*, *Eupatorium odoratum*, *Vitex negundo*, *Nyctanthes arbor-tristis* and bark of Terminalia arjuna were collected from the OUAT farm. These plant materials were dried at 100 °C for 7 days and extracted via hydro-distillation in methanol using a Clevenger apparatus at 50 °C (8, 9). The concentrated extracts were stored in glass vials for further use.

Methodology

Bioassays were conducted by topically applying the insecticides to the dorsal thoracic segments of the larvae using a Hamilton micro dispenser (1 µl/larva), with mortality recorded 24 hrs after application (10-12). Control groups were treated with acetone alone and mortality data were corrected using Abbott's formula (13). Acetone was used as a control in insecticide bioassays as it served as the solvent for preparing insecticide stock solutions. This ensured that any mortality observed in treated larvae was due to the insecticides rather than the solvent. Similarly, methanol was used as a control in synergism studies involving plant extracts, as it was the solvent used for botanical extractions (14). Including these controls allowed for an accurate assessment of insecticide and botanical efficacy by eliminating any confounding effects that the solvents might have on larval mortality. Synergism studies involved applying non-toxic doses of plant extracts or PBO (Piperonyl Butoxide) 15 min before applying insecticides (15), with doses tested ranging from 2 µg/larva to 0.5 µg/larva. Mortality was recorded 24 hours after treatment for each concentration. LD_{50} values were calculated using probit analysis in SPSS (v. 27.0) and synergism ratios (SR) were determined using the following formula:

$$\text{Synergistic ratio (SR)} = \frac{\text{LD50 of insecticide alone}}{\text{LD50 (insecticide + synergist)}}$$

This formula quantifies the enhancement of insecticidal efficacy due to the presence of the synergist, allowing for a comparative analysis of the effectiveness of different combinations.

Statistical analysis

All statistical analyses were conducted using SPSS (v. 27.0). Probit analysis was employed to calculate LD_{50} values and their respective confidence intervals. Synergism ratios (SR) were determined to assess the impact of plant extracts and PBO on insecticide efficacy.

The experiments were replicated across multiple batches of larvae collected from different field locations to validate the consistency and reliability of the results. Each treatment was conducted with at least three biological replicates and mortality data were subjected to ANOVA followed by Tukey's HSD test to determine significant differences among treatments ($p < 0.05$). This approach ensured the reproducibility of findings and minimized variations due to external factors.

Results

Acute toxicity experiments were conducted to estimate the LD_{50} values of several insecticides on 3rd instar larvae of *Leucinodes orbonalis* collected from Bhubaneswar during the *Rabi* 2022-23 (Table 1) and *Kharif* 2023 (Table 2) seasons. Using the topical application method, different concentrations were applied and mortality was recorded at 24 hours. Similar experiments were performed for tetraniliprole and emamectin benzoate.

The results from *Rabi* 2022-23 showed that spinetoram had the lowest LD_{50} value of 0.399 ng/larva, indicating the highest toxicity, followed by tetraniliprole with an LD_{50} value of 0.506 ng/larva. Emamectin benzoate exhibited a significantly higher LD_{50} value of 3.006 ng/larva, demonstrating lower toxicity than spinetoram and tetraniliprole.

In the *Kharif* 2023 season, spinetoram again demonstrated the highest toxicity with an LD_{50} of 0.141 ng/ larva, followed by tetraniliprole at 0.479 ng/larva and emamectin benzoate at 1.857 ng/larva. Across both seasons, spinetoram consistently showed the highest toxicity, followed by tetraniliprole, with emamectin benzoate being the least toxic.

Data in Table 3 revealed the non-toxic dosages of plant extracts and PBO. Toxicity estimation was performed using varying concentrations of plant extracts and PBO. Leaf extracts of *Alstonia scholaris*, *Spathodea campanulata*, *Eupatorium odoratum*, *Vitex negundo*, *Nyctanthes arbor-tristis* and bark extracts of *Terminalia arjuna*, along with PBO (a known inhibitor of mixed function oxidases), were used with methanol as a solvent.

Larvae collected from Bhubaneswar during *Rabi* 2022-23 were used for the synergistic study, with 30 larvae tested per concentration. The plant extracts and PBO were topically applied using a Hamilton micro applicator (1 µl/ larva) and mortality was recorded after 24 hours. PBO was tested at concentrations ranging from 100 to 10 ppm, corresponding to dosages of 0.1 to 0.01 µg/larva, with the lowest mortality recorded at 10 ppm (0.01 µg/larva). The plant extracts were tested at concentrations from 2000 to 500 ppm, equivalent to dosages of 2 to 0.5 µg/larva, with the lowest mortality observed at 500 ppm (0.5 µg/larva).

Similarly, larvae collected in *Kharif* 2023 were tested under the same conditions, with the lowest mortality for PBO recorded at a dosage of 0.01 µg/larva and for plant extracts at 0.5 µg/larva.

Table 4 presents the synergistic effects of various plant extracts and PBO with spinetoram, showing that the combination of PBO with spinetoram exhibited the highest synergistic ratio (SR) of 9.98, indicating a strong synergistic effect. *Nyctanthes arbor-tristis* followed with an SR of 8.67 and *Eupatorium odoratum* had an SR of 7.34. Other extracts, such as *Terminalia arjuna* and *Alstonia scholaris*, demonstrated SR values of 7.25 and 6.16, respectively, while *Spathodea campanulata* recorded an SR of 5.12. In contrast, *Vitex negundo* showed an antagonistic interaction with an SR of 0.82.

In the synergistic tests with tetraniliprole (Table 4), the combination with PBO recorded an SR of 3.16, indicating a synergistic effect. *Nyctanthes arbor-tristis* and *Eupatorium odoratum* also exhibited prominent synergistic effects with SR values of 3.72 and 3.64, respectively. Other extracts, such as *Spathodea campanulata*, *Terminalia arjuna* and *Alstonia scholaris*, showed synergistic interactions with SR values ranging from 1.73 to 3.03.

For emamectin benzoate (Table 4), PBO exhibited the highest SR of 14.31, indicating a strong synergistic effect. *Eupatorium odoratum* followed with an SR of 5.24, while *Spathodea campanulata*, *Nyctanthes arbor-tristis* and *Terminalia arjuna* demonstrated SR values between 2.61 and 3.32. *Vitex negundo* displayed a mild synergistic effect with an SR of 1.49.

Table 1. Toxicity of a few selected insecticides towards *L. orbonalis*, infesting brinjal collected from Bhubaneswar during *Rabi*, 2022-23

Sl.no	Name of the Insecticide	No of test insects	Slope ±SE	Regression Equation	LD_{50} (ng/larva)	Chi-square
1.	Spinetoram	30	0.458±0.17	Y=1.74+0.458x	0.141 (0.022-7.00)	0.812
2.	Tetraniliprole	30	0.525±0.19	Y=1.67+0.51x	0.479 (0.105-83.70)	0.682
3.	Emamectin benzoate	30	0.405±0.11	Y=1.12+0.4x	1.857 (0.244-0.272)	0.272

Data are represented as *Mean ± Standard Error (S.E)* and each experiment was replicated three times (*n = 3*) to ensure statistical reliability.

This table presents the toxicity levels of different insecticides against *L. orbonalis* larvae, evaluated using bioassay techniques. The toxicity is measured in terms of LD_{50} (ng/larva), with corresponding regression equations and Chi-square values.

Table 2. Toxicity of a few selected insecticides towards *L. orbonalis*, infesting brinjal collected from Bhubaneswar during *Kharif*, 2023

Sl.no	Name of the Insecticide	No of test insects	Slope ±SE	Regression Equation	LD_{50} (ng/larva)	Chi-square
1.	Spinetoram	30	0.511±0.18	Y=1.72+0.51x	0.399 (0.086-63.00)	0.083
2.	Tetraniliprole	30	0.591±0.20	Y=1.82+0.56x	0.506 (0.124-27.00)	1.123
3.	Emamectin benzoate	30	0.443±0.11	Y=1.10+0.43x	3.006 (0.462-58.51)	1.158

The data are represented as the *Mean ± Standard Error (S.E)* and each experiment was replicated three times (*n = 3*) to ensure statistical reliability.

This table presents the toxicity levels of different insecticides against *L. orbonalis* larvae collected during the *Kharif* season. The toxicity is measured in terms of LD_{50} (ng/larva), with corresponding regression equations and Chi-square values.

Table 3. Fixation of non-toxic doses of botanicals bringing mortality to 3rd instar larvae of *L. orbonalis* collected from Bhubaneswar during both the seasons of study (2022-23)

Sl.no	Name of the botanical/ Synergist	Dose ppm (mg/ml)	*Rabi*			*Kharif*		
			No. dead	No. Dosed	Corrected mortality (%)	No. dead	No. Dosed	Corrected mortality (%)
1	PBO	100 (0.1mg/ml)	13	30	41	12	30	37.9
		50 (0.05mg/ml)	9	30	27	10	30	31.05
		40 (0.04mg/ml)	7	30	20.3	8	30	24.09
		30 (0.03mg/ml)	5	30	13.03	4	30	10.3
		20 (0.02mg/ml)	2	30	2.79	2	30	2.79
		10 (0.01mg/ml)	1	30	0	1	30	0
	Control (Methanol)		1	30		0	30	
2	*Terminalia arjuna*	2000 (2mg/ml)	3	30	6.93	4	30	10.38
		1500 (1.5mg/ml)	3	30	6.93	4	30	10.38
		1000 (1mg/ml)	2	30	3.48	3	30	6.93
		750 (0.75mg/ml)	2	30	3.48	3	30	6.93
		500 (0.5mg/ml)	2	30	3.48	3	30	6.93
	Control (Methanol)		1	30		1	30	
3	*Alstonia scholaris*	2000 (2mg/ml)	2	30	3.48	3	30	6.93
		1500 (1.5mg/ml)	2	30	3.48	3	30	6.93
		1000 (1mg/ml)	2	30	3.48	3	30	6.93
		750 (0.75mg/ml)	2	30	3.48	3	30	6.93
		500 (0.5mg/ml)	1	30	0.03	2	30	3.48
	Control (Methanol)		1	30		1	30	
4	*Eupatorium odoratum*	2000 (2mg/ml)	3	30	6.9	3	30	6.9
		1500 (1.5mg/ml)	3	30	6.9	3	30	6.9
		1000 (1mg/ml)	3	30	6.9	3	30	6.9
		750 (0.75mg/ml)	2	30	3.45	2	30	3.45
		500 (0.5mg/ml)	2	30	3.45	2	30	3.45
	Control (Methanol)		1	30		1	30	
5	*Nyctanthes arbor-tristis*	2000 (2mg/ml)	2	30	6.67	3	30	10
		1500 (1.5mg/ml)	2	30	6.67	3	30	10
		1000 (1mg/ml)	2	30	6.67	3	30	10
		750 (0.75mg/ml)	1	30	3.33	2	30	6.67
		500 (0.5mg/ml)	1	30	3.33	2	30	6.67
	Control (Methanol)		0	30	0	0	30	
6	*Spathodea campanulata*	2000 (2mg/ml)	3	30	10	4	30	10.35
		1500 (1.5mg/ml)	3	30	10	4	30	10.35
		1000 (1mg/ml)	3	30	10	3	30	6.9
		750 (0.75mg/ml)	2	30	6.67	3	30	6.9
		500 (0.5mg/ml)	2	30	6.67	2	30	3.45
	Control (Methanol)		0	30		1	30	
7	*Vitex negundo*	2000 (2mg/ml)	3	30	6.9	4	30	13.33
		1500 (1.5mg/ml)	2	30	3.45	2	30	6.67
		1000 (1mg/ml)	2	30	3.45	2	30	6.67
		750 (0.75mg/ml)	2	30	3.45	2	30	6.67
		500 (0.5mg/ml)	2	30	3.45	2	30	6.67
	Control (Methanol)		1	30		0	30	

Data represent the mean number of larvae affected out of 30 per treatment and corrected mortality (%) was calculated. Each experiment was replicated thrice ($n = 3$) for statistical reliability.

Table 4. Interactive effect of tested plant extracts/PBO to selected insecticides during Rabi, 2022-23

Insecticide	Plant extract/PBO	Conc. in ppm (dosage mg/ ml)	LD_{50} (ng/ larva)	Chi-square	Slope ± SE	Regression equation	Fiducial limits		SR value	S/A
							Lower	Upper		
Spinetoram	Nil		0.399	0.083	0.448± 0.11	y=1.10+0.43x	0.086	63.00		
	Terminalia arjuna	500 (0.5 mg/ml)	0.055	0.393	0.332±0.110	y=1.42+0.33x	0.006	0.492	7.25	S
	Alstonia scholaris	500 (0.5 mg/ml)	0.065	0.184	0.304±0.117	y= 1.27+0.30x	0.005	1.000	6.14	S
	Eupatorium odoratum	500 (0.5 mg/ml)	0.054	0.357	0.243±0.011	y=1.04+0.24x	0.040	16.312	7.38	S
	Nyctanthes arbor-tristis	500 (0.5 mg/ml)	0.046	0.646	0.292±0.116	y=1.27+0.29x	0.002	1.000	8.67	S
	Spathodea campanulata	500 (0.5 mg/ml)	0.078	0.376	0.283±0.117	y=1.16+0.28x	0.006	3.620	5.12	S
	Vitex negundo	500 (0.5 mg/ml)	0.485	0.312	0.278±0.119	y=0.91+0.28x	0.072	5.346	0.82	A
	PBO	10(0.01mg/ml)	0.040	3.840	0.805±0.089	y=1.83+0.34x	0.158	0.506	9.98	S
Tetraniliprole	Nil		0.506	1.123	0.591± 0.20	y=1.82+0.56x	0.124	27.000		
	Terminalia arjuna	500 (0.5 mg/ml)	0.280	0.301	0.243±117	y=0.86+0.24x	0.029	65.540	1.81	S
	Alstonia scholaris	500 (0.5 mg/ml)	0.292	0.307	0.303±119	y=1.06+0.30x	0.050	155.730	1.73	S
	Eupatorium odoratum	500 (0.5 mg/ml)	0.139	0.137	0.267±0.11	y=1.03+0.27x	0.014	173.670	3.64	S
	Nyctanthes arbor-tristis	500 (0.5 mg/ml)	0.136	0.681	0.274±0.11	y=1.05+0.27x	0.014	68.390	3.72	S
	Spathodea campanulata	500 (0.5 mg/ml)	0.167	0.328	0.269±0.11	y=1.01+0.27x	0.019	39.720	3.03	S
	Vitex negundo	500 (0.5 mg/ml)	0.340	0.522	0.356±0.12	y=1.35+0.35x	0.076	21.360	1.49	S
	PBO	10(0.01mg/ml)	0.160	6.460	0.706±0.09	y=2.20+0.46x	0.265	0.617	3.1625	S
Emamectin benzoate	Nil		3.006	1.158	0.443± 0.11	y=1.10+0.43x	0.462	58.510		
	Terminalia arjuna	500 (0.5 mg/ml)	1.150	0.195	0.197±0.07	y=0.58+0.20x	0.061	190.860	2.61	S
	Alstonia scholaris	500 (0.5 mg/ml)	1.270	0.095	0.181±0.069	y=0.52+0.18x	0.049	89.720	2.37	S
	Eupatorium odoratum	500 (0.5 mg/ml)	0.574	0.245	0.169±0.69	y=0.55+0.17x	0.008	305.680	5.24	S
	Nyctanthes arbor-tristis	500 (0.5 mg/ml)	0.986	0.091	0.177±0.069	y=0.53+0.18x	0.030	65.379	3.05	S
	Spathodea campanulata	500 (0.5 mg/ml)	0.906	0.119	0.192±0.069	y=0.58+0.19x	0.039	145.690	3.32	S
	Vitex negundo	500 (0.5 mg/ml)	2.015	0.064	0.162±0.069	y=0.44+0.16x	0.057	177.125	1.49	S
	PBO	10(0.01mg/ml)	0.210	2.490	0.740±0.087	y=1.41+0.31x	0.136	0.477	14.31	S

Data are represented as *Mean ± Standard Error (S.E)*. Synergistic ratios (SR) indicate the enhancement effect of plant extracts and PBO on insecticides. Each experiment was replicated thrice (*n* = 3) for statistical validity.

Table 5 presents the synergistic effects observed during *Kharif* 2023. The combination of PBO with spinetoram showed an SR of 7.05, indicating significant synergy. Among plant extracts, *Alstonia scholaris* demonstrated an SR of 5.42 and *Spathodea campanulata* recorded an SR of 2.56. Other extracts, such as *Terminalia arjuna* and *Eupatorium odoratum*, exhibited SR values ranging from 1.36 to 1.80.

The combination of PBO with tetraniliprole in *Kharif* 2023 also recorded a high SR of 11.97. *Nyctanthes arbor-tristis* displayed an SR of 2.47, while *Alstonia scholaris* and *Eupatorium odoratum* showed SR values between 1.64 and 1.71. Other extracts exhibited mild synergistic effects with SR values between 1.17 and 1.41.

In the tests with emamectin benzoate, the combination with PBO recorded a notably high SR of 15.48, indicating a strong synergistic effect. *Nyctanthes arbor-tristis* and *Terminalia arjuna* followed with SR values of 7.80 and 7.49, respectively. *Eupatorium odoratum*, *Spathodea campanulata* and *Vitex negundo* demonstrated SR values ranging from 3.23 to 6.49.

Discussion

The results from this study demonstrate a clear pattern of synergistic interactions between plant extracts, PBO and the insecticides spinetoram, tetraniliprole and emamectin benzoate when applied to 3rd instar larvae of *Leucinodes orbonalis* collected from Bhubaneswar. Across both *Rabi* and *Kharif* seasons, spinetoram consistently exhibited the highest toxicity, followed by tetraniliprole and emamectin benzoate. The study also highlights the significant potential of using botanical synergists, along with PBO, to enhance the efficacy of insecticides and manage resistance in *L. orbonalis* populations.

Synergistic Potential of PBO and Plant Extracts

PBO, a well-known inhibitor of mixed function oxidases (MFO), showed a remarkable synergistic effect when combined with all three insecticides. The combination of PBO with spinetoram exhibited the highest synergistic ratio (SR) across both seasons, demonstrating the potential for enhanced insecticidal efficacy through metabolic inhibition. This aligns with previous studies that have emphasized the role of PBO in enhancing the toxicity of insecticides by inhibiting the metabolic enzymes responsible for insecticide detoxification (16). PBO's ability to block oxidative

Table 5. Interactive effect of tested plant extracts/PBO to selected insecticides during *Kharif*, 2023

Insecticide	Plant extract/PBO	Conc. in ppm (dosage mg/ml)	LD_{50} (ng/larva)	Chi-square	Slope±SE	Regression equation	Fiducial limits		SR value	S/A
							Lower	Upper		
Spinetoram	Nil		0.399	0.083	0.448± 0.11	y=1.10+0.43x	0.086	63		
	Terminalia arjuna	500 (0.5 mg/ml)	0.055	0.393	0.332±0.110	y=1.42+0.33x	0.006	0.492	7.25	S
	Alstonia scholaris	500 (0.5 mg/ml)	0.065	0.184	0.304±0.117	y= 1.27+0.30x	0.005	1.000	6.14	S
	Eupatorium odoratum	500 (0.5 mg/ml)	0.054	0.357	0.243±0.011	y=1.04+0.24x	0.040	16.312	7.38	S
	Nyctanthes arbor-tristis	500 (0.5 mg/ml)	0.046	0.646	0.292±0.116	y=1.27+0.29x	0.002	1.000	8.67	S
	Spathodea campanulata	500 (0.5 mg/ml)	0.078	0.376	0.283±0.117	y=1.16+0.28x	0.006	3.620	5.12	S
	Vitex negundo	500 (0.5 mg/ml)	0.485	0.312	0.278±0.119	y=0.91+0.28x	0.072	5.346	0.82	A
	PBO	10(0.01mg/ml)	0.040	3.840	0.805±0.089	y=1.83+0.34x	0.158	0.506	9.98	S
Tetraniliprole	Nil		0.506	1.123	0.591± 0.20	y=1.82+0.56x	0.124	27.000		
	Terminalia arjuna	500 (0.5 mg/ml)	0.280	0.301	0.243±117	y=0.86+0.24x	0.029	65.540	1.81	S
	Alstonia scholaris	500 (0.5 mg/ml)	0.292	0.307	0.303±119	y=1.06+0.30x	0.050	155.730	1.73	S
	Eupatorium odoratum	500 (0.5 mg/ml)	0.139	0.137	0.267±0.11	y=1.03+0.27x	0.014	173.670	3.64	S
	Nyctanthes arbor-tristis	500 (0.5 mg/ml)	0.136	0.681	0.274±0.11	y=1.05+0.27x	0.014	68.390	3.72	S
	Spathodea campanulata	500 (0.5 mg/ml)	0.167	0.328	0.269±0.11	y=1.01+0.27x	0.019	39.720	3.03	S
	Vitex negundo	500 (0.5 mg/ml)	0.340	0.522	0.356±0.12	y=1.35+0.35x	0.076	21.360	1.49	S
	PBO	10(0.01mg/ml)	0.160	6.460	0.706±0.09	y=2.20+0.46x	0.265	0.617	3.16	S
Emamectin benzoate	Nil		3.006	1.158	0.443± 0.11	y=1.10+0.43x	0.462	58.510		
	Terminalia arjuna	500 (0.5 mg/ml)	1.150	0.195	0.197±0.07	y=0.58+0.20x	0.061	190.860	2.61	S
	Alstonia scholaris	500 (0.5 mg/ml)	1.270	0.095	0.181±0.069	y=0.52+0.18x	0.049	89.720	2.37	S
	Eupatorium odoratum	500 (0.5 mg/ml)	0.574	0.245	0.169±0.69	y=0.55+0.17x	0.008	305.680	5.24	S
	Nyctanthes arbor-tristis	500 (0.5 mg/ml)	0.986	0.091	0.177±0.069	y=0.53+0.18x	0.030	65.379	3.05	S
	Spathodea campanulata	500 (0.5 mg/ml)	0.906	0.119	0.192±0.069	y=0.58+0.19x	0.039	145.690	3.32	S
	Vitex negundo	500 (0.5 mg/ml)	2.015	0.064	0.162±0.069	y=0.44+0.16x	0.057	177.125	1.49	S
	PBO	10(0.01mg/ml)	0.210	2.490	0.740±0.087	y=1.41+0.31x	0.136	0.477	14.31	S

Data are represented as *Mean ± Standard Error (S.E)*. Synergistic ratios (SR) indicate the enhancement effect of plant extracts and PBO on insecticides. Each experiment was replicated thrice (*n = 3*) for statistical validity.

detoxification pathways in insects has been well-documented, making it a powerful tool in managing resistance, especially in pests like *L. orbonalis* that have developed resistance to conventional insecticides.

The plant extracts tested in this study also exhibited varying degrees of synergistic activity. Among them, *Spathodea campanulata*, *Alstonia scholaris* and *Eupatorium odoratum* consistently showed strong synergistic effects when combined with spinetoram and emamectin benzoate. These findings suggest that the bioactive compounds in these plants may be interacting with the insecticides in ways that enhance their toxic effects. For instance, the bioactive compounds in *Spathodea campanulata*, such as rutin, could be affecting the insect's physiological processes in a manner that weakens their defence mechanisms against insecticides (17).

Seasonal Consistency in Toxicity Trends

The consistency in the order of toxicity across both *Rabi* and *Kharif* seasons-spinetoram > tetraniliprole > emamectin benzoate-provides valuable insight into the resistance dynamics of *L. orbonalis*. Spinetoram's superior toxicity in both seasons suggests that it remains highly effective in managing *L. orbonalis* populations despite the potential for seasonal variations in environmental factors such as temperature, humidity and resistant populations.

This seasonal consistency indicates that the metabolic mechanisms responsible for insecticide detoxification and resistance development in *L. orbonalis* may not vary significantly across different times of the year. The slightly higher resistance observed in the *Rabi* population compared to the *Kharif* population could be attributed to environmental factors, such as cooler temperatures and lower humidity during *Rabi*, which may affect the insect's metabolic rate and the efficiency of detoxification pathways. Studies shown that such environmental factors can influence the rate of insecticide metabolism and resistance development, with cooler temperatures often leading to reduced metabolic activity and a slower detoxification process (18).

Mechanisms of Resistance and Implications for Pest Management

The development of insecticide resistance in *L. orbonalis* is a growing concern, particularly with the increasing use of chemical insecticides in brinjal cultivation. In this study, the combination of spinetoram with PBO and plant extracts showed the greatest potential in overcoming resistance, as indicated by the high synergistic ratios observed in both seasons. This suggests that the primary mechanisms of resistance in *L. orbonalis*-such as enhanced detoxification enzyme activity and target site modifications-can be effectively countered by metabolic inhibitors like PBO and the bioactive compounds present in plant extracts.

Spinetoram had a lower LC_{50} value than other insecticides, indicating higher toxicity (19). It was about ten times more effective than Spinosad in topical and feeding bioassays (20). Spinetoram had low LC_{50} values at various time intervals for 4^{th} instar larvae (21). These findings are consistent with the results of the current study, where spinetoram outperformed tetraniliprole and emamectin benzoate in terms of toxicity.

Tetraniliprole LD_{50} on third-instar larvae of *Spodoptera litura* was lower than that of chlorantraniliprole (22). Tetraniliprole, although less toxic than spinetoram (23, 24, 25), showed a moderate level of synergism when combined with PBO and certain plant extracts, such as *Nyctanthes arbor -tristis* and *Spathodea campanulata*. The moderate synergism observed with tetraniliprole suggests that while this insecticide may be less effective on its own, its efficacy can be enhanced through synergists, potentially delaying the onset of resistance in *L. orbonalis* populations.

Emamectin benzoate, derived from *Streptomyces avermitilis*, disrupts insect nervous systems (26). The least toxic of the three insecticides, it showed a notable improvement in efficacy when combined with PBO, achieving a high SR of 14.31. This indicates that emamectin benzoate combined with synergists, could still play a valuable role in integrated pest management (IPM) strategies for *L. orbonalis*.

Role of Plant Extracts in Resistance Management

The use of plant extracts as synergists offers a promising avenue for enhancing the efficacy of chemical insecticides while reducing the overall reliance on synthetic chemicals. *Spathodea campanulata*, *Eupatorium odoratum* and *Alstonia scholaris* consistently showed strong synergistic effects across all tested insecticides. These plants are known to contain bioactive compounds that interfere with the insect's metabolic processes, thereby increasing the potency of chemical insecticides.

For example, *Eupatorium odoratum* is rich in chromolaenide, which shares structural similarities with chlorogenic acid. Chromolaenide and similar compounds can interfere with insect detoxification pathways, reducing their ability to metabolize insecticides (27). Similarly, *Spathodea campanulata* contains rutin, a compound shown to disrupt the insect's digestive and nervous systems, thereby enhancing the efficacy of insecticides like spinetoram and emamectin benzoate (17).

Using these plant extracts in conjunction with insecticides could provide a sustainable approach to managing resistance in *L. orbonalis*. By incorporating plant extracts into IPM strategies, farmers can reduce their reliance on chemical insecticides, potentially slowing the development of resistance and extending the useful lifespan of existing insecticide products.

Implications for Integrated Pest Management (IPM)

The findings of this study highlight the importance of incorporating synergists-both chemical (PBO) and botanical (plant extracts)-into pest management programs. The high synergistic ratios observed in this study suggest that combining these synergists with conventional insecticides can significantly enhance their efficacy, providing better control over *L. orbonalis* populations and reducing the likelihood of resistance development.

Incorporating botanical synergists into IPM strategies offers additional benefits, such as reduced environmental impact and lower risks to non-target organisms. Plant extracts are biodegradable and typically have lower toxicity to humans and wildlife than synthetic chemicals. Their use in pest management aligns with the principles of sustainable agriculture, which emphasize minimizing chemical inputs and promoting ecological balance.

Conclusion

This study highlights the effectiveness of novel insecticides, particularly spinetoram, tetraniliprole and emamectin benzoate, in managing *Leucinodes orbonalis* in brinjal crops, with spinetoram exhibiting the highest toxicity. The synergistic effects of piperonyl butoxide (PBO) and plant extracts like *Eupatorium odoratum* and *Nyctanthes arbor-tristis* significantly enhanced the insecticidal efficacy, offering promising solutions for resistance management. The seasonal consistency in toxicity patterns further emphasizes the potential of these combinations in integrated pest management (IPM). Incorporating botanical extracts with insecticides can lead to more sustainable and effective pest control strategies, reducing the reliance on chemical pesticides while mitigating resistance development in *L. orbonalis*.

Future research can explore the field applicability of these findings by testing the effectiveness of insecticide and botanical extract combinations under diverse agro-climatic conditions. Additionally, investigating the mechanisms behind the observed synergistic interactions could provide deeper insights into optimizing pest management strategies. Developing eco-friendly formulations incorporating these synergists could enhance their practical adoption, contributing to sustainable agriculture and improved crop yields.

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Authors' contributions

VSS and MK carried out the bioassay and synergist procedure and drafted the manuscript. SSD and MRK participated in the design of the study and performed the statistical analysis. BKP conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that there is no conflict of interest.

Ethical issues: None

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