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RESEARCH ARTICLE

Regional monitoring of insecticide resistance in brinjal fruit and shoot borer (*Leucinodes orbonalis* Guenée): A study of indoxacarb, spinosad and thiodicarb in Odisha

Bandana Pasayat[1*], Manoj Kumar Tripathy[1], Sajiya Quadri[1], Kabita Sethi[2] & Prachi Pattnaik[3]

[1]Department of Entomology, College of Agriculture, Odisha University of Agriculture and Technology 751 003, India
[2]Department of Fruit Science and Horticulture Technology, College of Agriculture, Odisha University of Agriculture and Technology 751 003, India
[3]Department of Horticulture, Institute of Agricultural Science, Banaras Hindu University 221 005, India

*Email: pasayatbandanaofs@gmail.com

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Abstract

The brinjal fruit and shoot borer (*Leucinodes orbonalis*) represents a significant pest of *Solanum melongena* (brinjal), adversely affecting its productivity. Current management approaches rely heavily on chemical insecticides, a dependency that has facilitated the development of resistance in various regions. A study conducted between November 2021 and September 2022 utilized a topical bioassay method to assess resistance trends in populations collected from four districts in Odisha against the insecticides Indoxacarb, Spinosad, and Thiodicarb. The results indicated a progressive increase in resistance to Indoxacarb until May 2022, as evidenced by LD_{50} values ranging from 0.525 to 0.752 µg/µl across districts. Subsequently, a decline in LD_{50} values was observed, potentially attributable to population turnover or a reduction in selection pressure. These trends were reflected in the resistance ratio (RR_{50}), which peaked in May 2022 and declined by September. Among the districts, the Bargarh population exhibited the highest resistance to Thiodicarb, with a 42.39-fold RR_{50}, underscoring localized resistance evolution. This study highlights significant heterogeneity in susceptibility across regions, emphasizing the urgent need for a revision of current pest management practices. It advocates for the judicious use of novel insecticides and adjustments in the application rates of established chemicals like Thiodicarb. Such measures are essential to mitigate resistance development and ensure the sustained productivity of *Solanum melongena*.

Keywords

bioassay; insecticide resistance; iso-female colony; regional monitoring; resistance level

Introduction

The brinjal fruit and shoot borer (*Leucinodes orbonalis* Guenée, Lepidoptera: Crambidae) is a significant pest endemic to tropical and subtropical regions worldwide. Brinjal (*Solanum melongena* Linn.), the primary host plant, is native to India, where the pest was first described. Currently, *L. orbonalis* is distributed across Asia, Africa, and parts of Europe (1, 2). In India, this pest is particularly destructive, causing yield losses ranging from 37–63% under general circumstances, with potential losses escalating to 70–90%, or even 100% in the absence of control measures (3–5). Its impact spans the crop lifecycle, from the nursery to the harvesting stage, necessitating extensive management efforts (6). The pest exhibits migratory behavior, with dispersion documented in Africa south of the Sahara, South-East Asia, including China, India, and the Philippines. Its movement is notably accelerated in hot and humid climates (7). Indian farmers reportedly apply insecticides as frequently as 84 times during a single six- to seven-month cropping season, translating to a pesticide load of 4.6 kg of active ingredient (a.i.) per hectare, second only to chili. This leads to residues in marketable brinjal fruits that are 40–400 times higher than the maximum residue limit (MRL), posing serious health risks (8–10). With high reproductive potential and overlapping generations, *L. orbonalis* has demonstrated significant resistance to insecticides due to indiscriminate and prolonged chemical use (11–13). Synthetic insecticides have been the predominant control strategy for decades. Although genetically modified Bt-brinjal has been developed, its adoption in India remains limited due to a government-imposed moratorium. Consequently, pesticide usage in non-Bt brinjal remains high (14, 15). In Odisha, farmers have identified *L. orbonalis* as one of the most challenging pests to manage, with an average annual insecticide application of 2.56 kg a.i./ha. Usage patterns vary significantly across the state's agroclimatic zones, with Bargarh registering the highest levels of insecticide application (16, 17).

Common insecticides for *L. orbonalis* management include older compounds such as Thiodicarb, alongside newer-generation chemicals like Spinosad, Indoxacarb, Flubendiamide, Chlorantraniliprole, Novaluron, and Cyantraniliprole. However, low market prices for brinjal and high input costs often compel farmers to rely on less expensive, older insecticides, exacerbating resistance development (18). Continuous exposure to pesticides, combined with the pest's large population size and year-round reproductive capacity, has accelerated resistance evolution in *L. orbonalis* (1, 17, 19). In Odisha, the trend of increasing insecticide application, rising from 2.45 kg a.i./ha in 2020 to 3.43 kg a.i./ha in 2023, highlights the intensification of pest control measures (16). Farmers' indiscriminate use of pesticides, often starting 15 days post-transplanting and continuing throughout the crop cycle, underscores a lack of awareness about the potential for pest resurgence, environmental contamination, and residual toxicity in brinjal (13).

This study aims to investigate the resistance development patterns of *L. orbonalis* to Indoxacarb, Spinosad, and Thiodicarb in key brinjal-growing districts of Odisha, including Khurda (Bhubaneswar), Cuttack, Keonjhar, and Bargarh. The findings offer critical insights into the dynamics of resistance evolution, elucidate potential causal factors, and emphasize the need for strategic interventions to address this growing agricultural challenge.

Materials and Methods

The study was conducted in the toxicology laboratory of the Department of Entomology at the College of Agriculture, Orissa University of Agriculture and Technology (OUAT), Bhubaneswar, from 2021 to 2022. The materials used and methods followed are detailed below:

Test Insect

Field populations of *L. orbonalis* larvae were collected from major brinjal cultivation areas in Odisha at two-month intervals from November 2021 to September 2022 (November 2021, January 2022, March 2022, May 2022, July 2022, and September 2022). These samples were used to evaluate resistance levels to selected insecticides. The study encompassed four key vegetable-growing districts representing three distinct agroclimatic zones: East & South Coastal Plains (Khurda and Cuttack), North-Central Plateau (Keonjhar), and Western Undulating Lands (Bargarh). These areas were chosen to assess regional variations in insecticide resistance. The susceptible iso-female line, derived from an untreated research plot at OUAT and maintained in the laboratory since 2019, served as the reference susceptible population (25^{th} generation) for resistance studies.

Insect Rearing

Field-collected populations from Bhubaneswar, Bargarh, Cuttack, and Keonjhar were reared under controlled laboratory conditions (27 ± 2°C, 60–70% relative humidity, and a 14:10 h light:dark photoperiod) with a natural diet of brinjal and potato. Rearing was conducted in plastic jars sealed with rubber bands and covered with plastic netting. The first filial (F1) generation of larvae was used for bioassays. After the last-instar larvae molted into pupae, food was removed to prevent contamination, as pupae do not require nourishment. Pupae were transferred to rearing cages. After adult emergence, the plastic jars were removed, and the cages were fitted with plastic nets, which had been previously preferred by adult females for egg-laying. A 10% honey solution was provided as a food source for the adults. Once eggs were laid on the nets, they were removed, and the newly emerged first-instar larvae were placed in separate plastic jars with fresh food. The third-instar larvae from the F1 generation were used for bioassays. The susceptible iso-female colony was maintained up to the 25th generation under identical rearing conditions.

Stock Solution and Serial Dilution

The insecticides used in the bioassay—Indoxacarb, Spinosad, and Thiodicarb—were selected for their prevalent use in brinjal cultivation by farmers, with each having a technical purity of 99% (Table 1). Stock solutions were prepared for each insecticide, and serial dilutions were made to achieve eight concentration levels, ensuring that mortality rates remained between 20% and 90%. Acetone was used as the solvent, and the stock solutions were prepared according to standard procedures (21).

Bioassay Technique

A topical bioassay method (22) was employed to test insecticide resistance in early third-instar *L. orbonalis* larvae. Insecticides were applied to the dorsal thoracic

Table 1. General information on insecticides used against *L. orbonlais* population in topical bioassay

Sl. No.	Name	Group	IRAC group	Mode of action	Chemical Formula
1	Indoxacarb (Technical grade,99% purity)	Oxadiazines	22A	Voltage dependent Sodium channel blocker Nerve action	$C_{22}H_{17}ClF_3N_3O_7$
2	Spinosad (Technical grade,99% purity)	Spinosyn	5	Nicotinic acetylcholine receptor (nAChR) allosteric modulator- Site-1	$C_{83}H_{132}N_2O_{20}$
3	Thiodicarb (Technical grade,99% purity)	Carbamate	1A	Acetylcholinesterase (AChE) inhibitor Carboxylesterase inhibitor	$C_{10}H_{18}N_4O_4S_3$

segments of the larvae using a Hamilton microapplicator (PB600-1, Hamilton Company), which has a 50 µl capacity and dispenses 1 µl at a time. Thirty larvae were treated per replication, with three replications for each dose. The treated larvae were transferred to plastic containers with netted tops containing fresh, untreated brinjal and potato pieces (Fig. 2). Mortality was assessed at 24-, 48-, and 72-hour post-treatment. Acetone was used for the control group. Larvae that did not move when touched with a fine brush were considered dead, and mortality data were recorded accordingly.

Statistical Analysis

Corrected mortality was calculated using the Abbott formula (23). Probit analysis was conducted using Polo Plus software version 2.0 (24) to determine the slope, LD_{50}, fiducial limits (95%), chi-square heterogeneity, and regression equations for each population. The resistance ratio (RR_{50}) was calculated by comparing the LD_{50} values of the field populations with the LD_{50} of the susceptible colony. The relative potency (REP) was used to assess the comparative effectiveness of the insecticides. REP is the ratio of the LD_{50} of the least toxic active ingredient to that of each active ingredient, providing a measure of insecticide potency (25). It is hypothesized that REP in resistant populations will show a distinct pattern compared to susceptible populations due to the development of resistance.

Results and Discussion

Resistance Trend to Indoxacarb

The current study revealed that *L. orbonalis* larvae exhibited moderate resistance to Indoxacarb, a voltage-dependent sodium channel blocker that interferes with the insect nervous system. The resistance to Indoxacarb increased from November 2021 to May 2022 across all

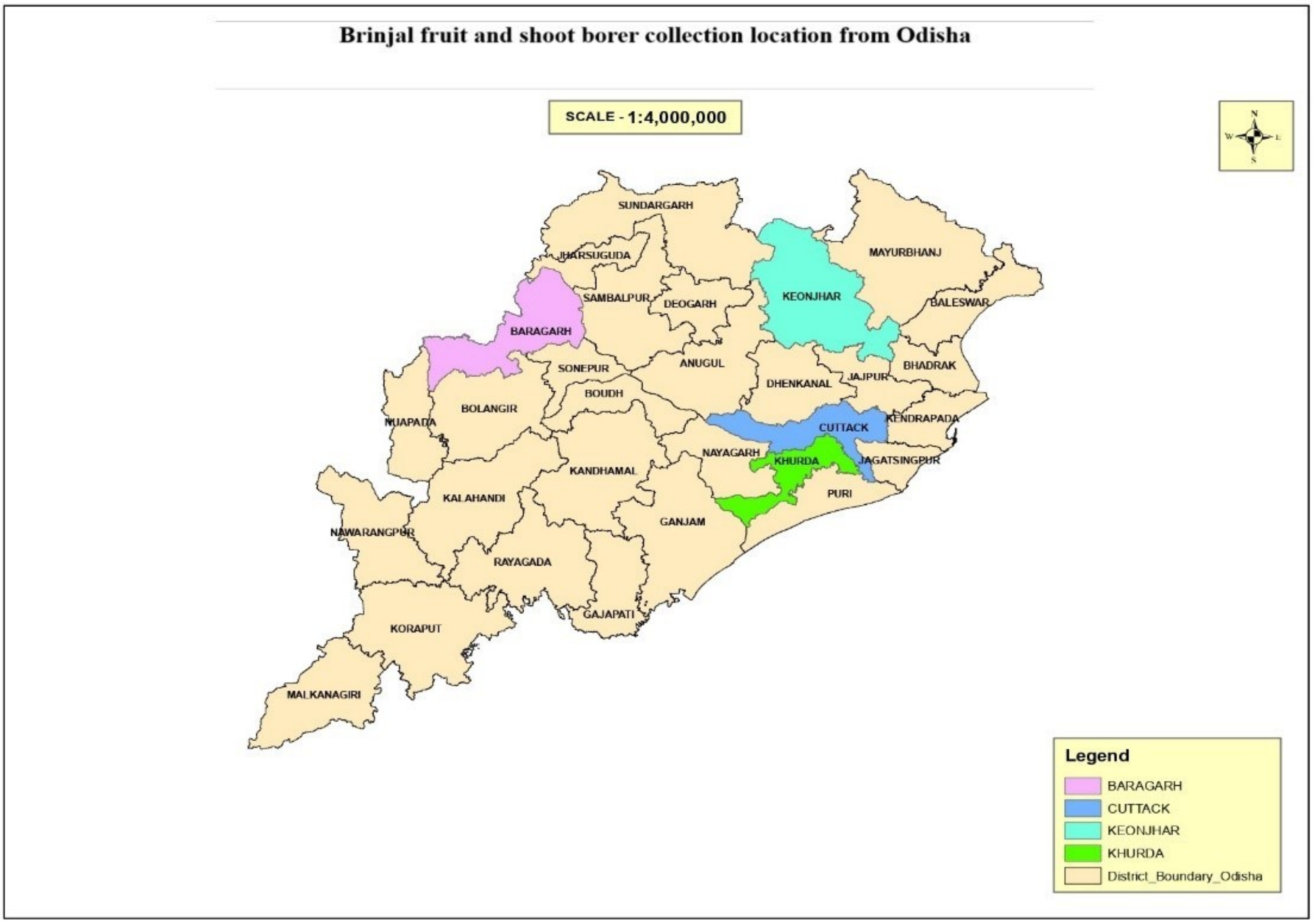

Fig. 1. Places of collection of *L. orbonalis* larvae (map created in https://www.arcgis.com).

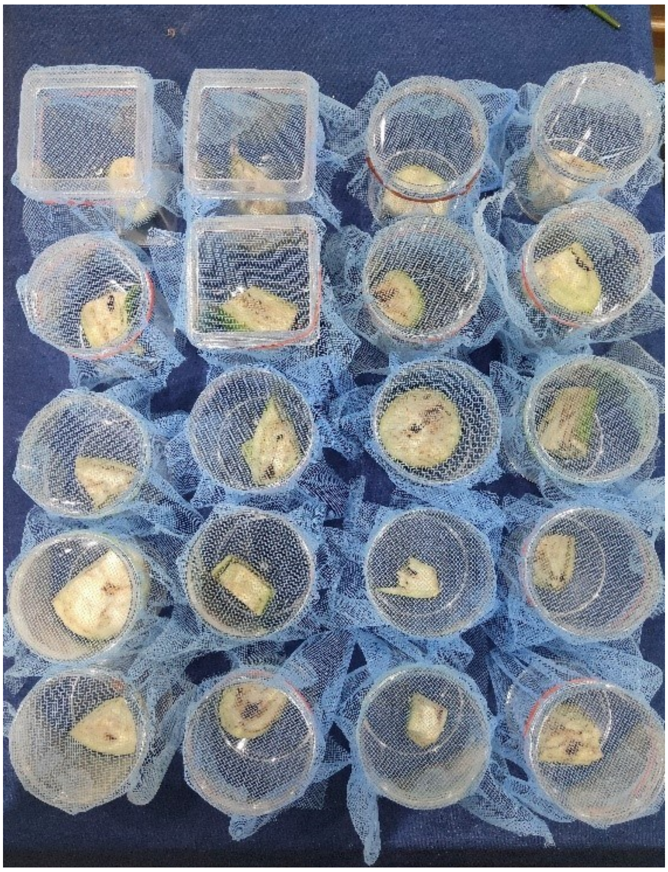

Fig. 2. Insecticide treated larvae.

study sites: Bhubaneswar (12.16-16.77-fold), Cuttack (12.72-15.84-fold), Keonjhar (14.60-16.44-fold), and Bargarh (15.98-17.49-fold). However, resistance levels decreased progressively during the months of July and September 2022, as shown in Table 2. The bioassay results from September 2022 revealed the lowest LD50 values for all populations: 0.604 µg/µl for Bhubaneswar, 0.611 µg/µl for Cuttack, 0.672 µg/µl for Keonjhar, and 0.697 µg/µl for Bargarh (Table 2). The resistance ratio was comparatively lower in the Bhubaneswar and Cuttack populations than in the Keonjhar and Bargarh populations. The overlapping fiducial limits across all populations throughout the study period suggested no significant statistical differences in their response to Indoxacarb. These findings align with previous studies (18, 20).

Indoxacarb, a new-generation insecticide with a novel mode of action, has led to the development of resistance in *L. orbonalis*, likely due to increased selection pressure and improper insecticide dosages. Similar trends of resistance have been reported for *Helicoverpa* species (22). Resistance mechanisms may include behavioral changes, cuticular thickening, and, more significantly, detoxification mechanisms involving increased hydrolysis and excretion, or elevated levels of detoxification enzymes such as carboxylesterase, acetylcholine esterase, glutathione S-transferase, and mixed-function oxidases (20). Bargarh, a major brinjal-growing area, experiences the highest insecticide usage (23 sprays per crop season), which likely contributes to the higher LD50 value (0.697 µg/µl) observed in this population compared to Bhubaneswar (0.604 µg/µl) in September 2022.

Resistance Trend to Spinosad

The resistance of *L. orbonalis* to Spinosad was confirmed by non-overlapping 95% fiducial limits across all study sites, indicating significant resistance in field populations compared to the susceptible colony. Resistance levels increased from November 2021 to May 2022 in all four districts: Bhubaneswar (8.35-13-fold), Cuttack (12.72-15.84 -fold), Keonjhar (14.60-16.44-fold), and Bargarh (15.98-17.49-fold). A similar trend was observed for RR90. After May 2022, a shift was noted, with increased toxicity of Spinosad to the field populations. LD_{50} values decreased to 0.205 µg/µl in July 2022 and 0.194 µg/µl in September 2022, compared to 0.221 µg/µl in May 2022, as shown in Table 3.

In Bhubaneswar, a lower LD_{50} value and a resistance ratio of less than 10-fold in November 2021 may be attributed to lower chemical pesticide usage and a preference for organic pesticides in some areas. This gradual increase in resistance suggests the potential carryover of resistant alleles to subsequent generations (22), warranting genetic studies to investigate the mechanisms of resistance and the role of resistant genes. Variations in spray patterns in different collection areas reflect the high selection pressure exerted, particularly in the Bargarh and Keonjhar populations, which may further contribute to the development of resistance (17).

Resistance Trend to Thiodicarb

The resistance ratio to Thiodicarb at the LD_{50} level for the Bargarh population ranged from 36.21 to 42.39-fold in the period from November 2021 to May 2022, with the highest resistance observed among the insecticides tested. This indicates a significant resistance development to Thiodicarb. However, subsequent tests in July 2022 (38.54-fold) and September 2022 (36.54-fold) showed a slight decrease in resistance levels. The overlapping fiducial limits for these months suggested no statistically significant difference in the field populations' response to this active ingredient during both observations (Table 4).

From Table 4, it is evident that resistance levels increased from November 2021 to May 2022, followed by a slight decline in July 2022 and a further decrease in September 2022 across all populations. The log dose probit regression slope for all field populations from Bhubaneswar, Cuttack, Keonjhar, Bargarh, and the susceptible colony (2.612 ± 0.077) varied significantly, indicating different toxicity levels to Thiodicarb over time. This further supports the development of resistance in *L. orbonalis* to this insecticide, which is consistent with prior studies on resistance in both *Helicoverpa* and *L. orbonalis* populations (22, 1, 9).

The observation of relatively consistent 95% fiducial limits (FL) across the study period suggests that the populations' responses were homogeneous, with no substantial differences in the resistance levels over time. However, large FL differences are typically observed when the highest dose in the bioassay results in less than 90% mortality, which may be attributed to the unavailability of

Table 2. Monitoring Insecticide resistance to Indoxacarb in different places of Odisha in 3rd instar larvae of *L. orbonalis* during Nov- 2021 to Sept- 2022

Place of collection	Month of observation	LD_{50} [b]	FL (95%)[e] Lower	Upper	χ^{2} [d]	Slope± SE[a]	RR_{50} [c]
Bhubaneswar	Nov, 2021	0.523	0.488	0.560	6.859**	0.927± 0.015	12.16
	Jan, 2022	0.545	0.439	0.677	7.000**	3.500± 0.048	12.67
	Mar, 2022	0.611	0.512	0.729	5.908*	4.304± 0.039	14.21
	May, 2022	0.635	0.609	0.852	5.705*	4.801± 0.037	16.77
	July, 2022	0.613	0.506	0.633	5.369*	0.951± 0.025	13.16
	Sept, 2022	0.604	0.404	0.659	6.995**	3.082± 0.054	12.00
Cuttack	Nov, 2021	0.547	0.467	0.640	4.840*	5.277± 0.035	12.72
	Jan, 2022	0.572	0.486	0.673	6.464**	4.999±0. 036	13.30
	Mar, 2022	0.618	0.551	0.692	4.982*	0.319± 0.052	14.37
	May, 2022	0.657	0.576	0.805	7.710**	4.559± 0.037	15.84
	July, 2022	0.632	0.517	0.700	4.664*	4.955± 0.034	14.00
	Sept, 2022	0.611	0.504	0.600	5.994*	0.164± 0.019	12.79
Keonjhar	Nov, 2021	0.628	0.539	0.732	6.753**	4.865± 0.034	14.60
	Jan, 2022	0.632	0.541	0.738	4.705*	4.792± 0.034	14.69
	Mar, 2022	0.692	0.633	0.756	5.398*	0.504±0. 020	16.09
	May, 2022	0.707	0.595	0.840	4.784*	4.440± 0.038	16.44
	July, 2022	0.684	0.599	0.799	7.994 **	5.499± 0.032	16.09
	Sept, 2022	0.672	0.629	0.753	4.234*	0.358± 0.020	16.02
Bargarh	Nov, 2021	0.687	0.596	0.792	6.996**	5.597± 0.031	15.98
	Jan, 2022	0.701	0.643	0.766	5.421*	0.611± 0.019	16.30
	Mar, 2022	0.731	0.642	0.833	6.785**	5.728± 0.029	17.01
	May, 2022	0.752	0.626	0.903	4.794*	4.441± 0.041	17.49
	July, 2022	0.718	0.644	0.801	7.438**	0.275± 0.024	16.69
	Sept, 2022	0.697	0.621	0.783	7.849**	0.777± 0.026	16.21
Susceptible	Sept, 2022	0.043	0.015	0.064	6.003**	0.408 ± 0.046	1.00

[a]SE = Standard error, [b]LD_{50} = Lethal dose expressed as µg of active ingredient per µl, [c]RR= Resistance ratio, LD_{50} of field population over the LD_{50} of the susceptible population, [d]χ^2 = Chi-square value, * = significant at P<0.05, ** = significant at P<0.01, [e]FL(95%)= 95%Fiducial limit of LD_{50} value

Table 3. Monitoring Insecticide resistance to Spinosad in different places of Odisha in 3rd instar larvae of *L. orbonalis* during Nov- 2021 to Sept- 2022

Place of collection	Month of observation	LD_{50} [b]	FL (95%)[e] Lower	Upper	χ^{2} [d]	Slope± SE[a]	RR_{50} [c]
Bhubaneswar	Nov, 2021	0.142	0.092	0.219	5.802*	1.776±0.802	8.35
	Jan, 2022	0.179	0.125	0.258	4.397*	2.108±0.080	10.53
	Mar, 2022	0.187	0.124	0.281	6.768**	1.878±0.090	11.00
	May, 2022	0.221	0.233	0.454	5.833*	2.318±0.074	13.00
	July, 2022	0.205	0.156	0.313	6.668**	2.200±0.077	12.06
	Sept, 2022	0.194	0.134	0.251	4.007*	2.499±0.069	10.76
Cuttack	Nov, 2021	0.193	0.136	0.273	6.571**	2.176±0.077	11.35
	Jan, 2022	0.198	0.117	0.249	7.484**	2.022±0.084	10.05
	Mar, 2022	0.208	0.143	0.302	5.861*	2.060±0.083	12.24
	May, 2022	0.232	0.246	0.517	7.839**	2.083±0.517	13.67
	July, 2022	0.220	0.169	0.286	6.517**	3.207 ±0.058	12.94
	Sept, 2022	0.210	0.148	0.284	7.453**	2.344±0.072	12.06
Keonjhar	Nov, 2021	0.306	0.226	0.412	6.065**	1.314±0.126	18.00
	Jan, 2022	0.313	0.243	0.403	5.066*	3.055±0.056	18.41
	Mar, 2022	0.337	0.261	0.434	5.004*	2.920±0.056	19.82
	May, 2022	0.369	0.372	0.588	7.995**	3.423±0.058	23.41
	July, 2022	0.358	0.234	0.490	6.042**	2.144±0.043	21.06
	Sept, 2022	0.353	0.264	0.487	7.784**	2.533±0.068	20.76
Bargarh	Nov, 2021	0.234	0.128	0.260	5.569*	2.166 ±0.079	10.71
	Jan, 2022	0.255	0.187	0.347	4.242*	2.457±0.069	15.00
	Mar, 2022	0.272	0.206	0.357	6.844**	2.931±0.061	16.00
	May, 2022	0.317	0.360	0.565	4.973*	3.485± 0.050	18.65
	July, 2022	0.305	0.223	0.382	4.897*	2.899± 0.059	17.18
	Sept, 2022	0.294	0.145	0.293	6.353**	2.427± 0.070	11.71
Susceptible	Sept, 2022	0.017	0.009	0.036	5.860*	1.745 ± 0.014	1.00

[a]SE = Standard error, [b]LD_{50} = Lethal dose expressed as µg of active ingredient per µl, [c]RR= Resistance ratio, LD_{50} of field population over the LD_{50} of the susceptible population, [d]χ^2 = Chi-square value, * = significant at P<0.05, ** = significant at P<0.01, [e]FL(95%)= 95%Fiducial limit of LD_{50} value

Table 4. Monitoring Insecticide resistance to Thiodicarb in different places of Odisha in 3rd instar larvae of *L. orbonalis* during Nov- 2021 to Sept- 2022

Place of collection	Month of observation	LD_{50}^{b}	FL (95%)^{e} Lower	Upper	χ2 d	Slope± SE^{a}	RR_{50}^{c}
Bhubaneswar	Nov, 2021	12.682	11.997	13.407	6.100**	4.604 ± 0.012	28.69
	Jan, 2022	13.090	12.767	13.421	5.306*	3.188± 0.006	29.62
	Mar, 2022	13.909	13.537	14.847	6.618**	7.841 ± 0.010	31.46
	May, 2022	14.615	14.791	16.440	7.008**	3.884 ± 0.012	35.28
	July, 2022	14.131	13.537	14.847	7.921**	0.845 ± 0.112	31.97
	Sept, 2022	13.993	13.095	14.953	6.912**	1.843 ± 0.015	31.65
Cuttack	Nov, 2021	14.032	12.826	15.351	5.970*	8.520± 0.020	31.74
	Jan, 2022	15.333	14.190	16.568	7.976**	0.345± 0.017	34.69
	Mar, 2022	15.921	15.224	16.650	8.626**	8.244 ± 0.010	36.02
	May, 2022	16.723	15.903	17.584	6.012**	4.719 ± 0.011	37.83
	July, 2022	16.342	15.265	16.731	5.925*	0.567 ± 0.010	36.16
	Sept, 2022	14.134	13.293	15.161	6.955**	2.012± 0.015	32.11
Keonjhar	Nov, 2021	14.231	12.910	15.723	7.990**	0.665± 0.022	32.23
	Jan, 2022	15.867	15.413	16.884	6.920**	0.225± 0.010	35.89
	Mar, 2022	15.969	14.940	17.069	5.914*	1.691 ± 0.015	36.13
	May, 2022	17.729	16.910	18.587	5.011*	5.628± 0.010	40.11
	July, 2022	17.453	15.134	17.155	7.997**	3.316± 0.013	36.34
	Sept, 2022	17.215	13.337	16.235	6.889**	1.854± 0.015	32.25
Bargarh	Nov, 2021	15.576	15.101	16.953	6.910**	3.642 ± 0.013	36.21
	Jan, 2022	16.219	16.082	17.659	5.997*	7.272 ± 0.010	38.13
	Mar, 2022	16.945	16.142	17.787	8.995*	6.378 ± 0.011	38.34
	May, 2022	18.564	17.917	19.590	7.010**	6.555 ± 0.010	42.39
	July, 2022	18.231	16.273	19.829	6.977**	7.569 ± 0.012	38.54
	Sept, 2022	17.982	15.413	18.884	5.929*	5.413±0.010	36.54
Susceptible	Sept, 2022	0.442	0.769	1.563	6.755**	2.612 ± 0.077	1.00

^{a}SE = Standard error, $^{b}LD_{50}$ = Lethal dose expressed as µg of active ingredient per µl, ^{c}RR= Resistance ratio, LD_{50} of field population over the LD_{50} of the susceptible population, $^{d}\chi^2$ = Chi-square value, * = significant at P<0.05, ** = significant at P<0.01, ^{e}FL(95%)= 95%Fiducial limit of LD_{50} value

additional insects or the necessity for significantly higher doses due to advanced resistance development.

Our findings highlight field-evolved resistance of *L. orbonalis* populations from four districts in Odisha to three insecticides with different modes of action: Acetylcholinesterase (AChE) inhibitor (Thiodicarb), Voltage -dependent sodium channel blocker (Indoxacarb), and Nicotinic acetylcholine receptor (nAChR) allosteric modulator-Site-1 (Spinosad). This development correlates with reports of poor performance of these active ingredients in the fields of Odisha. Similar findings have been documented in Mexico and Puerto Rico (26). While factors such as rainfall, inadequate pesticide formulation, and improper application methods can influence performance, the standard operating procedures of seed industries and farmers in Odisha mitigate these factors. Therefore, the reduced efficacy of these insecticides is primarily attributed to the resistance of *L. orbonalis*.

Furthermore, this study reveals a gradual increase in resistance to Spinosad, Thiodicarb, and Indoxacarb from November 2021 to May 2022, coinciding with a period of heavy pest infestation in the field (7, 27). The gradual decrease in susceptibility of *L. orbonalis* to both older (Thiodicarb) and newer (Indoxacarb and Spinosad) insecticides is concerning and warrants urgent attention. Previously, these insecticides were effective in controlling this pest at recommended field rates, as observed in Bhubaneswar in 2018 (14). However, after five years, the present study indicates that Thiodicarb is now the least effective compound against *L. orbonalis*, with resistance to Spinosad and Indoxacarb exceeding 10-fold, signaling an alarming need to revise pest control strategies in Odisha. Notably, a resistance ratio greater than 45-fold in the Bargarh population to Thiodicarb in May 2022 demonstrates the significant loss of susceptibility to this mode of action, further confirming that *L. orbonalis* in Odisha has developed considerable resistance to AChE inhibitors. These findings align with earlier research reports (22).

Currently, Spinosad is a key component in the control of *L. orbonalis* (14) and is widely utilized in management programs. All populations exhibited minimal resistance, with resistance levels lower than a 20-fold increase compared to susceptible strains. Furthermore, the non-overlapping 95% fiducial limits (FL) between the field and susceptible populations suggest that field populations have reduced susceptibility to this active ingredient. A recent study indicated that resistance to Spinosad and Thiodicarb in *L. orbonalis* is autosomal, polygenic, incompletely recessive, and associated with significant fitness costs (5). This finding aligns with similar observations of Spinosad resistance in Mexico (22). These characteristics of Spinosad resistance are critical for the development of Insect Resistance Management (IRM) programs, particularly for the Bargarh population, where the biological effectiveness of these insecticides may be compromised.

Statistically significant resistance was observed across all populations when compared to the susceptible strains. The evolution of resistance in *L. orbonalis* populations from Odisha to a broad spectrum of insecticides appears to be driven by a generalized mechanism. Detoxification enzymes such as carboxyl esterases, Glutathione S-transferases, and cytochrome P450s are key mechanisms of resistance in various polyphagous Lepidopteran species (28-33). Target-site insensitivity also contributes to resistance mechanisms alongside metabolic resistance to organophosphates, carbamates, and pyrethroids (28).

Genetic studies on *L. orbonalis* resistance to carbamates suggest that resistance is autosomal and incompletely recessive, implying the presence of one or more major genes in addition to minor genes, which can confer resistance to multiple insecticide classes in Lepidoptera (32, 34-38). In the *L. orbonalis* populations from Odisha, resistance to Spinosad and Thiodicarb may result from the overexpression of esterases, while in the Keonjhar population, overexpression of Glutathione S-transferases has been implicated (32). Although the resistance mechanisms may differ, they likely emerged concurrently due to strong selection pressure in the field (32, 39-42).

The populations of *L. orbonalis* from Bhubaneswar, Cuttack, Bargarh, and Keonjhar were screened in this study. Bargarh and Keonjhar account for 53% of the total brinjal cultivation area and 34% of the total production in Odisha (6, 16). These regions are characterized by the use of both local and hybrid brinjal seeds and heavy reliance on organophosphates and synthetic pyrethroids for pest control. Given the significant brinjal production area in Odisha, further comprehensive susceptibility studies involving a larger number of *L. orbonalis* populations are crucial.

Migration of *L. orbonalis* among agricultural regions within Odisha could also influence the maintenance of susceptibility to insecticides. Previous studies have described the migration of *L. orbonalis* carrying resistant alleles to new regions (7, 22). It is plausible that populations with susceptible alleles can contribute to reducing the frequency of resistance alleles in areas with intense insecticide selection pressure (38, 43-44). The diversity of agricultural practices, availability of numerous wild and cultivated hosts, and migration of *L. orbonalis* likely contribute to the relatively lower levels of pesticide resistance observed in the July and September 2022 populations from all four regions studied.

Additionally, *L. orbonalis* can utilize alternate host crops such as potatoes, tomatoes, and other solanaceous vegetables (7, 22). During the dry season, these pests may rely on wild hosts, which are typically not treated with insecticides. These wild hosts may serve as refuges for susceptible individuals, slowing the development of resistance, as observed in July and September 2022 across all study populations. While further research is needed, we hypothesize that these wild hosts play a critical role in preserving susceptibility. Conversely, the limited availability of alternative hosts may accelerate resistance development in Bargarh and Keonjhar (13, 14). Identifying potential native hosts of *L. orbonalis* is a critical area for future research. Incorporating these native hosts into agroecosystems could provide refuge for both susceptible *L. orbonalis* individuals and their natural predators, thereby mitigating resistance development. Furthermore, future studies on the inheritance of resistance in *L. orbonalis* from these regions are essential to understanding the nature of resistance—whether recessive or dominant—and to determining the number of genes involved.

REPs of Active Ingredients

In the current study, Thiodicarb, identified as the least potent insecticide among those tested on both the field and susceptible populations, was selected as the index insecticide for calculating Relative Efficacy Potency (REP) ratios. A lower REP value indicates reduced susceptibility of a population to an insecticide relative to other tested insecticides, and vice versa. Typically, older insecticides exhibit lower REP values than newer chemical classes. Field populations across all locations demonstrated lower REP values for each insecticide compared to the susceptible population.

As a member of the acetylcholine inhibitor class (IRAC Group 1A) and a carbamate, Thiodicarb exhibited the lowest potency against *L. orbonalis* across both field and susceptible populations when compared to Spinosad and Indoxacarb. The REP values for the susceptible population were consistently higher than those for the field populations for all insecticides. In May 2022, Thiodicarb had the highest LD_{50} (18.564 μg/μl) for the Bargarh population, and this value was used as the index for REP calculations. Since May 2022 saw the highest LD_{50} values for all populations, the REP for this month was selected for better clarification of the insecticide potency status. It was found that Spinosad exhibited a higher REP (58.56) compared to Indoxacarb (24.67) for the Bargarh population, indicating greater susceptibility of *L. orbonalis* to Spinosad relative to Indoxacarb, as shown in Figure 3. A similar trend was observed for the susceptible population when analyzing the REP of all three insecticides, as depicted in Figure 4.

Toxicological analysis utilizing REP ratios is common in fields such as environmental science, medicine, and agriculture. The REP ratio serves as a crucial parameter for assessing the potency of insecticides, allowing for the ranking of chemicals based on their relative toxicity compared to a standard (22). Further experiments involving additional field populations of *L. orbonalis* with varied susceptibility could help elucidate whether this variation is attributable to insecticide resistance or natural variation among populations with low resistance levels. This study provides valuable insights into the susceptibility of *L. orbonalis* to carbamates and newer insecticide classes, which is particularly relevant to the Indian subcontinent, where the pest has emerged as a significant threat to brinjal production.

Proactive measures such as rotating insecticides with different modes of action and periodically screening the

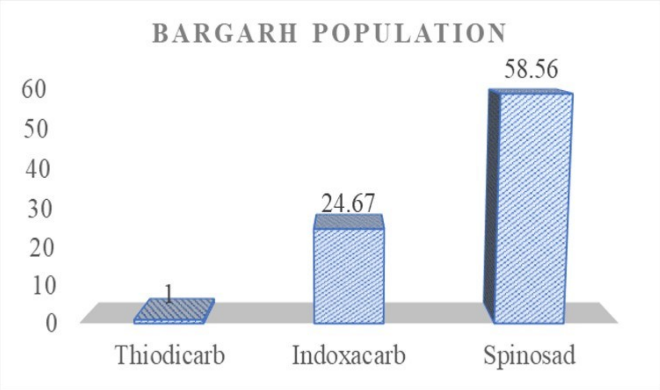

Fig. 3. Relative potency ratios tested for Bargarh population.

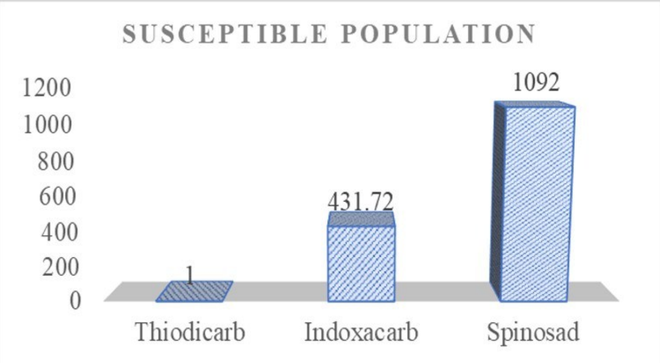

Fig. 4. Relative potency ratios tested for susceptible population.

susceptibility of *L. orbonalis* to these compounds should be considered. Additionally, the concept of migration must be taken into account, as it may influence pesticide susceptibility variations observed among populations collected at different times from the same location (22). Intense insecticide use, particularly in Bargarh, exerts strong selection pressure on *L. orbonalis*. Given the limited pesticide tolerance in hybrid brinjals, the status of pesticide resistance in *L. orbonalis* in Bargarh requires special attention. Alternative management strategies, such as regional crop rotation with non-host species, should be explored. Furthermore, ongoing monitoring of *L. orbonalis* susceptibility to insecticides across brinjal-growing areas in Odisha is recommended.

Conclusion

The field-evolved resistance of the Brinjal shoot and fruit borer (*L. orbonalis*) poses a significant threat to agricultural sustainability globally, including in India, particularly in Odisha. The spread of resistance to both older insecticides like carbamates and newer ones such as Indoxacarb and Spinosad jeopardizes pest control efforts. Therefore, it is crucial to assess the current resistance status by examining available control strategies, the pest's host preferences, migratory behavior, management practices, and the potential spread of the insect across various agroecological zones. As resistance continues to challenge traditional management methods, this study highlights the importance of exploring alternative solutions, including the use of biocontrol agents, development of resistant brinjal varieties, and the incorporation of synergistic insecticides with diverse modes of action. These measures can enhance insecticide efficacy and delay resistance onset. Moreover, the study recommends revising the application doses of chemicals like Indoxacarb, Spinosad, and Thiodicarb to better manage *L. orbonalis* infestations. The findings will help researchers understand resistance patterns and refine insect pest management strategies to tackle this ongoing issue effectively.

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Authors' contributions

BP carried out the bioassay and resistance monitoring studies and drafted the manuscript. BP and MKT participated in the methodology of the study. MKT, KS and PP performed the statistical analysis. Review writing and editing are done by BP, PP and SQ. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

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