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RESEARCH ARTICLE

Enhancement of thiodicarb efficacy using synergists and role of carboxylesterase in potentiation of synergistic activity in brinjal fruit and shoot borer (*Leucinodes orbonalis* Guenee)

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Abstract

The primary cause of low productivity in brinjal is the infestation by the fruit and shoot borer and field control failure is due to the evolution of insecticide resistance. In the current study, an investigation has been carried out in bimonthly intervals from Nov-2021 to Sept-2022 to assess the efficacy of some selected synergists with a carbamate, thiodicarb and the role of carboxylesterase enzyme in imparting insecticide resistance in Brinjal fruit and shoot borer from two locations of Odisha, Bhubaneswar and Bargarh. Out of all the synergists tested in the topical bioassay method, propargyl-oxy-phthalimide (PP) being a member of esterase hydrolase inhibitor group provided remarkable SR ratio (10.78) during May- 2022 in the Bhubaneswar population while that of Bargarh population indicated comparatively higher value (12.68) indicating efficacy of the synergist lowering the dose of thiodicarb to 1.464 µg/µl compared to 18.564 µg/µl when thiodicarb alone is used. This can be related to enhanced levels of detoxifying enzyme, Carboxylesterase in Bhubaneswar during May 2022 (4.52-fold) and Bargarh (5.36-fold) population compared to laboratory-reared susceptible population revealing the role of carboxylesterase enzyme in detoxification mechanism behind the efficacy of the synergists. This study highlights the importance of synergists like PP and Triphenyl phosphate (TPP) and suggests their inclusion in the existing pest management strategies of *L. orbonalis* in a wider agricultural area they reduce doses of insecticides and hence reduce impact on the environment which can lead to sustainable agriculture.

Keywords

carboxylesterase; enzyme assay; insecticide resistance; synergist; thiodicarb

Introduction

Brinjal (*Solanum melongena* L). is widely grown in India and many South Asian countries as well (1, 2). It is native to the Indian sub-continent (3) and its notorious pest, brinjal fruit and shoot borer, *Leucinodes orbonalis* prevalent in tropical and subtropical agricultural systems, where its rapid reproductive cycle allows for swift population increases, leading to substantial yield losses (4). Effective management of *L. orbonalis* is crucial for ensuring farmer's optimal production and economic viability.

Alarming resistance levels have been documented in earlier research on *L. orbonalis*, with studies indicating significant variation in resistance ratios among populations from different regions of the world (5-7). Additionally, the limited studies on the role of carboxylesterase in thiodicarb resistance in tropical pests report increased activities of carboxylesterases and glutathione S-transferases in resistant populations. These findings suggest metabolic pathways play a crucial role in a pest's

ability to withstand chemical treatments (8-9).

The global agricultural landscape is increasingly challenged by pest resistance, which undermines the effectiveness of chemical insecticides (10). Among the pests of significant concern is the brinjal fruit and shoot borer, *Leucinodes orbonalis*, a major threat to brinjal production (11). This pest not only causes substantial losses but also adversely affects the quality and yield of this important vegetable crop (12). The development of resistance in pest populations to commonly used insecticides has necessitated the exploration of alternative strategies to enhance insecticide efficacy and manage resistance (13).

In agricultural pest management, the reliance on chemical insecticides has been a predominant strategy. The indiscriminate and frequent use of pesticides has resulted in the development of resistance in pest populations, including *L. orbonalis* (14,15). Insecticide resistance typically arises from various physiological adaptations in pest populations, including increased metabolism, target site insensitivity and behavioural resistance often involves the enhanced expression of detoxification enzymes which facilitates the breakdown of insecticides before they reach their target sites within the insect (16-18). To counter these adaptations, researchers have focussed on using synergists-compounds that can enhance the activity of insecticides by inhibiting the resistance mechanisms. Synergists can effectively modify the pest's biochemical pathways, improving insecticide performance and potentially reversing resistance (19-21).

One of the most studied synergists, Piperonyl butoxide (PBO), functions primarily by inhibiting cytochrome P450 enzymes involved in detoxification. PBO has markedly increased the toxicity of various insecticides to resistant strains of insects by blocking the metabolic pathways that confer resistance. This dual approach- using PBO to enhance insecticidal potency while simultaneously targeting resistant pests- makes it an essential component in integrated pest management strategies (22). PP and TPP act as inhibitors of carboxylesterase enzyme. Hene more is the specific activity of TPP and PP, more likely is the synergistic ratio. Synergists lower the insecticide dose, hence reduce cost of insecticide and thus reduce the threat of environment pollution. Similarly, Diethyl maleate (DEM) has demonstrated synergistic effects by inhibiting glutathione S-transferase and other detoxifying enzymes. The objective of the study is to see the compatibility of synergists with thiodicarb and whether the carboxylesterase inhibiting synergists like N-Propargyl-oxy-phthalimide (PP) and Triphenyl phosphate (TPP) are having better synergistic ratio compared to other synergists used establishing a correlation with the enhanced production of carboxylesterase enzyme.

Materials and Methods

The current study was conducted in the toxicology laboratory of Department of Entomology, College of Agriculture, OUAT, Bhubaneswar from Nov 2021 to Sept 2022. The materials used for the study and the methods adopted are given as follows:

Test insects

Brinjal fruit and shoot borer, *L. orbonalis* (Lepidoptera: Crambidae) populations were collected from farmers' fields of brinjal growing regions of Khurda (Bhubaneswar) and Bargarh districts of Odisha as these two places witness heavy infestation by *L. orbonalis* and more frequent pesticide use to control this insect. The 3rd instar larvae of *L. orbonalis* were collected from the infested fruits of the selected places. All field populations collected from Bhubaneswar and Bargarh were reared following the standard methods (23) separately under laboratory conditions at 27±2ºC, 60-70% relative humidity (RH) and a photoperiod of 14:10h (L:D) on natural diet of brinjal and potato and in ventilated containers to minimize stress and desiccation covered with plastic net and mouth sealed with rubber band the F_1 individuals were used for bioassay study. The 3rd instar larvae of the F_1 generation were used for bioassay study (Fig. 1). Likewise, the susceptible iso-female colony was maintained up to the 5th generation in the laboratory using the same procedure.

Freshly cut and untreated brinjal and potato pieces were given as food sources to the growing larvae. 10% honey solution was provided as a source of food for adults. Food was replaced everyday with fresh food.

Preparation of insecticide solution and serial dilution

Technical grade thiodicarb (98% purity) was used for the dose-mortality bioassays based on their usage history on brinjal by the farmers. Test concentrations were prepared from the commercial formulation Sock solutions were further diluted to eight dilutions to dispense the required dose to keep the mortality in 20-90% range using acetone as the solvent. The stock solutions were prepared using the standard formula (24).

Bioassay techniques

Topical method of bioassay (24) was followed for insecticide resistance bioassay on early 3rd instar larvae of *L. orbonalis* as this method ensures exposure of the larvae to the treated insecticides compared to other methods of exposure as the insecticide is directly as the insecticides are applied to the dorsal thoracic segments of third instar larvae topically using a Hamilton micro applicator with a repeating dispenser (PB600-1, Hamilton company) of 50 µL capacity which is designed to dispense 1µL at a time. 30 larvae were treated per replication and three replications per dose were carried out. Treated larvae were transferred to a plastic container of similar size with net cloth sealed on the mouth and provided with fresh untreated cut

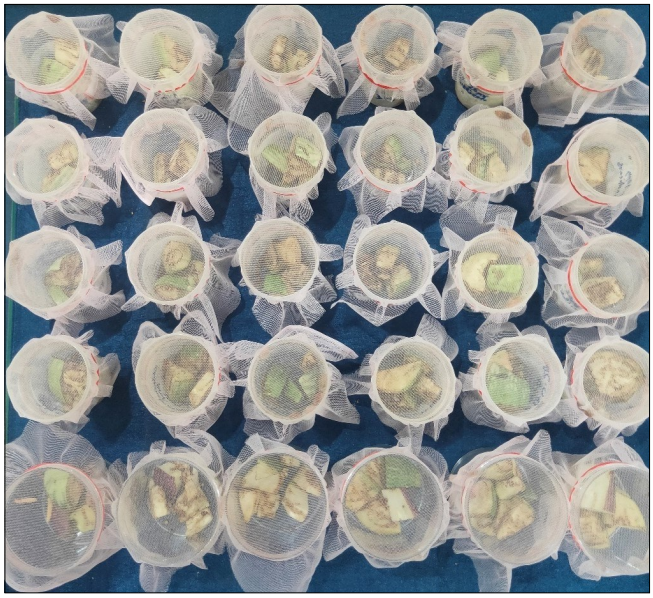

Fig. 1. Thiodicarb and synergist treated larvae.

brinjal and potato pieces (Fig. 1). Mortality count was recorded in 24 hrs, 48 hrs and 72 hrs. For control, acetone was used. Mortality was recorded at 24-, 48- and 72-hours post-exposure. Larvae were considered dead if they did not exhibit movement when gently probed with a fine brush. The experiment was replicated three times for each concentration to ensure reliability.

Synergists

Details of synergists used for testing synergistic activity procured from Sigma-Aldrich, St. Louis, Missouri, USA is given in Table 1. The solutions were prepared by weighing the required chemicals and dissolving in acetone as solvent. The non-toxic dose was determined as per standard procedure. Dose of synergists used: 10, 15, 10, 15, 10 and 10 µg/larvae for PBO, DEM, TPP, Hydroquinone, Resorcinol and POP, respectively

Synergists were applied topically 15 min prior to insecticide application. Synergistic ratio was determined using the standard formula (25).

Synergistic Ratio=LD_{50} of insecticide alone/LD_{50} of (insecticide+ synergist)

Preparation of midgut homogenate

The third instar larvae were kept starved and used for the preparation of midgut homogenate. Midguts were dissected and homogenized with homogenization buffer (0.1 M sodium phosphate buffer pH 7.8 containing 1 mM each of DDT, PTU and EDTA). The mixture was centrifuged at 10000 rpm for 20 min and the clear supernatant was used as enzyme source for estimating the carboxylesterase titres. The total protein content was assessed by using bovine serum albumin (BSA) as the standard following the standard procedure (26).

Estimation of Carboxylesterase activity

The estimation of carboxylesterase enzyme activity was conducted using α-Naphthyl Acetate as a substrate, following the standard method (27). The assay utilized a 20 mM phosphate buffer at pH 8.0, prepared by mixing solutions of dibasic sodium phosphate and monobasic potassium phosphate, with pH adjustment using a Labman auto digital pH meter. The substrate solution consisted of a 30 mM stock of α-Naphthyl Acetate, which was diluted to the working concentration with the phosphate buffer just before the assay. A coupling reagent was prepared by mixing Fast Blue B Salt and Sodium Lauryl Sulphate in a 2:5 ratio, stored at room temperature. Standard solutions of α-Naphthol were prepared and a standard graph was plotted by measuring the colour development at 605 nm after 30 min of incubation by systronics UV-108 double beam spectrophotometer.

For the enzyme assay 3rd instar larvae weighing 30-40 mg were homogenized in phosphate buffer (pH 8.0) at a ratio of 1mL buffer per 5mg of insect weight. The homogenate was centrifuged at 5000 rpm for 20 min and the supernatant was collected and stored at -4°C. The reaction mixture, consisting of 2.3 mL of phosphate buffer, 0.5 mL of α-Naphthyl Acetate and 0.2 mL of enzyme extract, was incubated at room temperature for 30 min. The reaction was terminated by adding 0.5 mL of the coupling reagent and the absorbance was recorded at 605 nm after another 30 min. Control without the enzyme extract was maintained and its absorbance was deducted from the sample absorbance to account for any non-enzymatic reactions and the enzyme activity was computed from α-naphthol standard curve.

Protein estimation

Materials required

Sodium carbonate solution (2%) in sodium hydroxide 0.1 N (Reagent A), $CuSO_4.5H_2O$ (0.5%) in potassium sodium tartrate (1%) (Reagent B), alkaline copper solution: 1 mL of reagent B and 50 mL of reagent A were mixed prior to use (Reagent C), Folin-ciocalteau reagent (Reagent D), protein solution (stock standard): Weighing accurately 50 mg of Bovine serum Albumin (fraction) and dissolving in distilled water and volume is made up to 50 mL in a standard flask, working standard: 10 mL of the stock solution diluted to 50 mL with distilled water.

Procedure

Working standards were pipetted out into a series of test-tubes (0.2, 0.4, 0.6, 0.8 and 1.00 mL). 0.1 mL and 0.2 mL of the sample extract were pipetted out into other test-tube. In all the test-tubes the volume was made up to 1 mL. After that reagent C (5 mL) of was added in each test-tube including the test-tube containing blank. The solution allowed to mix well and kept undisturbed for 5 min., 0.5 mL of reagent D was added, incubated at room temp. in the dark for 30 min. Blue colour development was observed and measured at 660 nm wavelength by systronics UV-108 double beam spectrophotometer against the blank (1 mL of water). The sample mixture contained (enzyme extract 1 mL, Folin-ciocalteu reagent 0.5 mL, analytical reagent 5 mL). 3-5 replication of each was taken. A standard graph was drawn (Fig. 2) and the amount of protein in the sample was calculated and expressed in µg/ mg fresh weight. This protein quantity was used to estimate the carboxylesterase quantity per mg of protein.

Statistical analysis

Corrected mortality was computed using the Abbot formula (28). Probit analysis was conducted using Polo Plus software, version 2.0, developed by LeOra Software Inc., Berkeley, CA (29) to calculate values of LD_{50} value, slope, fiducial limit and standard

Table 1. Details of synergists used in the study

Sl. No.	Name	Group	Mode of action	Chemical formula
1	Piperonyl butoxide (PBO)	Methylenedioxy phenyl	MFO inhibitor	$C_{19}H_{30}O_5$
2	Diethyl maleate (DEM)	Maleate ester	Glutathion inhibitor	$C_8H_{12}O_4$
3	Triphenyl phosphate (TPP)	Aromatic organophosphate	Acetylcholinesterase (AChE) inhibitor	$C_{18}H_{15}O_4P$
4	Hydroquinone	Benzenediol	Antioxidant	$C_6H4(OH)_2$
5	Resorcinol	Benzenediol	Antioxidant	$C_6H_6O_2$
6	N-propargyl-oxy-phthalimide (PP)	Dicarboximide	Esterase hydrolase inhibitor	$C_{11}H_7NO_3$

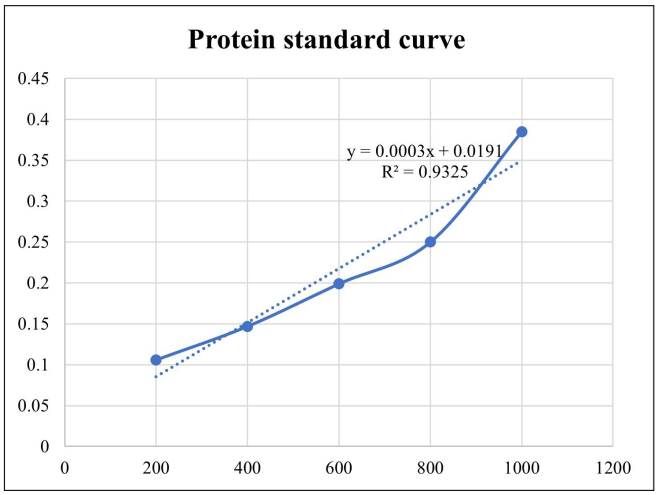

Fig. 2. Protein standard curve.

error of the bioassay conducted. Mann-Whiteney U-test was conducted to find out significant difference in mean value in enzyme analysis (30).

Results and Discussion

The efficacy and toxicity of thiodicarb, a carbamate insecticide was assessed in the presence of various synergists on the third instar larvae of *L. orbonalis* collected from two distinct geographic locations: Bhubaneswar and Bargarh in Odisha over several months from Nov-2021 to Sept-2022. The results are summarized in Table. 2 and Table 3. focussing on key parameters such as lethal dose (LD_{50}), synergistic ratio (SR) and slope in dose-response analysis of thidiocarb alone and in combination with the studied synergists. The result revealed significant insights into the interactions between thiodicarb and various synergists, offering implications for pest management strategies tailored to local conditions.

Table 2. Effect of studied synergists on efficacy and toxicity of thiodicarb on 3rd instar larvae of *L. orbonalis* collected from Bhubaneswar during different months of observation from Nov-2021 to Sept-2022

Month of observation	Synergist	LD_{50}[b]	SR[c]	Slope ±SE[d]	FL[e]	
					lower	upper
Nov-2021	Nil (12.082) [a]	-	-	0.604±0.01*	11.997	13.407
	PBO	3.730	3.24	0.564±0.02	4.008	6.102
	DEM	5.726	2.11	0.665±0.22*	1.342	6.119
	TPP	2.648	4.57	1.343±0.01*	1.344	3.457
	Hydroquinone	11.845	1.02	0.564±0.11	3.247	12.336
	Resorcinol	10.416	1.16	1.342±0.22*	3.546	11.102
	PP	1.668	7.24	0.328±0.03	1.045	2.443
Jan-2022	Nil (13.109) [a]	-	-	2.118±0.06*	12.767	13.421
	PBO	4.122	3.18	1.332±0.02*	2.497	7.234
	DEM	5.878	2.23	0.458±0.34	2.768	6.225
	TPP	3.174	4.13	0.453±0.11	2.545	5.005
	Hydroquinone	9.856	1.33	0.444±0.32	4.654	10.342
	Resorcinol	9.232	1.42	1.231±0.25*	4.342	10.221
	PP	1.574	8.33	1.454±0.06*	0.434	3.237
Mar-2022	Nil (14.209) [a]	-	-	0.841±0.04*	13.537	14.847
	PBO	4.332	3.28	0.554±0.07	3.665	6.568
	DEM	5.550	2.56	0.563±0.11	1.094	6.218
	TPP	2.518	5.64	0.562±0.22	2.234	3.136
	Hydroquinone	8.993	1.58	0.776±0.32*	3.127	9.995
	Resorcinol	8.717	1.63	1.554±0.11*	3.106	9.004
	PP	1.468	9.67	1.561±0.03*	1.006	2.106
May-2022	Nil (14.915) [a]	-	-	0.884±0.02*	14.791	16.440
	PBO	4.492	3.32	0.569±0.06	2.192	6.783
	DEM	5.608	2.66	0.665±0.02*	2.108	6.837
	TPP	2.607	5.72	1.443±0.12*	2.347	3.768
	Hydroquinone	8.984	1.66	2.562±0.14*	3.124	9.178
	Resorcinol	8.722	1.71	0.654±0.11*	3.254	9.452
	PP	1.384	10.78	1.238±0.14*	1.006	2.145
Jul-2022	Nil (14.131) [a]	-	-	0.845±0.11*	13.537	14.847
	PBO	4.362	3.24	1.453±0.33*	3.234	6.874
	DEM	5.456	2.59	0.665±0.09*	2.118	6.679
	TPP	3.013	4.69	0.563±0.11	2.678	3.674
	Hydroquinone	9.001	1.57	0.554±0.11	3.245	10.318
	Resorcinol	8.564	1.65	0.548±0.15	3.237	10.201
	PP	1.736	8.14	0.668±0.12	1.126	2.116
Sept-2022	Nil (13.993) [a]	-	-	0.843±0.01	13.095	14.953
	PBO	4.254	3.29	0.675±0.11	2.236	7.226
	DEM	5.424	2.58	1.778±0.22*	2.006	8.262
	TPP	3.036	4.61	0.563±0.12	2.786	4.004
	Hydroquinone	9.266	1.51	2.334±0.11*	3.231	10.107
	Resorcinol	8.638	1.62	0.567±0.14	3.108	10.266
	PP	1.850	7.56	0.558±0.12	1.054	2.234

[a]=LD_{50} (Lethal dose) of Thiodicarb alone expressed as µg of active ingredient per µl, [b]LD_{50} = Lethal dose of Thiodicarb and synergists expressed as µg of active ingredient per µl, [c]SR= Resistance ratio, LD_{50} of Thiodicarb alone over the LD_{50} of Thiodicarb and synergist combinedly, [d]SE = Standard error, *= significant at P⊠0.05, [e]FL(95%)= 95%Fiducial limit of LD_{50} value

Table 3. Effect of Studied synergists on efficacy and toxicity of thiodicarb on 3rd instar larvae of *L. orbonalis* collected from Bargarh during different months of observation from Nov-2021 to Sept-2022

Month of observation	Synergist	LD_{50}[b]	SR[c]	Slope ±SE[d]	FL[e]	
					lower	upper
Nov-2021	Nil (15.576) [a]	-	-	0.642±0.21*	15.101	16.953
	PBO	4.663	3.34	1.224±0.22*	2.876	7.334
	DEM	7.016	2.22	0.662±0.31*	2.134	8.654
	TPP	3.500	4.45	0.642±0.37*	2.676	4.776
	Hydroquinone	10.315	1.51	1.228±0.11*	5.444	12.343
	Resorcinol	11.854	1.34	1.126±0.23*	5.564	12.115
	PP	1.863	8.36	2.108±0.12*	1.667	3.554
Jan-2022	Nil (16.219) [a]	-	-	0.272±0.11	16.082	17.659
	PBO	4.930	3.29	0.565±0.11	3.343	8.233
	DEM	7.439	2.18	0.472±0.14	2.765	8.665
	TPP	2.998	5.41	0.442±0.29	1.655	5.565
	Hydroquinone	11.034	1.47	0.612±0.11*	5.667	12.429
	Resorcinol	12.872	1.26	0.565±0.19	6.344	14.334
	PP	1.714	9.46	0.446±0.12	1.665	3.255
Mar-2022	Nil (16.945) [a]	-	-	1.378±0.21*	16.142	17.787
	PBO	4.939	3.37	0.565±0.34	2.454	8.298
	DEM	7.206	2.31	1.443±0.28*	2.554	8.334
	TPP	3.021	5.51	1.334±0.34*	2.654	5.654
	Hydroquinone	10.534	1.58	2.124±0.29*	5.443	11.445
	Resorcinol	11.402	1.46	2.128±0.11*	5.876	12.332
	PP	1.576	10.56	2.342±0.12*	0.786	3.554
May-2022	Nil (18.564) [a]	-	-	0.555±0.11	17.917	19.590
	PBO	5.380	3.45	1.234±0.33*	3.448	8.453
	DEM	7.671	2.42	0.442±0.23	3.786	8.443
	TPP	3.162	5.87	1.238±0.11*	2.554	5.545
	Hydroquinone	9.980	1.86	0.774±0.16*	5.454	10.453
	Resorcinol	10.608	1.75	0.662±0.12*	5.664	12.773
	PP	1.464	12.68	1.108±0.16*	1.008	3.528
Jul-2022	Nil (18.231) [a]	-	-	0.569±0.33	16.273	19.829
	PBO	5.346	3.41	0.551±0.36	2.443	8.342
	DEM	7.792	2.34	1.104±0.27*	3.766	8.435
	TPP	3.226	5.65	0.552±0.12	2.665	5.665
	Hydroquinone	11.184	1.63	2.112±0.11*	5.432	12.454
	Resorcinol	11.762	1.55	1.102±0.11*	6.568	12.865
	PP	1.934	9.43	0.118±0.15	1.776	3.223
Sept-2022	Nil (17.982) [a]	-	-	0.413±0.31	15.413	18.884
	PBO	5.335	3.37	1.008±0.22*	5.565	4.554
	DEM	7.886	2.28	1.225±0.38*	3.665	8.234
	TPP	3.264	5.51	1.346±0.12*	2.768	6.554
	Hydroquinone	11.526	1.56	0.444±0.36	6.332	12.443
	Resorcinol	12.753	1.41	1.118±0.24*	6.564	14.342
	PP	2.156	8.39	2.102±0.18*	1.887	3.345

[a]=LD_{50} (Lethal dose) of Thiodicarb alone expressed as µg of active ingredient per µl, [b]LD_{50} = Lethal dose of Thiodicarb and synergists expressed as µg of active ingredient per µl, [c]SR= Resistance ratio, LD_{50} of Thiodicarb alone over the LD_{50} of Thiodicarb and synergist combinedly, [d]SE = Standard error, *= significant at P<0.05, [e]FL(95%)= 95%Fiducial limit of LD_{50} value

The LD_{50} values provide a quantitative measure of the toxicity of thiodicarb in each location. In Bhubaneswar, the LD_{50} of thiodicarb alone fluctuated between 12.082 µg/larvae in Nov-2021 and 14.915 µg/larvae in May-2022. This variation indicates a potential increase in larval susceptibility during the observed period, possibly linked to environmental factors such as humidity which can influence larval physiology. Conversely, the baseline toxicity of thiodicarb in Bargarh consistently remained higher, ranging from 15.576 µg/larvae in Nov-2021 to 18.564 µg/larvae in May-2022. This may be due to the consistent and indiscriminate use of pesticides in Bargarh and this persistent elevation in LD_{50} values may suggest underlying resistance mechanisms in the local *L. orbonalis* population or differences in environmental conditions that affect the insect's vulnerability to thiodicarb. This data corroborates the findings of earlier workers (31-34).

Effects of synergists

The application of synergists demonstrated varying degrees of efficacy across both locations, significantly enhancing the effectiveness of thiodicarb (31). The introduction of synergists resulted in significantly lower LD_{50} values, with the most pronounced effects observed with the synergist Triphenyl phosphate (TPP) and N-Propargyl-oxy-phthalimide (PP) as in Nov 2021, in Bhubaneswar population, the LD_{50} with TPP was 2.648 µg/larvae, reflecting a 4.57-fold decrease in the required dose for lethality compared to thiodicarb alone. Similar result was obtained for Bargarh population as in Nov 2021 the addition of TPP decreased the LD_{50} to 3.500 µg/larvae, resulting in a synergistic ratio (SR) of 4.45.

PP proved to be particularly effective in Bhubaneswar, where the LD_{50} was significantly lowered in Nov 2021 to 1.668 µg/larvae (SR= 7.24). This substantial reduction indicates a strong synergistic effect, likely due to PP's ability to inhibit metabolic detoxification pathways (35) Bargarh recorded a higher LD_{50} of 1.863 µg/larvae (SR=8.36), but the compound still maintained a

notable synergistic effect. The comparative results suggests that while the effectiveness of PP remains robust, local resistance factors may mitigate its impact in Bargarh.

TPP showed highest efficacy during May-2022 with LD_{50} of 2.607 µg/larvae in Bhubaneswar and 3.162 µg/larvae in Bargarh with both sites showing SR exceeding 4.00. The significant reductions in LD_{50} values reflect TPP's capability to enhance the insecticidal action of thiodicarb, potentially by disrupting neuronal signaling Acetylcholinesterase (AChE) and carboxylesterase inhibition pathways (31, 36)

Hydroquinone and Resorcinol both synergists exhibited consistent efficacy across locations, with similar LD_{50} reductions and SR values. This suggests that they function effectively in both environments, enhancing thiodicarb's lethality through mechanisms that may involve the inhibition of detoxification enzymes (37).

The synergistic ratios varied among the synergists, with PP showing the highest SR (12.68) in Bargarh, during May-2022, reducing the LD_{50} to 1.464 µg/larvae. In Bhubaneswar, the LD_{50} was lower at 1.384 µg/larvae. This suggests that PP is highly effective amongst all the synergists tested in enhancing thiodicarb's activity, particularly in Bhubaneswar, where environmental conditions may favour its action.

The slopes derived from the dose-response curves offer insights into the consistency of the larvae's response to thiodicarb and its synergists. Statistical robustness is affirmed by narrow confidence intervals, making these findings valuable for guiding pest management strategies. The slope values, indicative of the steepness of the dose-response curve, ranged from approximately 0.328 to 2.562. Higher slope values correspond to a more sensitive response of the larvae to the insecticide-synergist combination (38). The slope for PP, particularly in Bhubaneswar, was noted to be 0.328, suggesting a less predictable response, while Hydroquinone exhibited slope of 2.562, indicating a highly sensitive response.

Statistical significance was assessed through the fiducial limits (FL) provided for each LD_{50} estimate. The confidence intervals demonstrate variability in the effectiveness of the synergists, with narrower limits suggesting more reliable estimates. For instance, the fiducial limits for PBO in Nov-2021 ranged from 4.008 to 6.102 µg/larva, reflecting a robust efficacy range. A comparative analysis of the two locations revealed that the synergistic effects varied, possibly due to environmental factors, larval population dynamics, or differences in local pest resistance.

Seasonal Variation in synergistic efficacy

The analysis also highlighted seasonal variations in the efficacy of thiodicarb and its synergists. In Bhubaneswar, the LD_{50} values showed a downward trend from Nov-2021 to May-2022, suggesting that larvae became increasingly susceptible over this period. This could be due to several factors, including changes in the larvae's developmental stage, population dynamics or seasonal shifts in environmental stressors (39). In Bargarh, however, the LD_{50} values exhibited a consistent increase from November to September, indicating that the larvae may be developing resistance to thiodicarb or that environmental temperature is imposing greater stress on the population, reducing their overall susceptibility as temperature imposes some effect on the efficacy of the synergists (32).

The findings from this study illustrate the complex interactions between thiodicarb and various synergists, with significant implications for pest management strategies. The consistent efficacy of synergists like PBO, DEM and TPP across both Bhubaneswar and Bargarh suggests that integrating these compounds could optimize thiodicarb's effectiveness. Thiodicarb is a carbamate and inhibits carboxylesterase and PP and TPP are also known as carboxylesterase inhibitors. The biochemical mechanism involves increased metabolism of carboxylesterase enzyme by upregulating enzymes that degrade it or through reactive metabolites that modify enzyme's structure (32). However, the higher baseline toxicity of thiodicarb in Bargarh raises concerns regarding potential resistance mechanisms. Seasonal fluctuations and local ecological factors must be further explored to develop targeted pest management approaches that effectively mitigate resistance and enhance insecticidal efficacy.

Previous research has indicated a correlation between the overproduction of detoxification enzymes and increased resistance in *L. orbonalis* (40). For instance, studies have demonstrated elevated levels of Carboxylesterase in resistance strains, suggesting that the enzyme plays a critical role in the pest's ability to survive chemical exposure (41). Furthermore, the investigation of synergistic interactions among insecticides and chemical inhibitors has revealed potential pathways to overcome resistance by reducing the effective dosage of insecticides required for control (42).

The integration of synergists into insecticide formulations represents a critical advancement in the fight against insecticide resistance. By effectively reversing resistance mechanisms, synergists not only enhance the efficacy of existing chemical controls but also prolong the useful life of the insecticides (31, 36). The strategic use of synergists can lead to reduced application rates, lower overall pesticide usage and diminished environmental impacts, aligning with the principles of sustainable agriculture.

Despite the promising role of synergists, there is a lack of comprehensive studies focussing on their effectiveness in overcoming insecticide resistance specifically in *Leucinodes orbonalis*. This gap in research highlights the need for targeted investigations to access the potential of various synergists in improving the performance of insecticides against resistant strains of the brinjal fruit and shoot borer (33-34). By systematically evaluating the synergistic effects of PBO, DEM, TPP, Resorcinol, Hydroquinone and N- Propargyl-oxy-phthalimide in enhancing insecticide efficacy against resistant populations of brinjal fruit and shoot borer this study aims to provide valuable insight s into their mechanisms of action and their synergistic effects when combined with commonly used carbamate insecticide thiodicarb. By elucidating the mechanisms through which these compounds operate, this research seeks to provide critical insights into developing more effective pest management strategies.

Carboxylesterase enzyme assay

The carboxylesterase titres in third instar larvae of *L. orbonalis* revealed significant differences between populations from Bhubaneswar and Bargarh over the observation period as shown in Table 4. The specific activity of carboxylesterase in Bhubaneswar population increased from 6.98±0.53 µmoles/mg

Table 4. Quantification of carboxylesterase activity in 3rd instar larvae of *L. orbonalis* collected from Bhubaneswar and Bargarh during different observation period

Population	Month of observation	Specific activity (µmoles/mg of protein/min) ±SE	Fold variation as compared to susceptible population
Bhubaneswar	Nov 2021	6.98±0.53*	3.79
	Jan 2022	7.21±0.28*	3.91
	May 2022	8.32±0.25*	4.52
Bargarh	Nov 2021	8.12±0.24*	4.41
	Jan 2022	9.13±0.06*	4.96
	May 2022	9.86±0.18*	5.36
Susceptible population		1.84±0.11	1.00

*indicates significant differences by Mann-Whitney U- test test ($p < 0.05$) as compared to the laboratory-reared susceptible population

of protein/min in Nov 2021 to 8.32±0.25 µmoles/mg of protein/ min in May 2022. The rise corresponds to a fold variation relative to the susceptible population which was 3.79 in November and reached 4.52 by March.

In comparison, the Bargarh population consistently exhibited higher enzymatic activity, starting at 8.12±0.24 µmoles/mg of protein/min in Nov 2021 and culminating at 9.86±0.18 µmoles/mg of protein/min in May 2022. The fold variation for this population increased from 4.41 to 5.36, indicating a robust enhancement in carboxylesterase activity compared to the susceptible baseline of 1.84±0.11 µmoles/mg of protein/min.

These findings suggest a clear correlation between the observed carboxylesterase activity and the synergists lowering the doses of thiodicarb indicating a detoxification mechanism by expression of carboxylesterase enzyme. The increased activity in both populations points to an adaptive response to the selective pressure imposed by insecticide applications (43). The elevation in enzyme levels, particularly in the Bargarh population, may reflect a more significant exposure to insecticides, leading to enhanced metabolic detoxification capabilities.

The progressive increase in carboxylesterase activity over time highlights the dynamic nature of resistance development (42). The Bargarh population's higher baseline activity and greater fold increase suggest that this population may possess genetic or physiological traits that confer a more substantial capacity for detoxification. This aspect is crucial, as it emphasizes the necessity for region-specific pest management strategies that account for the differing resistance profiles among populations (44).

Moreover, the findings underscore the importance of continuous monitoring of enzymatic activity as a predictive measure of resistance evolution. As *L. orbonalis* adapts to insecticidal pressure, understanding the biochemical pathways involved in resistance, such as carboxylesterase activity, will be essential for developing sustainable management practices and as synergists enhance the efficacy of insecticides by stimulating the production of carboxylesterase enzymes (45-46). These insights can guide future research aimed at exploring the complexities of resistance mechanisms, ultimately contributing to more effective and environmentally responsible pest control strategies. Synergist itself is not toxic but when used with insecticides enhances the effectiveness of insecticides. As synergists lower the doses of insecticides used, incorporating synergists into pest management programs could reduce overall insecticide use, aligning with sustainable agriculture goals

Conclusion

The escalating issue of insecticide resistance necessitates innovative and effective management strategies. The role of synergists in augmenting insecticide efficacy offers a promising avenue to overcome resistance challenges, ensuring the sustainable production of brinjal and other crops. The interaction between insecticides and synergists is complex and often depends on the specific biochemical pathways targeted by each compound. Understanding these interactions and the role of carboxylesterase enzyme in insecticide detoxification is crucial for developing effective pest management strategies. The results indicate that incorporating synergists such as TPP and PP can significantly enhance the effectiveness of thiodicarb against *L. orbonalis*, offering a promising approach for the improvement of pest control strategies in agricultural settings. Use of synergists should be in non-toxic doses and it should be applied 10-15 min prior to application of insecticides. Further research into the mechanisms underlying these synergistic interactions and the long-term impacts on pest populations and resistance management in wider agricultural regions is warranted.

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Authors' contributions

BP carried out the synergistic bioassay and enzymatic studies and drafted the manuscript. BP and MKT participated in the methodology of the study. MKT performed the statistical analysis. Review writing and editing are done by BP and MKT. Both the authors read and approved the final manuscript.

Compliance with ethical standards

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

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