



RESEARCH ARTICLE

Detoxification enzyme inhibitor mediated synergism of insecticide toxicity in *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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Abstract

Insecticide efficacy against the maize fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), is closely linked to metabolic detoxification processes that can be influenced by the use of synergists. This study assessed the effect of five selected synergists on the toxicity of five commonly used insecticides against third-instar larvae collected from 2 locations in Odisha, namely Bhubaneswar and Bhawanipatna, under laboratory conditions. In the Bhubaneswar population (BBPL), lambda-cyhalothrin exhibited the highest synergism with piperonyl butoxide (PBO) and N-(propargyloxy) phthalimide with synergistic ratios (SR) of 6.50 and 4.41 respectively. Acephate recorded the highest synergism with triphenyl phosphate (TPP) (SR = 5.85). Diethyl maleate (DEM) showed greater synergism with emamectin benzoate (SR = 3.91) and spinetoram responded notably to PBO (SR = 4.20). In contrast, the Bhawanipatna population (BPPL) showed a stronger and more consistent response to DEM with all five insecticides, with SR values of 6.71 for lambda-cyhalothrin, 6.20 for acephate, 5.10 for spinetoram and 4.76 for emamectin benzoate. Chlorantraniliprole exhibited comparatively lower synergistic responses with most synergists, although DEM produced a substantially higher synergistic ratio in BPPL (SR = 4.39). The least effective synergist was 5-nitouracil (1.25–1.72) in both populations, indicating a minimal role of uridine diphosphate glycosyltransferases (UGTs) mediated detoxification pathways. The difference in synergistic response between populations suggests involvement of different detoxification enzymes in both populations. Cytochrome P450 monooxygenases and carboxyl esterases appear more influential in BBPL while glutathione S-transferases (GST) are more significant in BPPL. These findings highlight the potential of synergists to improve insecticide efficacy and strengthen insecticide resistance management (IRM) strategies.

Keywords: cytochrome P450; diethyl maleate; piperonyl butoxide; population variability; resistance management strategies; synergistic ratio

Introduction

Cultivated extensively across India and worldwide, *Zea mays* L. (maize) serves as a cornerstone cereal crop that provides essential resources for human food, animal feed and industrial production. The invasive fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), consistently jeopardises maize yields due to its exceptional reproductive potential, polyphagous nature and high migratory capacity. Originating from the Americas, this pest has spread to pivotal maize production zones in Asia, triggering substantial economic setbacks and posing serious threats to global food security. Environmental factors such as temperature, rainfall and shifts in vegetation patterns govern its seasonal occurrence and spread, leading to marked variations in infestation intensity over time (1). In mainland Southeast Asia, a sharp drop in maize output has been linked directly to widespread FAW

outbreaks, underscoring the pest's enduring destructiveness long after its first arrival (2, 3). Moreover, prolonged contact between FAW populations and insecticides has fostered resistance development which intensifies the challenges of effective control. Consequently, a more profound understanding of the pest's ecological traits and reactions to interventions emerges as essential for crafting robust sustainable management approaches.

Chemical insecticides have been widely favoured in FAW management owing to their rapid action and practical benefits. Resistance has developed in various populations through repeated exposure to multiple insecticide groups. Significant variations in susceptibility to different insecticides across regions have been reported reflecting differences in selection pressures and usage patterns. Newer options such as emamectin benzoate, chlorantraniliprole and spinetoram have proven effective against

larval stages while lambda-cyhalothrin and other conventional ones have shown comparatively lower efficiency (4, 5). Field studies have indicated substantial reductions in larval numbers and crop damage following applications of emamectin benzoate and chlorantraniliprole, though performance can vary under diverse agro-climatic conditions (6, 7). Increasing resistance to spinetoram and chlorantraniliprole has been noted in some populations hinting at declining susceptibility and potential cross-resistance between groups (8). Such findings underscore the importance of ongoing monitoring of insecticide efficacy across FAW populations.

Insecticide resistance in many cases has been closely linked to enhanced metabolic detoxification processes. The diminished efficacy of insecticides across various chemical classes can be mainly attributed to their degradation via phase I functionalisation and phase II conjugation pathways mediated by enzymes such as cytochrome P450 monooxygenases, glutathione S-transferases (GST), esterases and amidases (8). These metabolic pathways effectively shield the pest from the lethal effects of consecutive insecticide exposures. Field conditions influenced by environmental factors like temperature have been found to affect these detoxification routes thereby altering resistance levels (9). Detoxification enzymes especially cytochrome P450 monooxygenases are considered pivotal in metabolising insecticides through oxidative reactions prior to their arrival at target sites (10). Esterases and amidases contribute via hydrolytic breakdown of insecticide structures while GST and uridine diphosphate glycosyltransferases (UGTs) facilitate conjugation essential for detoxification. These enzymes often function in combination to confer resistance (11). Efforts to counter these mechanisms have involved testing enzyme inhibitors including piperonyl butoxide (PBO), N-(propargyloxy) phthalimide, triphenyl phosphate (TPP), diethyl maleate (DEM) and 5-nitouracil aimed at boosting insecticide performance. Piperonyl butoxide and N-(propargyloxy) phthalimide, for instance, inhibits cytochrome P450 activity, reducing oxidative breakdown and thereby improving insecticidal impact whereas TPP target esterase-driven metabolism and DEM disrupts glutathione conjugation, making insecticides more potent against FAW (12–15). Similarly, 5-nitouracil has been reported to inhibit UGT-mediated detoxification pathways, thereby reducing conjugation-based metabolism and enhancing insecticide toxicity in resistant insect populations (16).

Several studies have demonstrated that the toxicity of insecticides can be increased when they are applied along with enzyme inhibitors, indicating the significant contribution of detoxification mechanisms to the development of insecticide resistance (17). The level of toxicity expressed by an insecticide is largely governed by its interaction with the physiological and biochemical systems of the insect. Therefore, understanding the biochemical responses of pest populations becomes essential. Differences in metabolic processes and enzyme activity among FAW populations can markedly alter the effectiveness of insecticidal treatments, thereby influencing the success of pest management under both laboratory and field conditions.

A promising strategy to address reduced sensitivity in FAW involves the use of enzyme inhibitors along with insecticides to target key metabolic resistance mechanisms. These inhibitors block specific detoxification pathways, thereby enhancing insecticide

toxicity and improving control efficacy. Based on this approach, the present study was undertaken to evaluate the synergistic interactions of enzyme inhibitors such as PBO, N-(propargyloxy) phthalimide, TPP, DEM and 5-nitouracil with 5 insecticides including emamectin benzoate, chlorantraniliprole, spinetoram, acephate and lambda-cyhalothrin. The extent of synergistic enhancement was assessed to understand the role of major detoxification enzymes and to determine improvements in insecticide efficacy following enzyme inhibition. The findings are expected to improve understanding of resistance mechanisms and contribute to the development of more sustainable management strategies for FAW.

Materials and Methods

This study was conducted in the Toxicology Laboratory of the Department of Entomology, College of Agriculture, Odisha University of Agriculture and Technology (OUAT), Bhubaneswar from June to November, 2024. The experiment aimed to evaluate the effect of selected synergists on the toxicity of various insecticides against FAW under controlled conditions.

Insect culture

Around 200 late-instar FAW larvae were collected from maize fields in 2 locations, Bhubaneswar and Bhawanipatna, Odisha, where insecticides are regularly applied. The collected larvae were brought to the laboratory and maintained separately to retain the characteristics of each field population. Rearing was carried out under controlled conditions at 27 ± 2 °C temperature, 60–70 % relative humidity and a 14 : 10 hr (L : D) photoperiod. To prevent cannibalism, each larva was kept individually in cells of rearing trays and supplied daily with fresh, untreated maize leaves. After completion of larval development, the insects were allowed to pupate and the pupae were transferred to plastic containers for adult emergence. The emerged adults were maintained in plastic containers and fed with 10 % honey solution enriched with vitamin E using a cotton wick. Paper sheets were placed inside the containers as an oviposition substrate and the egg masses laid on these sheets were collected at regular intervals and transferred to clean containers for hatching. The newly hatched larvae were reared on fresh, untreated maize leaves under the same controlled conditions. The F₁ generation was used for bioassay studies to obtain a more uniform test population and to reduce variation that could arise from differences in age, physiological condition, feeding history and previous field exposure of the field collected larvae.

Insecticides

Technical grade insecticides of high purity were used in this study. Information on the selected insecticides including their chemical group, mode of action and chemical formula is provided in Table 1. The insecticides were chosen because they belong to different chemical groups namely avermectins, diamides, spinosyns, organophosphates and pyrethroids having different modes of action, so that the effect of synergists could be examined across a range of target sites.

Preparation of insecticide solutions

Stock solutions of each insecticide were prepared by dissolving the required amount in acetone. Further concentrations were obtained from these stocks through serial dilution to cover a range capable of producing 20–90 % mortality. Fresh solutions were prepared for each set of bioassays to ensure uniformity and

Table 1. Details of insecticides and synergists used in the study

Sl. No.	Name of insecticide	Purity (%)	Chemical group	Mode of action	Chemical formula
1	Emamectin benzoate	98	Avermectin	Activates glutamate-gated chloride channels leading to paralysis	$C_{49}H_{75}NO_{13}$
2	Chlorantraniliprole	98	Diamide	Ryanodine receptor modulator disrupting Ca^{2+} release	$C_{18}H_{14}BrCl_2N_5O_2$
3	Spinetoram	97	Spinosyn	Allosteric activator of nicotinic acetylcholine receptors causing neuromuscular disruption	$C_{42}H_{69}NO_{10}$
4	Acephate	98	Organophosphate	Acetylcholinesterase inhibitor	$C_4H_{10}NO_3PS$
5	Lambda-cyhalothrin	98	Pyrethroid	Voltage-gated sodium channel modulator	$C_{23}H_{19}ClF_3NO_3$
6	N-(Propargyloxy)phthalimide	97	Dicarboximide	Cytochrome P450 monooxygenase inhibitor	$C_{11}H_7NO_3$
7	Triphenyl phosphate (TPP)	99	Aromatic organophosphate	Carboxylesterase inhibitor	$C_{18}H_{15}O_4P$
8	Piperonyl butoxide (PBO)	98	Methylenedioxyphenyl compound	Cytochrome P450 monooxygenase inhibitor	$C_{19}H_{30}O_5$
9	Diethyl maleate (DEM)	97	Maleate ester	Glutathione-S-transferase inhibitor	$C_8H_{12}O_4$
10	5-Nitrouracil	98	Nitrogenous heterocyclic compound	Uridine diphosphate glycosyltransferases Inhibitor	$C_4H_3N_3O_4$

to minimise any loss of activity due to degradation.

Bioassay procedure

Insecticide toxicity was evaluated using a topical application method on early third-instar larvae, ensuring direct contact of the test compound with the insect body. A Hamilton micro-applicator (PB600-1, Hamilton Company) fitted with a repeating dispenser was used to apply 1 μ L of the insecticide solution onto the dorsal thoracic region of each larva.

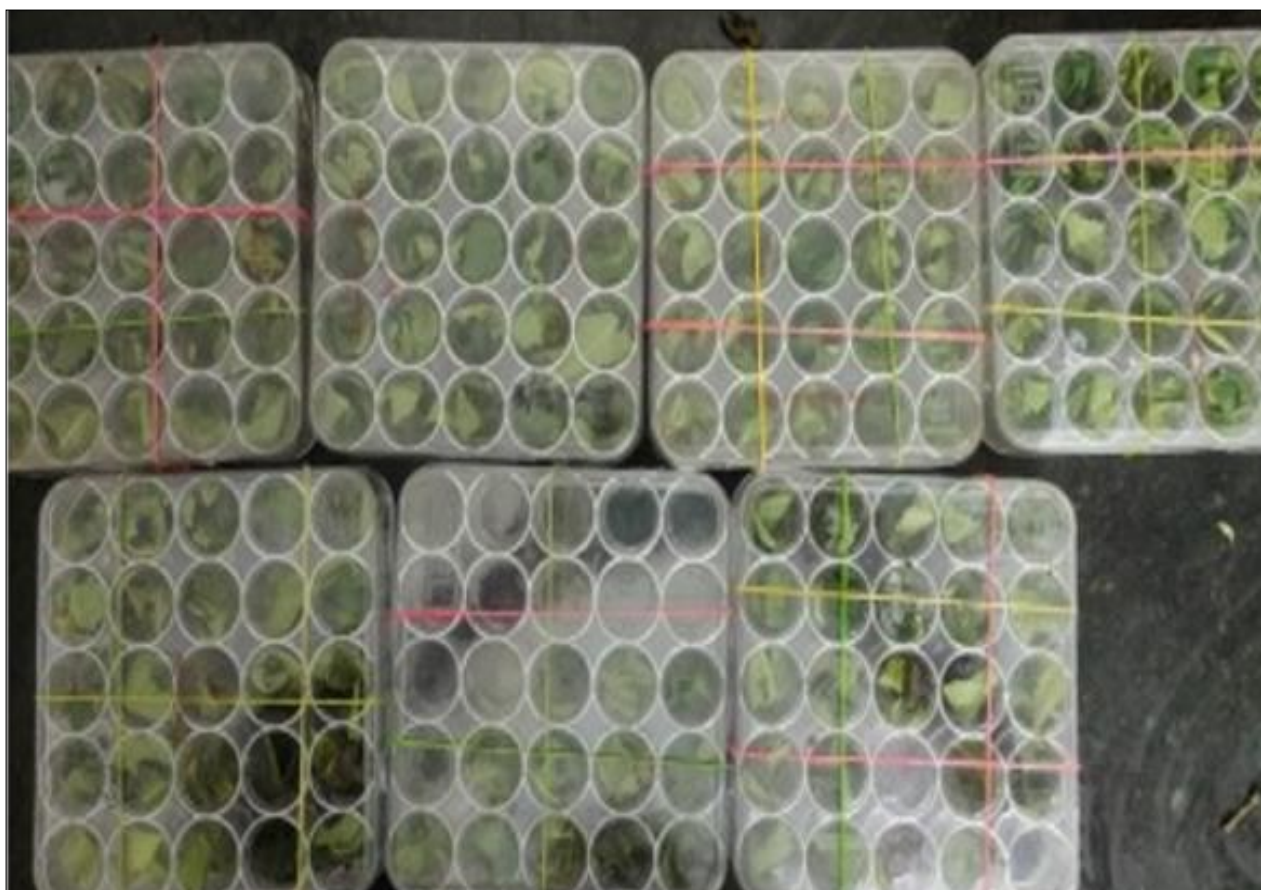
For each concentration, 30 larvae were treated per replication and the experiment was repeated three times as independent biological replicates using separate batches of larvae. Following treatment, larvae were kept individually in 25-celled rearing trays (Fig. 1) and provided with fresh, untreated maize leaves as diet (18). Mortality observation was taken at 24, 48 and 72 hr after treatment. Larva that failed to react to gentle probing

with a fine brush were recorded as dead. For control, larvae were treated with acetone only meant for comparative purposes. To ensure consistency in observation the whole experiment was conducted under uniform laboratory conditions.

Procedure for synergism assessment

Five synergists selected for this study were based on their known interference with key detoxification enzyme systems. These include PBO and N-(propargyloxy) phthalimide are associated with the inhibition of cytochrome P450 monooxygenases, whereas DEM, TPP and 5-nitrouracil inhibit GST, carboxylesterases and UGT mediated pathways respectively (Table 1).

Prior to the initiation of experiment, stock solutions for each synergist were prepared using acetone and appropriate sublethal dosages being carefully standardised. The specific doses utilised for treatment were 10 μ g / larva for PBO, 15 μ g / larva for DEM

**Fig. 1.** Bioassay setup for assessing insecticide-synergist interactions in fall armyworm.

and a consistent 10 µg / larva for TPP, N-(propargyloxy) phthalimide and 5-nitouracil. Topical application of the synergists was carried out 15 min before insecticide exposure to allow for a sufficient time for interaction with the targeted enzyme systems. All subsequent insecticide applications and experimental observations were recorded according to the same protocols detailed for the toxicity bioassay (19, 20).

Statistical analysis

Mortality data were corrected using Abbott's formula (21). Probit analysis of dose-mortality response data was carried out using Polo Plus (version 2.0, LeOra Software Inc., Berkeley, CA) to obtain LD₅₀ values, slope and standard error of the regression line (22). Synergistic ratio (SR) was expressed as the ratio of LD₅₀ of insecticide alone to the LD₅₀ of insecticide in combination with synergist.

Results

Effect of synergists on insecticide toxicity in Bhubaneswar population

The data presented in Table 2, show that toxicity of 5 insecticides against BBPL increased upon the addition of metabolic synergists. This pattern appeared clearly through higher SR and lower LD₅₀ values. With emamectin benzoate the strongest synergism came from DEM at a SR of 3.91 while N-(propargyloxy) phthalimide and PBO gave 2.77 and 2.34. 5-nitouracil exhibited the lowest synergistic effect, with a SR of 1.55. Chlorantraniliprole responded with more moderate toxicity shifts. The PBO produced the highest recorded SR at 2.10, the remaining

synergists showed a narrow range of 1.33-1.99. Spinetoram improved most when combined with PBO and N-(propargyloxy) phthalimide with SRs of 4.20 and 3.11 respectively. Synergistic ratios value with TPP was 2.50 followed by DEM (1.97) and then 5-nitouracil only 1.44. Lambda-cyhalothrin had the biggest boosts from PBO at 6.50 and N-(propargyloxy) phthalimide at 4.41 while TPP and DEM offered 2.60 and 2.34. 5-nitouracil lagged at 1.32. For acephate TPP stood out best at 5.85 ahead of N-(propargyloxy) phthalimide at 4.18 but with DEM, PBO and 5-nitouracil much weaker at 2.08, 1.56 and 1.43 respectively. Overall, synergistic responses differed among insecticides; however, PBO, DEM and TPP generally produced higher synergistic ratios than the other synergists while 5-nitouracil yielded the lowest synergistic enhancement.

Effect of synergists on insecticide toxicity in Bhawanipatna population

The susceptibility of the Bhawanipatna population (BPPL) to the tested insecticides was markedly altered by the addition of the 5 synergists (Table 3). For emamectin benzoate the strongest boost came from DEM with a SR of 4.76 while N-(propargyloxy) phthalimide and PBO trailed at 4.0 and 3.45 respectively. 5-nitouracil offered only a modest lift of 1.72. Chlorantraniliprole exhibited comparatively lower synergistic responses with most synergists, although DEM produced a markedly higher SR (4.39), whereas the other compounds remained within a tighter range of 1.52 to 1.92, with 5-nitouracil again trailing at 1.25. Spinetoram exhibited substantial synergistic enhancement especially with DEM at 5.10 and PBO at 4.22 over N-(propargyloxy) phthalimide at 3.51 and TPP at 2.61 but with 5-nitouracil again weakest at 1.46.

Table 2. Influence of selected synergists on the toxicity of insecticides against third instar larvae of fall armyworm collected from Bhubaneswar

Insecticide	Synergist	LD ₅₀ (µg/larva)	SR ^a	χ ²	Slope ± SE ^b	FL ^c (Lower)	FL ^c (Upper)
Emamectin benzoate	Nil	0.0180	–	0.985	1.517 ± 0.127	0.0144	0.0216
	PBO	0.0077	2.34	2.986	0.901 ± 0.213	0.0062	0.0092
	N-(Propargyloxy) phthalimide	0.0065	2.77	4.916	0.961 ± 0.182	0.0052	0.0078
	TPP	0.0086	2.09	4.059	0.939 ± 0.166	0.0069	0.0103
	DEM	0.0046	3.91	5.908	1.892 ± 0.201	0.0037	0.0055
Chlorantraniliprole	5-Nitouracil	0.0116	1.55	5.229	1.396 ± 0.229	0.0093	0.0139
	Nil	0.0342	–	2.975	1.195 ± 0.074	0.0274	0.0410
	PBO	0.0163	2.10	5.093	1.674 ± 0.204	0.0130	0.0196
	N-(Propargyloxy) phthalimide	0.0172	1.99	5.886	2.038 ± 0.236	0.0138	0.0206
	TPP	0.0187	1.83	3.754	2.019 ± 0.236	0.0150	0.0224
Spinetoram	DEM	0.0201	1.70	4.869	1.817 ± 0.261	0.0161	0.0241
	5-Nitouracil	0.0258	1.33	2.146	1.382 ± 0.267	0.0206	0.0310
	Nil	0.0252	–	1.918	1.265 ± 0.113	0.0202	0.0302
	PBO	0.0060	4.20	5.874	1.833 ± 0.132	0.0048	0.0072
	N-(Propargyloxy) phthalimide	0.0081	3.11	4.962	1.391 ± 0.192	0.0065	0.0097
Lambda-cyhalothrin	TPP	0.0101	2.50	0.849	1.700 ± 0.183	0.0081	0.0121
	DEM	0.0128	1.97	5.954	1.723 ± 0.199	0.0102	0.0154
	5-Nitouracil	0.0175	1.44	3.812	1.187 ± 0.173	0.0140	0.0210
	Nil	0.1371	–	0.909	1.207 ± 0.071	0.1097	0.1645
	PBO	0.0211	6.50	2.910	1.174 ± 0.202	0.0169	0.0253
Acephate	N-(Propargyloxy) phthalimide	0.0311	4.41	1.054	1.263 ± 0.321	0.0249	0.0373
	TPP	0.0527	2.60	4.053	1.111 ± 0.459	0.0422	0.0632
	DEM	0.0585	2.34	2.102	1.009 ± 0.482	0.0468	0.0702
	5-Nitouracil	0.1038	1.32	3.106	2.021 ± 0.689	0.0830	0.1246
	Nil	0.2901	–	5.782	1.282 ± 0.156	0.2321	0.3481
	PBO	0.1859	1.56	0.823	1.417 ± 1.233	0.1487	0.2231
	N-(Propargyloxy) phthalimide	0.0694	4.18	2.249	1.331 ± 0.524	0.0555	0.0833
	TPP	0.0496	5.85	4.741	1.363 ± 0.455	0.0397	0.0595
	DEM	0.1394	2.08	2.214	1.118 ± 1.016	0.1115	0.1673
	5-Nitouracil	0.2028	1.43	3.802	1.156 ± 1.196	0.1622	0.2434

PBO: piperonyl butoxide; TPP: triphenyl phosphate; DEM: diethyl maleate; Nil: indicates insecticide applied alone (without synergist); SR^a (synergistic ratio): LD₅₀ of insecticide applied alone / LD₅₀ of insecticide in combination with synergist; SE^b: standard error of the slope estimate; FL^c: 95 % fiducial limits of the LD₅₀ estimate.

Table 3. Influence of selected synergists on the toxicity of insecticides against third instar larvae of fall armyworm collected from Bhawanipatna

Insecticide	Synergist	LD ₅₀ (µg/larva)	SR ^a	χ ²	Slope ± SE ^b	FL ^c (Lower)	FL ^c (Upper)
Emamectin benzoate	Nil	0.0176	–	0.800	1.309 ± 0.071	0.0124	0.0250
	PBO	0.0051	3.45	1.327	2.165 ± 0.155	0.0042	0.0069
	N-(Propargyloxy) phthalimide	0.0044	4.00	2.159	1.182 ± 0.086	0.0021	0.0064
	TPP	0.0075	2.35	2.971	1.712 ± 0.116	0.0058	0.0101
	DEM	0.0037	4.76	0.964	1.531 ± 0.195	0.0029	0.0079
Chlorantraniliprole	5-Nitroureacil	0.0102	1.72	0.776	1.692 ± 0.122	0.0080	0.0142
	Nil	0.0514	–	2.554	1.451 ± 0.123	0.0367	0.0720
	PBO	0.0268	1.92	3.645	1.199 ± 0.226	0.0185	0.0388
	N-(Propargyloxy) phthalimide	0.0281	1.83	3.241	1.561 ± 0.117	0.0220	0.0359
	TPP	0.0338	1.52	4.668	0.926 ± 0.199	0.0223	0.0513
Spinetoram	DEM	0.0117	4.39	3.387	2.162 ± 0.225	0.0098	0.0140
	5-Nitroureacil	0.0411	1.25	0.579	0.905 ± 0.175	0.0269	0.0628
	Nil	0.0418	–	0.313	1.668 ± 0.087	0.0326	0.0536
	PBO	0.0099	4.22	2.243	0.907 ± 0.131	0.0065	0.0150
	N-(Propargyloxy) phthalimide	0.0119	3.51	1.215	1.516 ± 0.184	0.0092	0.0153
Lambda-cyhalothrin	TPP	0.0160	2.61	1.284	1.465 ± 0.194	0.0115	0.0222
	DEM	0.0082	5.10	3.370	1.110 ± 0.162	0.0056	0.0120
	5-Nitroureacil	0.0286	1.46	2.210	1.529 ± 0.230	0.0221	0.0368
	Nil	0.1563	–	1.427	1.492 ± 0.159	0.1115	0.2190
	PBO	0.0326	4.80	3.457	2.121 ± 0.165	0.0271	0.0392
Acephate	N-(Propargyloxy) phthalimide	0.0289	5.41	2.118	1.961 ± 0.205	0.0235	0.0355
	TPP	0.0539	2.90	0.986	1.251 ± 0.235	0.0368	0.0789
	DEM	0.0233	6.71	1.342	1.829 ± 0.313	0.0180	0.0300
	5-Nitroureacil	0.1158	1.35	0.522	0.952 ± 0.478	0.0748	0.1790
	Nil	0.3248	–	0.643	1.621 ± 0.125	0.2520	0.4190
	PBO	0.1911	1.70	1.199	1.356 ± 0.784	0.1325	0.2758
	N-(Propargyloxy) phthalimide	0.0755	4.30	2.453	1.668 ± 0.342	0.0588	0.0969
	TPP	0.0580	5.60	2.855	1.933 ± 0.315	0.0461	0.0730
	DEM	0.0524	6.20	0.969	2.145 ± 0.689	0.0428	0.0641
	5-Nitroureacil	0.2165	1.50	0.817	1.795 ± 0.841	0.1692	0.2770

PBO: piperonyl butoxide; TPP: triphenyl phosphate; DEM: diethyl maleate; Nil: indicates insecticide applied alone (without synergist); SR^a (synergistic ratio): LD₅₀ of insecticide applied alone / LD₅₀ of insecticide in combination with synergist; SE^b: standard error of the slope estimate; FL^c: 95 % fiducial limits of the LD₅₀ estimate.

Lambda-cyhalothrin showed the sharpest rise notably with DEM at 6.71, N-(propargyloxy) phthalimide at 5.41 and PBO at 4.80 far ahead of TPP at 2.90 and 5-nitroureacil at 1.35. Similar patterns held for acephate, where DEM at 6.20 and TPP at 5.60 drove major increases compared with the milder shifts from N-(propargyloxy) phthalimide at 4.30, PBO at 1.70 and 5-nitroureacil at 1.50. Overall, DEM exhibited the highest synergistic activity across most insecticides in BPPL while 5-nitroureacil proved least influential.

Comparative synergistic effects in Bhubaneswar population and Bhawanipatna population

As shown in Fig. 2, a pronounced disparity was observed between the two FAW populations, with BPPL generally exhibiting higher synergistic ratios (SRs) than BBPL across most insecticide-synergist combinations. The influence of DEM stands out particularly in BPPL where it produced greater toxicity enhancement for emamectin benzoate, spinetoram, lambda-cyhalothrin and acephate. However, its impact is considerably more subdued within BBPL. A parallel trend is observed for PBO and N-(propargyloxy) phthalimide both of which drive higher levels of synergism in BPPL especially regarding the efficacy of lambda-cyhalothrin and spinetoram. Triphenyl phosphate shows a marginal increase in activity in BPPL mainly with acephate and its performance remains largely consistent across both populations. Chlorantraniliprole represents a unique case where a relatively stagnant and moderate response is maintained in both populations regardless of the synergist applied. Finally, 5-nitroureacil is distinguished by its lack of efficacy consistently

showing a negligible influence on insecticide performance in both populations.

Discussion

The enhanced toxicity of insecticides following synergist treatment in both BBPL and BPPL populations of *S. frugiperda* demonstrates the involvement of metabolic detoxification enzymes in resistance development. Synergists suppress specific detoxification pathways and thereby restore insecticidal activity, providing indirect evidence of the biochemical mechanisms associated with resistance expression (23, 24).

In the BBPL population, pronounced synergism with PBO against lambda-cyhalothrin and spinetoram indicates a substantial role of cytochrome P450 monooxygenases in oxidative detoxification. Similar PBO-mediated enhancement has been documented in FAW populations from Hainan (China) and Hosur (Tamil Nadu, India), where SR for chlorantraniliprole reached 5.7 and 6.44 respectively, confirming the importance of oxidative metabolism in geographically distinct populations (25, 26). The enhanced response of emamectin benzoate with DEM suggests the involvement of GST-mediated conjugation, whereas stronger synergism of acephate with TPP indicates participation of esterase-related detoxification pathways. Collectively, these observations suggest that resistance in BBPL is associated with the combined action of multiple detoxification systems rather than a single metabolic pathway (27, 28).

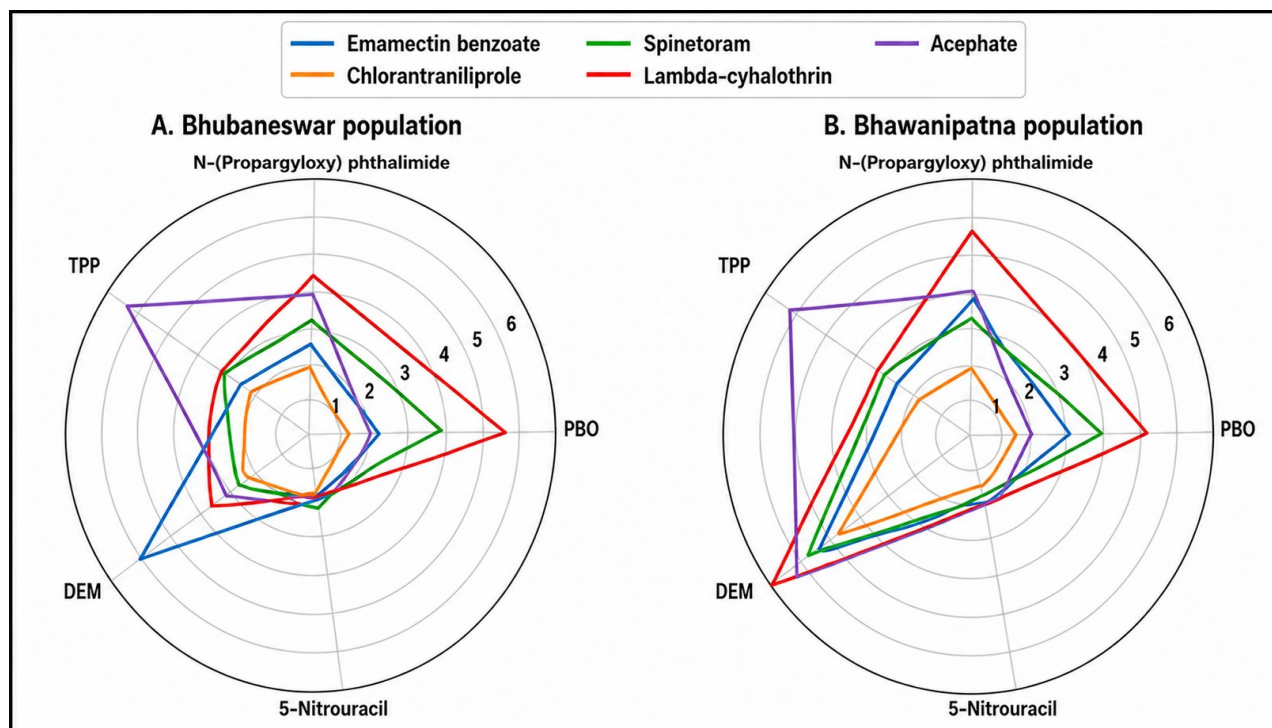


Fig. 2. Comparative radar plots of synergistic ratios (SR) of insecticides. (A) in Bhubaneswar population (BBPL); (B) in Bhawanipatna population (BPPL) with selected synergists. PBO: piperonyl butoxide; DEM: diethyl maleate; TPP: triphenyl phosphate.

Compared with BBPL, the BPPL population exhibited comparatively stronger synergistic responses with DEM across most insecticides, particularly lambda-cyhalothrin, spinetoram, emamectin benzoate and acephate, indicating a greater contribution of GST-mediated detoxification in this population. Similar population-dependent variation in GST-associated synergism has been reported in FAW populations from China, where synergistic responses differed considerably according to local insecticide selection pressure and resistance background (8, 25). Comparable locality-based differences in synergistic responses have also been observed in *Leucinodes orbonalis* populations from Odisha, indicating that detoxification-mediated resistance can vary substantially across geographically separated populations exposed to different insecticide use patterns (29). Moderate enhancement with PBO and N-(propargyloxy) phthalimide in BPPL additionally suggests partial involvement of oxidative detoxification pathways. In contrast, 5-nitouracil produced consistently weak synergistic responses in both populations, indicating that UGT mediated detoxification contributes less prominently to resistance relative to monooxygenase, GST and esterase-mediated pathways. Similar enhancement of insecticide toxicity following inhibition of UGT activity has previously been reported in resistant *Nilaparvata lugens* populations (16).

A notable outcome of the present investigation was the comparatively modest synergistic response of chlorantraniliprole in both populations irrespective of the synergist used. In BBPL, synergistic enhancement remained within a narrow range, while BPPL showed only moderate improvement compared with the stronger responses observed for lambda-cyhalothrin and spinetoram. Similar weak synergistic responses for chlorantraniliprole were reported from Sichuan (China) where PBO, DEM and TPP produced SR of only 1.43, 1.08 and 0.93 respectively, suggesting that metabolic detoxification contributes only partially to chlorantraniliprole tolerance in some FAW populations (8). In contrast, stronger PBO-mediated

synergism reported from Hainan and Hosur populations indicates that the contribution of oxidative detoxification to chlorantraniliprole resistance may vary geographically depending on insecticide exposure history and resistance intensity. The comparatively stronger synergism observed with lambda-cyhalothrin in the present study suggests that pyrethroid resistance in these Odisha populations is more strongly linked with metabolic enzyme activity than resistance to chlorantraniliprole. This difference may reflect variation in the underlying resistance mechanisms of pyrethroids and diamides, with chlorantraniliprole resistance possibly involving additional non-metabolic mechanisms such as alterations in target-site sensitivity.

The contrasting synergistic patterns observed between BBPL and BPPL further indicate that resistance evolution in FAW is highly population specific. Differential dominance of oxidative, conjugative and esterase-mediated detoxification pathways likely reflects variation in local insecticide use patterns and selection pressure. Such population-level variability highlights the importance of localised resistance monitoring for the development of effective resistance management strategies. Although synergists are primarily used as diagnostic tools, identification of dominant detoxification pathways may help improve insecticide rotation strategies and support the selection of compounds less vulnerable to existing resistance mechanisms. However, practical field application of synergist-based formulations may be influenced by factors such as formulation stability, environmental persistence, cost-effectiveness and regulatory approval. Therefore, synergist studies are valuable for improving understanding of resistance mechanisms and supporting resistance-informed management strategies for sustainable FAW control in maize ecosystems (30–33).

Conclusion

The present investigation conducted during June–November 2024 demonstrated clear population-specific variation in metabolic detoxification mechanisms in FAW populations from Odisha. Strong

synergistic responses with piperonyl butoxide (PBO) and N-(propargyloxy) phthalimide in the Bhubaneswar population (BBPL) indicated greater involvement of cytochrome P450 monooxygenases, whereas higher enhancement with diethyl maleate (DEM) in the Bhawanipatna population (BPPL) suggested a dominant role of glutathione S-transferase (GST)-mediated detoxification. Triphenyl phosphate also produced notable synergism with certain insecticides, indicating the contribution of esterase-mediated pathways. In contrast, 5-nitouracil showed consistently weak synergistic effects in both populations, suggesting a limited role of UDP-glycosyltransferase (UGT) mediated detoxification. The study highlights the importance of localised resistance monitoring and mechanism-based insecticide selection for sustainable FAW management. Further studies involving biochemical and molecular characterisation of resistance mechanisms along with field validation of synergist-based approaches are needed to strengthen insecticide resistance management (IRM) strategies.

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Authors' contributions

SQ carried out the experimental work, gathered the data and prepared the preliminary draft of the manuscript. MKT designed and planned the study, supervised its execution and contributed substantially to data analysis and manuscript refinement. SQ and AZ undertook statistical analysis, interpreted the findings and supported the writing and editing process. KCS, AKS and MD were involved in study conceptualisation, provided technical guidance on bioassays and critically reviewed the manuscript for scientific accuracy. AS and SNM assisted with field activities, including sampling and data collection and contributed to discussion development and final manuscript preparation. All authors read and approved the final manuscript.

Compliance with ethical standards

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used OpenAI's ChatGPT to assist in language refinement. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the final manuscript.

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