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Baseline toxicity evaluation of new insecticide molecules against *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) in cole crops

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ABSTRACT: *Spodoptera litura* (F.), poses a significant threat to the productivity and quality of cole crops such as cabbage, cauliflower, and broccoli. In this study evaluated the baseline toxicity of seven novel insecticides was evaluated against *S. litura* populations from the F_1 and F_6 generations using the leaf dip bioassay method. The insecticides evaluated were emamectin benzoate, indoxacarb, spinosad, Lambda cyhalothrin, chlorfenapyr, cyantraniliprole, and chlorfluazuron. Among the F_1 generation, emamectin benzoate exhibited the highest toxicity (LC_{50}: 0.93 µg/mL), followed closely by Spinosad (LC_{50}: 1.03 µg/mL) and Indoxacarb (LC_{50}: 1.13 µg/mL), whereas Lambda cyhalothrin showed the lowest toxicity (LC_{50}: 60.21 µg/mL). In the F_6 generation, indoxacarb emerged as the most toxic insecticide (LC_{50}: 0.39 µg/mL), followed by emamectin benzoate (LC_{50}: 0.57 µg/mL) and Cyantraniliprole (LC_{50}: 0.68 µg/mL), with Lambda cyhalothrin remaining the least effective (LC_{50}: 34.76 µg/mL). The baseline toxicity data generated contributing to resistance management programs.

Keywords: *Spodoptera litura*, cole crops, baseline toxicity, insecticides, bioassay

INTRODUCTION

Cole crops such as cabbage, cauliflower, kale, and broccoli, belonging to the family Brassicaceae, are extensively cultivated in temperate and tropical regions worldwide, including India. Among the states, West Bengal ranks first in cole crop production. Bihar, another significant producer, ranks sixth in cabbage production. However, the cultivation of these economically important crops is hampered by severe pest infestations that occur from the early stages of crop growth to harvest. In India, cole crops are attacked by a total of 37 insect pests. Notable among these are the cabbage leaf webber (*Crocidolomia binotalis* Zell), painted bug (*Bagrada hilaris* Burmeister and *Bagrada cruciferarum* Kirk.), cabbage butterfly (*Pieris brassicae* L.), cabbage semilooper (*Trichoplusia ni* Hubner), diamond back moth (*Plutella xylostella* L.), and tobacco cutworm (*Spodoptera litura* Fabricius). Overall, pest infestations in cole crops can result in yield losses ranging from 7% to 90% (Choudhuri *et al*., 2001; Rao & Lal, 2005). These pests not only reduce yield but also degrade produce quality through mining, skeletonization, and discoloration of leaves.

Spodoptera litura (Fabricius) is one of the most polyphagous and economically important pests. Belonging to the family Noctuidae, it infests over 120 host plants globally, including tobacco, peanuts, tea, cotton, pulses, cauliflower, cabbage, potatoes, castor, sunflower, and tomato. Despite the widespread use of insecticides, the pest has developed resistance to conventional and novel insecticides with diverse modes of action, resulting in frequent outbreaks and control failures (Ahmad *et al*., 2011).

To combat the rising issue of insecticide resistance, insecticidal resistance management (IRM) strategies are crucial. These include the rotation and combination of insecticides from different chemical groups with distinct modes of action. Synergists such as piperonyl butoxide, diethyl maleate, and triphenyl phosphate can enhance insecticide efficacy by inhibiting metabolic resistance mechanisms. Piperonyl butoxide blocks mixed-function oxidases, diethyl maleate inhibits glutathione S-transferase, and triphenyl phosphate acts as an esterase inhibitor (Raffa and Priester, 1985). Despite these measures, the need for alternative pesticides with low resistance potential, minimal environmental impact, and reduced risks to human health remains critical. Given the economic importance of cole crops and the significant impact of pests like *S. litura*, it is imperative to conduct baseline toxicity studies. These studies assess the susceptibility of pest populations to various insecticides, providing critical data for developing effective pest management strategies and delay resistance development. Establishing baseline susceptibility levels enables the monitoring of resistance trends over time and informs the selection of appropriate control measures to ensure sustainable crop production.

MATERIALS AND METHODS

Collection and rearing of *Spodoptera litura*

Fifth and sixth instar larvae of *Spodoptera litura* were collected from fields of cabbage, cauliflower, and broccoli (cole crops) through a zigzag sampling method, ensuring representation of populations from different non-identical localities. The larvae were transferred to glass jars for rearing under controlled laboratory conditions (26°C and 65% RH). Fresh food was provided daily, and the larvae's excreta was removed to maintain hygiene and prevent diseases. After pupation, adult moths were transferred to transparent plastic jars with mesh on one side to ensure proper ventilation. The moths were fed a 10% sugar solution, supplemented with methyl-4-hydroxybenzoate (2 g/L) and vitamins, soaked in cotton wool balls. After mating, moths laid eggs, which were then hatched under optimal conditions. This process continued upto the F_6 generation.

Leaf dip bioassay

The baseline toxicity of various newer insecticides was assessed using the leaf dip bioassay method. Third-instar larvae (weighing 0.06–0.08 g and measuring 0.8–1.0 cm in length) from the F_1 and F_6 generations were used for the bioassay. Newer insecticides recommended by the CIBRC for the control of *S. litura* on cole crops, including emamectin benzoate 5% SG, indoxacarb 14.5%, spinosad 2.8% EC, Lambda cyhalothrin 5% EC, chlorfenapyr 10% SC, cyantraniliprole 10.26% OD, and chlorfluazuron 5.4% EC, were used for the study (Table 1). The bioassay procedure followed the FAO-recommended methods for resistance monitoring (FAO Plant Protection Bulletin, FAO Method No. 17, 18, 1979).

Initially, a preliminary assay was conducted by applying arbitrary concentrations of each insecticide without replication to identify the concentration range that caused 0–100% mortality. Serial dilutions of six concentrations, with one control (water), were then prepared for each insecticide (Table 1), and three replications were made for each concentration. In each replication, ten larvae were used.

Fresh, pesticide-free leaves from cabbage, cauliflower, and broccoli plants were collected from the field. Leaf discs (4–5 cm in diameter) were cut on both side of the midrib using a sterilized punch, taking care to avoid contamination. Test concentrations were prepared through serial dilution, and the leaf discs were immersed in the solutions for approximately 1 minute. Excess liquid was removed by air drying. After drying, the leaf discs were placed in separate Petri dishes using sterilized forceps. A total of ten third-instar larvae were placed in each Petri dish, which was then covered with white muslin cloth and labelled according to the diluted concentrations. This procedure was repeated for three replicates of each concentration.

Larval mortality was recorded 24 hours after treatment. Larvae were considered dead if they showed signs of growth retardation, such as cessation of feeding, body size reduction, or the inability to return to an upright position when poked (moribund). The LD_{95} values of the F_6 generation were considered as the discriminative dose value for every tested insecticide.

Data Analysis

The mortality data were analysed using probit analysis through the POLOPLUS program (LeOra Software,

Table 1. Insecticides used, along and their concentrations upon serial dilution

Treatment	Insecticides	Dose (ml/L)	Concentration (in PPM)	Toxicity labels
T1	Emamectin Benzoate 5% SG	0.002	8, 4, 2, 1, 0.5, 0.25, 0	Blue
T2	Indoxacarb 14.5% SC	0.003	8, 4, 2, 1, 0.75, 0.50, 0	Yellow
T3	Spinosad 2.8% EC	0.003	24.32, 12.16, 6.08, 3.04, 1.52, 0.076, 0	Blue
T4	Lambda cyhalothrin 5 % EC	0.6	960, 480, 240, 120, 60, 30, 0	Yellow
T5	Chlorfenapyr 10 % SC	0.02	72, 36, 18, 9, 4.5, 2.3, 0	Blue
T6	Cyantraniliprole 10.26 % OD	0.01	10.08, 5.04, 2.52, 1.26, 0.63, 0.315, 0	Green
T7	Chlorfluazuron 5.40% EC	0.01	50, 30, 10, 7, 5, 3, 0	Green
T8	Control (with water)	-	-	-

2003), based on Finney's (1971) method. The following formulae were used to calculate the susceptibility index and rate of resistance decline.

- Susceptibility index = LC_{50} or LD_{95} of 1st generation/ LC_{50} or LD_{95} of last generation
- Rate of resistance decline (R) = log(final LC_{50}) – log(intial LC_{50}) / n
  (where, n = no. of generation)
- Generation (G) = 1/R

RESULTS AND DISCUSSION

The LC_{50} values and toxicity ratios of various insecticides against the F_1 and F_6 generation populations of *S. litura* are presented in tables 2 and 3, respectively. The chi-square analysis of mortality data confirmed a good fit to the probit regression model, validating accuracy of our estimates. In the F_1 generation, population of *S. litura*, the pest was highly susceptible to emamectin benzoate and least susceptible to Lambda cyhalothrin. Emamectin benzoate exhibited the highest toxicity with the LC_{50} value of 0.93 µg/mL, making it 65 times more toxic than the least toxic insecticide. The LC_{50} values of spinosad, indoxacarb, cyantraniliprole, chlorfluazuron, and chlorfenapyr were 1.03, 1.13, 1.34, 7.58, and 9.22 µg/mL, respectively, and these were 58.45, 53.28, 45.00, 8.00, and 6.60 times more toxic than least toxic insecticide. Lambda cyhalothrin recorded as the least toxic insecticide, with the LC_{50} value of 60.21 µg/mL.

In the F_6 generation, population of *S. litura*, the pest showed highsusceptibility to Indoxacarb and low susceptibility to Lambda cyhalothrin. Indoxacarb was the most toxic insecticide, with an LC_{50} value of 0.39 µg/mL, making it 89.12 times more toxic than the least toxic insecticide. The LC_{50} values of emamectin benzoate, cyantraniliprole, spinosad, chlorfluazuron and chlorfenapyr were 0.57, 0.68, 0.85, 5.28, and 6.76 µg/mL, respectively, which were 61.00, 51.00, 41.00, 6.10,

Table 2. Dosage mortality response of *S. litura* against different insecticides through leaf dip method in F_1 generation at 24 h

Treatments		LC_{50} (µg/ml)	Fiducial LD_{50} limit (ppm)		X^2	Slope ±SE	LD_{95} (µg/ml)	Fiducial LD_{95} limit (ppm)		Toxicity ratio
			Lower	upper				Lower	Upper	
T1	Emamectin Benzoate 5% SG	0.93	0.62	1.32	1.30	1.351±0.215	15.47	7.92	51.89	65.00
T2	Indoxacarb 14.5% SC	1.13	0.77	1.53	3.51	1.475+-0.260	14.74	7.71	52.64	53.28
T3	Spinosad 2.8% EC	1.03	0.31	2.18	4.3	0.941+-0.143	57.78	18.50	778.20	58.45
T4	Lambda cyhalothrin 5 % EC	60.21	26.94	97.06	1.3	1.007±0.206	2590	1033.1	19872	1.00
T5	Chlorfenapyr10 % SC	9.22	6.97	11.95	0.82	1.929±0.253	65.65	42.33	131.69	6.60
T6	Cyantraniliprole 10.26 % OD	1.34	0.56	2.63	5.52	1.038±0.200	30.58	11.65	301.95	45.00
T7	Chlorfluazuron 5.40 % EC	7.58	5.76	9.72	1.36	1.924±0.288	54.28	33.89	122.98	8.00

Note: LC50.: Lethal concentration 50, LD95.: Lethal dose 95, SE.: Standard error, X2.: Chi sqaure

Table 3: Dosage mortality response of *S. litura* against different insecticides through leaf dip method in F_6 generation at 24 h

Treatments		LC_{50} (µg/ml)	Fiducial LD_{50} limit (ppm)		X^2	Slope ±SE	LD_{95} (µg/ml)	Fiducial LD_{95} limit (ppm)		Toxicity ratio
			Lower	upper				Lower	Upper	
T1	Emamectin Benzoate 5% SG	0.57	0.39	0.76	1.99	1.773±0.258	4.82	3.06	10.2	61.00
T2	Indoxacarb 14.5% SC	0.39	0.10	0.68	0.57	1.093±0.274	12.63	5.56	119	89.12
T3	Spinosad 2.8% EC	0.85	0.31	1.66	3	0.993±0.146	38.75	14.94	254.91	41.00
T4	Lambda cyhalothrin 5 % EC	34.76	9.05	64.67	0.61	0.8930.209	2420.1	883.15	3102	1.00
T5	Chlorfenapyr 10 % SC	6.76	4.13	10.15	1.2	1.185+0.210	50.68	77.38	774	5.10
T6	Cyantraniliprole 10.26 % OD	0.68	0.35	1.05	2.37	1.117+0.211	20.33	9.17	103.7	51.00
T7	Chlorfluazuron 5.40 % EC	5.28	2.47	8.17	5.13	1.764+0.295	45.28	22.58	340	6.10

Note: LC50.: Lethal concentration 50, LD95.: Lethal dose 95, SE.: Standard error, X2.: Chi sqaure

Table 4: Susceptibility index and rate of resistance decline based on F_1 and F_6 generationsat 24 h

Treatments	Generation	LC_{50} (µg/ml)	LD_{95} (µg/ml)	Susceptibility index		Rate of resistance decline and generation	
				LC_{50} (µg/ml)	LD_{95} (µg/ml)	R	G
T_1 Emamectin Benzoate 5% SG	F_1 F_6	0.93 0.57	15.47 4.82	1.64 1.00	3.20 1.00	-0.03	27.70
T_2 Indoxacarb 14.5% SC	F_1 F_6	1.13 0.39	14.74 12.63	2.86 1.00	1.16 1.00	-0.07	13.13
T_3 Spinosad 2.8% EC	F_1 F_6	1.03 0.85	57.78 38.75	2.60 1.00	1.49 1.00	-0.01	74.86
T_4 Lambda cyhalothrin 5 % EC	F_1 F_6	60.21 34.76	2590 2420	1.72 1.00	1.07 1.00	-0.03	25.14
T_5 Chlorfenapyr10 % SC	F_1 F_6	9.22 6.76	65.65 50.68	1.36 1.00	1.29 1.00	-0.02	43.40
T_6 Cyantraniliprole 10.26 % OD	F_1 F_6	1.34 0.68	30.58 20.33	1.96 1.00	1.50 1.00	-0.01	55.74
T_7 Chlorfluazuron 5.40 % EC	F_1 F_6	7.58 5.28	54.28 45.28	1.43 1.00	1.19 1.00	-0.02	38.35

Note: LC50.: Lethal concentration 50, LD95.: Lethal dose 95, R.: Rate of resistance decline, G..: No. of generations

and 5.10 times more toxic than least toxic insecticide. Lambda cyhalothrin emerged as the least toxic insecticide, with an LC_{50} value of 34.76 µg/mL.

Indoxacarb showed the highest susceptibility index (2.86 µg/mL), followed by spinosad (2.60), cyantraniliprole (1.96), Lambda cyhalothrin (1.72), emamectin benzoate (1.64), chlorfluazuron (1.43), and chlorfenapyr (1.36). Our findings indicate that indoxacarb requires 13.13 generations to reduce resistance by 0.07-fold. Lambda cyhalothrin requires 25.14 generations to reduce resistance by 0.03-fold. Emamectin benzoate necessitates 27.70 generations for a 0.03-fold decrease in resistance. Chlorfluazuron requires 38.35 generations to reduce resistance by 0.02-fold. Chlorfenapyr needs 43.40 generations to decrease resistance by 0.02-fold. Cyantraniliprole requires 55.74 generations to reduce resistance by 0.01-fold. Finally, Spinosad requires 74.86 generations for a 0.01-fold reduction in resistance (Table 4).

Previous research, including Ishtiaq *et al*. (2012), has indicated that indoxacarb and emamectin benzoate are more toxic than Spinosad, and our study corroborates these findings. In our results, Indoxacarb and emamectin benzoate exhibited significantly higher toxicity than Spinosad against *S. litura*, corroborating Ishtiaq *et al*. (2012) findings on *S. exigua*. These results highlight the higher efficacy of newer insecticides, such as indoxacarb and emamectin benzoate, compared to older insecticides like Spinosad, which is widely used but less effective in our study.

Li *et al*. (2015) reported that spinosad is highly effective in controlling Lepidopteran pests, including *Plutella xylostella*, but in our study, emamectin benzoate and indoxacarb outperformed spinosad in terms of toxicity. Similarly, findings by Karuppaiah *et al*. (2017) and Bird (2015) support the higher toxicity of emamectin benzoate in several pest species. The discrepancy in our study can likely be attributed to different experimental conditions, such as pest populations and environmental factors.

Cyantraniliprole, which was introduced for the control of Lepidopteran pests, also demonstrated good efficacy, though it was less toxic than the aforementioned insecticides. Sang *et al*. (2016) found that cyantraniliprole had moderate efficacy against *S. litura* in China, which is similar with our results where it was less effective than indoxacarb and emamectin benzoate but still more effective than older insecticides like Lambda cyhalothrin. Other studies, such as by Sang *et al*. (2016), have also reported that cyantraniliprole is an effective alternative to conventional insecticides, although its toxicity varies depending on the pest species and resistance levels.

Dash *et al*. (2020) reported that chlorfluazuron is less effective than emamectin benzoate and Indoxacarb in controlling *S. litura*, suggesting that insecticides in the same chemical class, such as chlorfenapyr, may have limitations in pest control.This observation aligns with our findings, as both chlorfluazuron and chlorfenapyr exhibited relatively low toxicity in both F_1 and F_6 generations.Previous studies by Su and Sun (2014), have similarly highlighted that the effectiveness of chlorfluazuron is often compromised due to the development of pest resistance.

Furthermore, Lambda cyhalothrin emerged as the least toxic insecticide in both F_1 and F_6 generations, consistent with the results of Sreelakshmi *et al*. (2019), who found reduced efficacy of Lambda cyhalothrin against *S. litura*. The reduced effectiveness of Lambda cyhalothrin in our study may be attributed to the overuse of pyrethroids in pest management, leading to resistance development in pest populations, as reported by Guillem□Amat *et al*. (2022). This variability in insecticide effectiveness underscores the need for continuous monitoring of pest susceptibility and resistance levels. Our findings, in line with previous studies (Ahmad *et al*., 2018; El-Sheikh., 2015), highlight the importance of establishing baseline susceptibility in pest populations before implementing long-term pest management strategies.

CONCLUSION

This study highlights the baseline toxicity of various insecticides against *Spodoptera litura* in cole crops. Indoxacarb and emamectin benzoate demonstrated superior efficacy in controlling pest populations, with significantly higher toxicity compared to other insecticides tested. These findings underscore the importance of integrating effective, low-resistance insecticides into pest management strategies. Continuous monitoring of insecticide susceptibility is essential to mitigate resistance development and ensure the sustainable cultivation of cole crops. The baseline data provided will serve as a valuable reference for future resistance management and pest control efforts.

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