



RESEARCH ARTICLE

Safety of some biopesticides towards ladybird beetles and rove beetle on cowpea, *Vigna unguiculata* (L.)

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Abstract

Field trials were conducted at the Agricultural Research Station, Faculty of Agricultural Sciences, SOADU, Binjhagiri, Khurdha, during the rainy season of 2022 and winter of 2022-23 to evaluate the safety of five biopesticides on predators in cowpea crops. Four species of ladybird beetles and one species of rove beetle (Paederus fuscipes Curtis) were observed. Among the ladybird beetles, Coccinella repanda Thunberg was the predominant species, accounting 43.84% of the total population, followed by Cheilomenes sexmaculata Fab. (31.51%), Micraspis discolour Fab. (15.07%) and Brumoides suturalis Fab. (9.59%). The treatments included Bacillus thuringiensis var. kurstaki (16000 i.u./mg) at 1.0 Kg/ha, Beauveria bassiana (108 cfu/g) at 2.5 Kg/ha, Metarrhizium anisopliae (108 spores/g) at 2.5 Kg/ha, Lecanicillium lecani (108 cfu/g) at 2.5 L/ha, azadirachtin (300 ppm) at 2.5 L/ha, chlorantraniliprole (18.5% active ingredient) at 0.25 L/ha (chemical check) and an untreated control. All the biopesticides proved to be safe for ladybird beetles and the rove beetle. B. thuringiensis var. kurstaki was the safest, recording only 3.94% and 3.40% reductions in ladybird population five days after application during the rainy season of 2022 and winter of 2022-23, respectively. It was followed by B. bassiana (6.35% and 4.45% reduction), M. anisopliae (8.97% and 7.33% reduction), L. lecani (10.28% and 8.90% reduction) and azadirachtin (19.04% and 26.96% reduction). A similar trend was observed in rove beetle, with respective reduction of 5.07% and 3.79%, 5.67% and 4.92%, 6.87% and 6.06%, 7.76% and 6.82% and 17.31% and 26.14%.

Keywords

biopesticides; field trials; ladybird beetles; rove beetle; safety

Introduction

Cowpea, *Vigna unguiculata* (L.), is one of the principal vegetable crops farmed in India. It is one of the most widely adapted and highly nutritious legumes. It can be used as a vegetable, pulse or fodder crop. Cowpea consists of protein (23%), fats (1.3%), fibre (1.8%), carbohydrates (67%) and water (8-9%) (1). It is an efficient nitrogen-fixing, heat- and drought-tolerant legume (2). It is susceptible to a wide range of insect pests that attack the crop at all stages of growth. The major insect pests that severely damage cowpea crops during different growth stages include the gram pod borer (*Helicoverpa armigera* Hub.), the spotted pod borer (*Maruca testulalis* Geyer), the plume moth (*Exelastis atomosa* Wals.), the blue butterfly (*Lampides boeticus* Linn.), the blister beetle (*Mylabris phalerata* Pallas.), bean aphids (*Aphis craccivora* Koch.), the lablab bug or stink bug (*Coptosoma cribraria* Fab.) and the whitefly (*Bemisia tabaci* Genn.). These pests can severely reduce the yield of the cowpea plant. Yield losses ranging from 10 to 100% and crop failure occur due to these pests (3).

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Different predators are observed in the cowpea field, contributing to natural pest suppression. Predators belonging to four orders and seven families of insects were recorded from the cowpea fields of South Rajasthan. Of these, ladybird beetles were the most predominant, comprising 30-40% of the total predator population, followed by the geocorid bug, pentatomid bug, reduviid bug and syrphid flies (4). These predators are killed when chemical pesticides are applied to control pests. On the other hand, biopesticides are considered safe for predators (5). The application of biopesticides not only suppresses insect pests but also poses no residual risks to the environment. Most of the time, biopesticides only affect the target pests and are less hazardous to non-target natural enemies. Biopesticides are effective even at small doses. Hence, in this experiment, five biopesticides were evaluated for their safety to predators.

Materials and Methods

Field experiments were conducted at the Agricultural Research Station, Faculty of Agricultural Sciences, SOADU Binjhagiri, Khordha, Odisha, during the rainy season of 2022 and the winter season of 2022-23, following a randomized block design with three replications. The research station is located at a latitude of 20°23' N and a longitude of 85°83' E. The cowpea variety 'Utkal Manik' was grown as the test variety.

The field was prepared by cross-ploughing with a tractordrawn cultivator, followed by harrowing and planking to obtain well-pulverized soil. The seeds were sown in finely tilled soil, maintaining a planting distance of 45 cm (row to row) and 20 cm (plant to plant). Fertilizers were applied at 25:50:25 Kg of N:P₂O₅:K₂O per hectare. Five biopesticides (B. thuringiensis var. kurstaki 16,000 IU/mg at 1.0 Kg/ha, Beauveria bassiana 108 CFU/g at 2.5 Kg/ha, M. anisopliae 108 spores/g at 2.5 Kg/ha, L. lecani 108CFU/g at 2.5 L/ha and azadirachtin 300 ppm at 2.5 L/ha were evaluated against one chemical check (chlorantraniliprole 18.5% active ingredient at 0.25 L/ha) and an untreated control. Pesticides were applied as a foliar spray using a high-volume knapsack sprayer fitted with a hollow-cone nozzle. The quantity of spray fluid was 500 L/ha (6). The pesticides were applied twice, first, at the initiation of flowering and second, 20 days after the first application. Four species of ladybird beetles and one species of rove beetle were observed in the experimental field. These predators were identified by Dr. S. M. A. Mandal, Professor (retired), Entomology, Odisha University of Agriculture and Technology, Bhubaneswar. The population of the predators was recorded one day before spraying (DBS) and at 5, 10 and 15 days after spraying (DAS) from five randomly selected plants per plot. The population data were subjected to square -root transformation. The transformed data were subjected to analysis of variance (ANOVA) (7).

Results

Ladybird beetles

Relative abundance of species: Four species of ladybird

beetles and one species of rove beetle were found in the experimental field. The ladybird beetles prey on sucking insects in general and aphids in particular. The rove beetles, in addition to feeding on soil insects, consume small larvae and eggs of many insect pests on the plants. The relative abundance of four species of ladybird beetles (calculated over two seasons) is illustrated in Fig. 1. The data indicate that *C. repanda* was the predominant species, contributing 43.84% of the total ladybird beetle population, followed by *C. sexmaculata* (31.51%), *Micraspis discolor* (15.07%) and *B. suturalis* (9.59%), as shown in Fig. 2-5, respectively.

Safety of biopesticides during rainy season, 2022: It is revealed that there was no significant difference in the ladybird beetle population among the treatments, which ranged from 3.95 to 4.33 grubs and adults per five plants at one DBS (Table 1). At five DAS, the highest population of 4.39 grubs and adults per five plants was observed in *B. thuringiensis* var. *kurstaki* (T_1)-treated plots, followed by *B. bassiana* (T_2) (4.28), *M. anisopliae* (T_3) (4.16), *L. lecani* (T_4) (4.10) and azadirachtin (T_5) (3.70) among the biopesticides. These treatments were at par with the untreated control (T_7), which recorded the highest population of ladybird beetles (4.57) and were significantly superior to the chemical check, chlorantraniliprole (T_6), which recorded the lowest population (1.20).

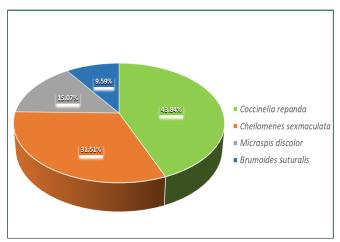


Fig. 1. Relative abundance of ladybird beetle species.



Fig. 2. Ladybird beetle, C. repanda.



Fig. 3. Ladybird beetle, C. sexmaculata.



Fig. 5. Ladybird beetle, B. suturalis.



Fig. 4. Ladybird beetle, M. discolor.

The reduction in the ladybird beetle population compared to the untreated control was the lowest in T_1 (3.94%), followed by T_2 (6.35%), T_3 (8.97%), T_4 (10.28%) and T_5 (19.04%). The highest reduction, 73.74% was recorded in T_6 . Similar trends in population reduction were observed at 10 and 15 DAS across the treatments. However, the toxicity of the chemical check, chlorantraniliprole, reduced gradually at 10 and 15 DAS, with reductions of 62.23% and 35.04% in the ladybird beetle population, respectively.

Safety of biopesticides during winter, 2022-23: It is observed that there was no significant difference in the ladybird beetle population among the treatments, which ranged from 3.37 to 3.80 grubs and adults per five plants at one DBS (Table 2). At five DAS, the highest population of 3.69 grubs and adults per five plants was observed in *B. thuringiensis* var. *kurstaki* (T_1)-treated plots, followed by *B. bassiana* (T_2) (3.61), *M. anisopliae* (T_3) (3.54), *L. lecani* (T_4) (3.48) and azadirachtin (T_5) (2.79) among the biopesticides. These treatments were at par with the untreated control (T_7), which recorded the highest population of the ladybird

Table 1. Population of ladybird beetles under different treatments in cowpea at Bhubaneswar during the rainy season, 2022

			Dose of – commercial products / ha	Population of ladybird beetles# (grub and adult)							
	Treatments	Strength of		1 DBS	5 [5 DAS		DAS	15 DAS		
Tr. no.		commercial products		No. of ladybird beetle / 5 plants	No. of ladybird beetle / 5 plants	Reduction (%) over untreated control	No. of ladybird beetle / 5 plants	Reduction (%) over untreated control	No. of ladybird beetle / 5 plants	Reduction (%) over untreated control	
T ₁	B. thuringiensis var. kurstaki	16000 i.u./mg	1.0 Kg	4.09 (2.14)	4.39 (2.21)	3.94	4.39 (2.21)	5.79	4.26 (2.18)	4.91	
T_2	B. bassiana	10 ⁸ CFU/g	2.5 Kg	4.12 (2.15)	4.28 (2.19)	6.35	4.24 (2.18)	9.01	4.03	10.04	
T ₃	M. anisopliae	10 ⁸ spores/g	2.5 Kg	3.95 [°] (2.11)	4.16 (2.16)	8.97	4.15 (2.16)	10.94	3.90 (2.10)	12.95	
T ₄	L. lecani	10 ⁸ CFU/g	2.5 L	3.98 (2.11)	4.10 (2.14)	10.28	4.09 (2.14)	12.23	3.83 (2.08)	14.51	
T ₅	Azadirachtin	300 ppm (0.03% active ingredient)	2.5 L	4.28 (2.19)	3.70 (2.05)	19.04	3.80 (2.07)	18.45	3.85 (2.08)	14.06	
T_6	Chlorantranilip role	18.5% active ingredient	0.25 L	4.33 (2.20)	1.20 (1.30)	73.74	1.76 (1.50)	62.23	2.91 (1.85)	35.04	
T_7	Untreated control	-	-	4.20 (2.17)	4.57 (2.25)	-	4.66 (2.27)	-	4.48 (2.23)	-	
	SE (m)± CD (0.05)			(0.04) (NS)	(0.17) (0.52)		(0.13) (0.40)		(0.06) (0.18)		

^{*} Ladybird beetles: C. repanda Thunberg, C. sexmaculata Fab., M. discolor Fab. and B. suturalis Fab.

[•] DBS: Day before spraying • DAS: Days after spraying • Figures in parentheses are $\sqrt{(x+0.5)}$ values.

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Table 2. Population of ladybird beetles under different treatments in cowpea at Bhubaneswar during the winter season, 2022-23

		Strength of commercial products	Dose of commercial products / ha	Population of ladybird beetles# (grub and adult)							
				1 DBS 5 DAS			10 DAS			15 DAS	
Tr. no.	Treatments			No. of ladybird beetles / 5 plants	No. of ladybird beetles / 5 plants	Reduction (%) over untreated control	ladybird	Reduction (%) over untreated control	No. of ladybird beetles / 5 plants	Reduction (%) over untreated control	
T_1	B. thuringiensis var. kurstaki	16000 i.u./mg	1.0 Kg	3.43 (1.98)	3.69 (2.05)	3.40	3.25 (1.94)	4.13	3.01 (1.87)	7.38	
T_2	M. bassiana	108 CFU/m	2.5 Kg	3.37 (1.97)	3.61 (2.03)	4.45	3.20 (1.92)	5.60	2.97 (1.86)	8.62	
T_3	M. anisopliae	10 ⁸ spores/g	2.5 Kg	3.80 (2.07)	3.54 (2.01)	7.33	3.13 (1.90)	7.67	2.89 (1.84)	11.08	
T_4	L. lecani	10 ⁸ CFU/g	2.5 L	3.74 (2.06)	3.48 (1.99)	8.90	3.02 (1.88)	10.91	2.82 (1.82)	13.23	
T ₅	Azadirachtin	300 ppm (0.03% active ingredient)	2.5 L	3.65 (2.04)	2.79 (1.81)	29.96	2.55 (1.75)	24.78	2.50 (1.73)	23.08	
T_6	Chlorantraniliprole	18.5% active ingredient	0.25 L	3.59 (2.02)	1.06 (1.25)	72.25	1.18 (1.30)	65.19	2.18 (1.64)	32.93	
T_7	Untreated control	-	-	3.60 (2.02)	3.82 (2.08)		3.39 (1.97)		3.25 (1.94)	-	
	SE (m)± CD (0.05)			(0.05) (NS)	(0.10) (0.31)	-	(0.11) (0.34)	-	(0.04) (0.12)	-	

^{*} Ladybird beetles: C. repanda Thunberg, C. sexmaculata Fab., M. discolor Fab. and B. suturalis Fab.

beetles (3.82) and were significantly superior to the chemical check, chlorantraniliprole (T_6), which recorded the lowest population (1.06).

The reduction in the ladybird beetle population compared to the untreated control was the lowest in T_1 (3.40%), followed by T_2 (4.45%), T_3 (7.33%), T_4 (8.90%) and T_5 (26.96%). The highest reduction, 72.25% was recorded in T_6 . Similar trends in population reduction were observed at 10 and 15 DAS across the treatments. However, the toxicity of the chemical check, chlorantraniliprole, reduced gradually at 10 and 15 DAS, with reductions of 65.19% and 32.93% in the ladybird beetle population, respectively.

Rove beetle

Safety of biopesticides during rainy season, 2022: The roves beetle population among the treatments, which ranged from 3.08 to 3.29 adults per five plants at one DBS, showed no significant difference among them (Table 3, Fig. 6). The

highest population of 3.18 adults per five plants was observed in *B. thuringiensis* var. *kurstaki* (T_1)-treated plots at 5 DAS, followed by *B. Bassiana* (T_2) (3.16), *M. anisopliae* (T_3) (3.12), *L. lecani* (T_4) (3.09) and azadirachtin (T_5) (2.77) among the biopesticides. These treatments were at par with the untreated control (T_7), which recorded the highest population of the rove beetles (3.35) and were significantly superior to the chemical check, chlorantraniliprole (T_6), which recorded the lowest population (0.98) at five DAS.

The reduction in the rove beetle population compared to the untreated control was the lowest in $T_1(5.07\%)$, followed by $T_2(5.67\%)$, $T_3(6.87\%)$, $T_4(7.76\%)$ and $T_5(17.31\%)$. The highest reduction, 70.75% was recorded in T_6 . Similar trends in population reduction were observed at 10 and 15 DAS across the treatments. However, the toxicity of the chemical check, chlorantraniliprole, reduced gradually at 10 and 15 DAS, with reductions of 56.20% and 28.24%, in rove beetle population, respectively.

Table 3. Population of rove beetle under different treatments in cowpea at Bhubaneswar during the rainy season, 2022

		Strength of commercial products	Dose of commercial products / ha	Population of rove beetle# (adult)							
				1 DBS	5 DAS		10 DAS		15 DAS		
Tr. no.	Treatments			No. of rove beetle / 5 plants	No. of rove beetle / 5 plants	Reduction (%) over untreated control	rove	Reduction (%) over untreated control	No. of rove beetle / 5 plants		
T ₁	B. thuringiensis var. kurstaki	16000 i.u./mg	1.0 Kg	3.24 (1.93)	3.18 (1.92)	5.07	3.28 (1.94)	5.48	3.20 (1.92)	5.88	
T_2	B. bassiana	10 ⁸ CFU/g	2.5 Kg	3.29 (1.95)	3.16 (1.91)	5.67	3.23 (1.93)	6.92	3.09 (1.89)	9.12	
T_3	M. anisopliae	10 ⁸ spores/g	2.5 Kg	3.08 (1.89)	3.12 (1.90)	6.87	3.15 (1.91)	9.22	3.06 (1.89)	10.00	
T_4	L. lecani	108 CFU/g	2.5 L	3.15 (1.91)	3.09 (1.89)	7.76	3.11 (1.9)	10.37	3.00 (1.87)	11.76	
T ₅	Azadirachtin	300 ppm (0.03% active ingredient)	2.5 L	3.11 (1.90)	2.77 (1.81)	17.31	2.94 (1.85)	15.27	3.13 (1.90)	7.94	
T ₆	Chlorantraniliprole	18.5% active ingredient	0.25 L	3.20 (1.92)	0.98 (1.22)	70.75	1.52 (1.42)	56.20	2.44 (1.71)	28.24	
T_7	Untreated control	-	-	3.16 (1.91)	3.35 (1.96)	-	3.47 (1.99)	-	3.40 (1.97)	-	
	SE (m)± CD (0.05)			(0.04) (NS)	(0.11) (0.34)		(0.10) (0.31)		(0.03) (0.09)		

^{*} Rove beetle: *P. fuscipes* Curtis • DBS: Day before spraying • DAS: Days after spraying • Figures in parentheses are √(x+0.5) values.

[•] DBS: Day before spraying • DAS: Days after spraying • Figures in parentheses are $\sqrt{(x+0.5)}$ values.



Fig. 6. Rove beetle, P. fuscipes.

Safety of biopesticides during winter, 2022-23: It is perceived that there was no significant difference in the rove beetle population among the treatments, which ranged from 2.62 to 2.88 adults per five plants at one DBS (Table 4). At five DAS, the highest population of 2.54 adults per five plants was observed in *B. thuringiensis* var. *kurstaki* (T_1)-treated plots, followed by *B. bassiana* (T_2) (2.51), *M. anisopliae* (T_3) (2.48), *L. lecani* (T_4) (2.46) and azadirachtin (T_5) (1.95) among the biopesticides. These treatments were at par with the untreated control (T_7), which recorded the highest population of the rove beetles (2.64) and were significantly superior to the chemical check, chlorantraniliprole (T_6), which recorded the lowest population (0.94).

The reduction in the rove beetle population compared to the untreated control was the lowest in T_1 (3.79%) at 5 DAS, followed by T_2 (4.92%), T_3 (6.06%), T_4 (6.82%) and T_5 (26.14%). The highest reduction, 64.39%, was recorded in T_6 . Similar trends in population reduction were observed at 10 and 15 DAS across the treatments. However, the toxicity of the chemical check, chlorantraniliprole, reduced gradually at 10

and 15 DAS, with reductions of 47.12% and 32.16% in the rove beetle population, respectively.

Discussion

The elytra cover a major portion of the dorsum of adult ladybird beetles and thus hinder the entomofungal pathogens from coming into contact with the integument. The wings primarily serve a protective function in insects with elytra, so the effects of contamination are of minor importance in these insects (8). As the predators do not feed on plants, they do not accumulate B. thuringiensis (which acts as a stomach poison) in their gut. Only in the alkaline medium of the gut is the crystal protein (which is a protoxin) produced by B. thuringiensis is transformed into a toxin, killing the insects (9). These may be the reasons why entomofungal and entomobacterial pathogens have been proven safe. Ladybird beetles feed on aphids, which in turn feed on plant sap contaminated with azadirachtin. This may explain why slightly fewer ladybird beetles were found in the plots treated with azadirachtin compared to those treated with microbial pesticides.

B. bassiana 1.15% WP, applied at 1500, 2000, 2500 and 3000 g/ha to control chickpea pod borer, was found to be safer for natural enemies including, coccinellids (10). The biopesticides, B. thuringiensis var. kurstaki, B. bassiana, M. anisopliae and L. lecani were safe for the ladybird beetles, recording only 14.29% and 16.67%, 14.29% and 12.50%, 12.70% and 15.28% and, 9.52% and 11.11% reductions in population compared to the control in two field experiments, respectively (11). Azadirachtin at 0.03% was safer for coccinellids, with a 32.11% reduction in population when used to control cabbage aphid in cruciferous vegetables (12). Azadirachtin 0.03 EC was comparatively safer for predatory coccinellids than the chemical pesticides, with only 19.64 to 24.13% reduction in population on black gram (13). An increase in the coccinellid population over the control was observed even after application of azadirachtin, M. anisopliae and B. bassiana in the cowpea ecosystem (14). The safety of L. lecani 1.15 WP at 0.004% and azadirachtin 10,000 ppm at 0.003% (applied for controlling *A. craccivora* in cowpea) against the ladybird beetles (grubs and adults) was recorded

Table 4. Population of rove beetle under different treatments in cowpea at Bhubaneswar during the winter season, 2022-23

			Dose of commercial products / ha	Population of rove beetle" (adult)							
		Strength of		1 DBS	5 DAS		10 DAS		15 DAS		
Tr. no.	Treatments	commercial		No. of rove beetle / 5 plants	No. of rove beetle / 5 plants	Reduction (%) over untreated control	rove	Reduction (%) over untreated control	No. of rove beetle / 5 plants	Reduction (%) over untreated control	
T_1	B. thuringiensis var. kurstaki	16000 iu/mg	1.0 Kg	2.71 (1.79)	2.54 (1.74)	3.79	2.77 (1.81)	6.10	2.68 (1.78)	5.30	
T_2	B. bassiana	108 CFU/g	2.5 Kg	2.68 (1.78)	2.51 (1.73)	4.92	2.76 (1.80)	6.44	2.66 (1.78)	6.01	
T_3	M. anisopliae	108 spores/g	2.5 Kg	2.62 (1.77)	2.48 (1.72)	6.06	2.74 (1.8)	7.12	2.63 (1.77)	7.07	
T ₄	L. lecani	108 CFU/g	2.5 L	2.85 (1.83)	2.46 (1.72)	6.82	2.68 (1.78)	9.15	2.57 (1.75)	9.19	
T ₅	Azadirachtin	300 ppm (0.03% active ingredient)	2.5 L	2.88 (1.84)	1.95 (1.57)	26.14	2.24(1.65)	24.07	2.28 (1.67)	19.43	
T_6	Chlorantraniliprole	18.5% active ingredient	0.25 L	2.80 (1.82)	0.94 (1.2)	64.39	1.56 (1.43)	47.12	1.92 (1.55)	32.16	
T ₇	Untreated control	-	-	2.78 (1.81)	2.64 (1.77)	-	2.95 (1.86)	-	2.83 (1.82)		
	SE (m)±			(0.03)	(0.08)	-	(0.06)	-	(0.04)	-	
	CD (0.05)			(NS)	(0.25)	-	(0.18)	-	(0.12)	-	
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^{*} Rove beetle: P. fuscipes Curtis • DBS: Day before spraying • DAS: Days after spraying • Figures in parentheses are √(x+0.5) values.

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as 1.50 and 1.44 grubs and adults per plant, respectively, compared to 1.70 grubs and adults in the untreated control (15). All these reports from previous studies are in agreement with the present research findings.

The reasons explained for the safety of microbial and botanical pesticides against the ladybird beetles also apply to the rove beetle, except the fact that the elytra partially cover the abdomen of the rove beetle. The vegetable extract (*Capsicum oleoresin* extract), oils (garlic oil and soybean oil) and one fungal formulation (*Isaria fumosorosea*) were harmless to the rove beetle, *Dalotia coriaria* (Kraatz), recording 80 - 100% adult survival (16). A transgenic rice line harbouring the Cry2Aa gene showed effective resistance to some lepidopteran rice pests, but no harmful effect was detected on the life table parameters of the rove beetle, *P. fuscipes*, in this tritrophic chain (17). All these results from previous research are in agreement with the present findings.

Conclusion

Four species of the ladybird beetles and one species of rove beetle (*P. fuscipes*) were observed in the cowpea crop. Among the ladybirds, *C. repanda* was the most predominant, followed by *C. sexmaculata*, *M. discolor* and *B. suturalis*. All five biopesticides evaluated were found to be safe for both the ladybird beetles and the rove beetle. *B. thuringiensis* var. *kurstaki* was the safest, followed by *B. bassiana*, *M. anisopliae*, *L. lecani* and azadirachtin.

There is ample scope for future research on this topic. In the present investigation, the safety of the biopesticides for predators was evaluated separately. Further studies may be conducted to assess the safety of the combined application of different group of biopesticides *viz.*, bacterial (*B. thuringiensis*), fungal (*B. bassiana, M. anisopliae* and *L. lecani*) and botanical (azadirachtin) towards predators, as well as the effectiveness of their combined application in managing the insect pests.

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Authors' contributions

BM carried out all the experiments. SMAM supervised and BA performed the statistical analysis. SKP planned the study and participated in its design and coordination. All 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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