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### FIELD EVALUATION AND ECONOMICS OF DIFFERENT BIO-RATIONAL INSECTICIDES AGAINST PINK BOLLWORM IN COTTON

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The field experiment was conducted at the Division of Crop Protection, ICAR-CICR, Nagpur, Maharashtra during the cotton growing season 2022-23, which aimed to assess the effectiveness of various biorational insecticides against pink bollworm under field conditions and found that, after the first spray, spinetoram 11.7 SC, lambda-cyhalothrin 5 EC and emamectin benzoate 5 SG recorded lowest flower damage of 7.71, 7.84 and 8.07 per cent, respectively followed by *B. bassiana* + lambda-cyhalothrin (half dose) and *M. anisopliae* + lambda-cyhalothrin (half dose). However, after second and third spray, spinetoram 11.7 SC, lambda-cyhalothrin 5 EC and emamectin benzoate 5 SG were superior in reducing larval incidence, locule and green bool damage, followed by *B. bassiana* + lambda-cyhalothrin (half dose) and *M. anisopliae* + lambda-cyhalothrin (half dose) which displayed a synergistic effect. The plots treated with Spinetoram 11.7 SC, lambda-cyhalothrin 5 EC and emamectin benzoate 5 SG demonstrated the highest B:C ratio of 1.98, 1.95 and 1.89, respectively. Whereas, the lowest B:C ratio (1.31) was observed in azadirachtin 0.15 EC treated plots. This study provides valuable insights into the effective management of pink bollworm in cotton crops by using biorational insecticides.

Key words : Pink bollworm, Cotton, Biorationals, Green boll damage, Larval incidence, Yield economics.

#### Introduction

Cotton (*Gossypium* spp.) also known as the "King of fibres" or "White gold", it plays an important role in the commercial and industrial activity of the nation, both in terms of employment generation in the production and processing sector but also in terms of the foreign market exchange. Cotton is primarily grown for its fibre, which is used to make clothes. It plays a significant part in both the domestic and global economy (Paslawar and Deotalu, 2015). In India, cotton is cultivated on an area of 130.61 lakh ha with production of 343.47 lakh bales of 170 kg and lint productivity of 447.0 kg per ha as against the world average of 800 kg lint per ha (Anonymous, 2022). Pink bollworm is a very destructive insect pest which causes significant yield losses in cotton (Anonymous,

2001). It causes rosette symptoms, malformation of flowers and premature boll opening. It feeds on the developing buds, flowers, bolls and seeds of cotton. Damage translates in heavy shedding of infested flowers and bolls, reduction in fiber length and poor quality of lint due to staining (Kranthi, 2015).

There is overwhelming evidence that the use of chemical pesticides has created many other serious problems like ecological backlashes in pest species, environmental pollution and degradation, threat to biodiversity conservation, loss of beneficial fauna (predators, parasitoids and pollinators) and human beings in particular. Keeping in view, the new generation of pesticides has attracted attention in pest management in recent years, Biorational pesticides as "third-generation pesticides" are derived from some natural source and impose minimum or no adversarial threats on the environment or beneficial organisms (Bhumika and Kritika, 2020). This warrants screening of certain biorational insecticides for their efficacy against pink bollworm. Biorational insecticides often derived from natural sources and environmentally friendly are being investigated as alternatives to traditional chemical pesticides. Evaluating their effectiveness against the pink bollworm, a significant cotton pest, is crucial for sustainable cotton farming practices. The present study suggests that biorational insecticides may offer a promising solution for managing pink bollworm infestations while minimizing the environmental impact associated with chemical pesticides.

#### **Materials and Methods**

The field study was conducted to evaluate the bioefficacy of listed biorational insecticides along with untreated check against pink bollworm at the Division of Crop Protection, ICAR-CICR, Nagpur, Maharashtra during cotton growing season 2022-23. The Cotton variety, Suraj non-Bt sown on 22nd June 2022 at a spacing of  $60 \times 90$  cm in a plot size of  $4.5 \times 6$  m. The experiment laid out in a randomized block design (RBD) with nine treatments replicated thrice. The treatments were imposed three times commencing from 60 Days after sowing (DAS) up to 150 DAS. Where, first spray was taken at 80 DAS, second spray at 100 DAS and third spray at 120 DAS. The spraying was done with pneumatic knapsack sprayer using 1000 liters of spray fluid per hectare.

The observations were recorded on randomly selected 5 plants per replication in each treatment with 10 bolls were picked from the selected plants, then the bolls were dissected and mean worked out on percent infestation. The observations on flower damage, green boll damage, locule damage, open boll damage and larval incidence was recorded one day before each spray, and 3, 7 and 10 DAT. Thus, all the observations were subjected to one way analysis of variance (ANOVA) and the mean values were compared by Duncan Multiple Range Test (DMRT) (P = 0.05) and the preliminary calculations were made by using the formulas given below.

Flower damage (%) = 
$$\frac{\text{Number of rosette flowers}}{\text{Total number of flowers observed}} \times 100$$
  
Boll infestation (%) =  $\frac{\text{Number of infested bolls}}{\text{Total number of bolls}} \times 100$   
Locule damage (%) =  $\frac{\text{Total number of damaged locules}}{\text{Total number of locules}} \times 100$ 

Total no. of GOB's/plant Per cent good opened boll damage (%) =  $\frac{1}{\text{Total opened bolls}} \times 100$ 

#### **Results and Discussion**

#### **Rosette flower**

On the basis of Pretreatment count the spray was done and post treatment observations were recorded at 3, 7 and 10 DAT. Data of all the post treatment observation of per cent infestation of rosette flower during 1<sup>st</sup> spray (Table 1) indicated that all the treatments were significantly superior to untreated control (24.11%). Treatments spinetoram 11.7 SC, lambda-cyhalothrin 5 EC and emamectin benzoate 5 SG were found highly effective and significantly superior to other treatments by recording lowest flower damage of 7.71, 7.84 and 8.01%, respectively and which were on par with each other. Next best treatments were, the combination of the EPF's with half dose of lambda-cyhalothrin 5 EC viz., B. bassiana + lambda-cyhalothrin and M. anisopliae + lambdacyhalothrin with 9.86 and 10.18 %, respectively. Whereas, the control plots recorded highest flower damage (24.11%) followed by azadirachtin 0.15 EC sprayed plots with 11.83 % flower damage. The present findings are corroborated with the results of Pathan et al. (2021) where spinetoram 11.7 SC sprayed plots recorded 8.40% flower damage and lambda-cyhalothrin 5 EC recorded 9.60% followed by emamectin benzoate 5 SG with 12.44% flower damage. Similarly, Gosavi et al. (2019) reported that lambda-cyhalothrin 5 EC was recorded minimum rosette flower (%) among the treated insecticides and azadirachtin recorded highest rosette flower.

#### Green Boll damage

Significantly least green boll damage was observed in the treatment sprayed with spinetoram 11.7 SC (18.61%) and it was on par with the treatments sprayed with lambda-cyhalothrin 5 EC (19.17%) and emamectin benzoate 5 SG (20.22%). Next best treatments were B.  $bassiana10^8$  + half dose of lambda-cyhalothrin and M. anisopliae  $10^8$  + half dose of lambda-cyhalothrin which recorded green boll damage of 26.39 and 27.78 %, respectively (Table 2). The current findings are in line with the results of Pathan et al. (2021), where spinetoram 11.7 SC recorded lower per cent green boll damage of 9.74% followed by lambda-cyhalothrin 5 EC (10.78%) and emamectin benzoate 5 SG (12.73%). Moreover, the obtained results strengthened by Ghure et al. (2008) and Gosavi et al. (2020) they observed that lambdacyhalothrin was recorded minimum per cent boll damage. Similarly, Sharma et al. (2020) who reported the efficacy of spinetoram individually and in combination with

	<b>Table 1</b> : Effect of biorational insecticides on flower damage caused by pink bollworm in cotton after first spray	cides on I	lower damage ca	aused by pink bol	Iworm in cotton ?	utter turst spray.			
E	Tr. Treatments	D	Dosage /ha		Ŗ	Rosette flowers (%)	()		Reduction over
no.		a.i/ha (g/ml)	Formulation	PTC	3DAT	7 DAT	10 DAT	Mean	control (%)
$\mathbf{I}_{1}$	<sup>1</sup> Spinetoram 11.7 SC	50	450 ml	13.00(21.09) <sup>a</sup>	10.07(18.48)	7.03(15.36)ª	6.03(14.19) <sup>a</sup>	7.71(16.03) <sup>a</sup>	68.03
E.	T <sub>2</sub> Emamectin benzoate 5 SG	11	200g	13.10(21.21) <sup>a</sup>	$10.37(18.78)^{a}$	7.38(15.75) <sup>a</sup>	6.28(14.51) <sup>a</sup>	8.01(16.35) <sup>a</sup>	66.77
E.	T <sub>3</sub> Lambda-cyhalothrin 5 EC	20	500 ml	12.96(21.01) <sup>a</sup>	$10.07(18.48)^{a}$	7.17(15.52) <sup>a</sup>	6.27(14.50) <sup>a</sup>	7.84(16.18) <sup>a</sup>	67.48
E.	T <sub>4</sub> Beauveria bassiana 1x10 <sup>8</sup>	1	2500 g	13.10(21.21) <sup>a</sup>	12.63(20.80) <sup>b</sup>	11.20(19.53) <sup>c</sup>	10.83(19.19)°	11.56(19.86)°	52.05
E	T <sub>5</sub> Metarhizium anisopliae 1x10 <sup>8</sup>	I	2500 g	13.22(21.25) <sup>a</sup>	12.78(20.94) <sup>b</sup>	11.71(19.90) والم	10.90(19.24)°	11.79(20.08)°	51.10
E	T6B. bassiana 108 + Lambda-cyhalothrin 5 EC (half dose)	1	2500 g + 250 ml	13.23(21.30) <sup>a</sup>	11.48(19.77) <sup>ab</sup>	9.60(18.00) <sup>b</sup>	8.49(16.90) <sup>b</sup>	9.86(18.27) <sup>b</sup>	59.10
L	T7M. anisopliae 108 + Lambda- cyhalothrin 5 EC (half dose)	1	2500 g + 250 ml	13.10(21.21) <sup>a</sup>	11.80(20.04) <sup>ab</sup>	9.97(18.34) <sup>b</sup>	8.78(17.21) <sup>b</sup>	10.18(18.58) <sup>b</sup>	57.77
H	T <sub>8</sub> Azadirachtin 0.15 EC           (1500 ppm)	1	2500ml	13.23(21.30) <sup>a</sup>	12.55(20.66) <sup>b</sup>	11.80(20.04)	11.13(19.40)°	11.83(20.11)	50.93
$\mathbf{T}_{9}$	, Control	I	I	13.00(21.09) <sup>a</sup>	18.28(25.30)°	27.86(31.84) <sup>d</sup>	26.19(30.77) <sup>d</sup>	24.11(29.53) <sup>d</sup>	ı
	Ftest			NS	Sig	Sig	Sig	Sig	
-									

cotton after first sur . ș ansed hy nink hollworm down on flower Table 1 : Effect of hiorational incertividee sulfoxaflor against PBW effective in reducing the green boll damage.

#### Locule damage

Figures in parenthesis are arcsine transformed values

Means followed by same alphabet in columns did not differ significantly (p=0.05) by DMRT.

PTC - Pre-Treatment Count; DAT - Days after Treatment; NS - Non-Significant

0.85 2.56

> 2.46 8.72

> 2.43

11.25

ï

CD at 5% (p=0.05)

S. Em (±)

CV at 5%

0.82

0.93 2.79 9.09

0.81

9.67

The treatment sprayed with spinetoram 11.7 SC recorded lowest locule damage (12.16%) and it was on par with the treatments sprayed with lambda-cyhalothrin 5 EC (12.32%) and emamectin benzoate 5 SG (13.58%). Next best treatments were *B*.  $bassiana10^8$  + half dose of lambda-cyhalothrin and M. anisopliae  $10^8$  + half dose of lambda-cyhalothrin which recorded green boll damage of 16.34 and 16.92 %, respectively (Table 2). Whereas, M. anisopliae  $10^8$ sprayed plots recorded highest locule damage (20.33 %). The current results are in accordance with the findings of Pathan et al. (2021), who reported lowest locule damage in the treatments sprayed with chlorantraniliprole 18.5 SC followed by spinetoram 11.7 SC and lambda-cyhalothrin 5 EC. Moreover, our results strengthened by Ghure et al. (2008) and Gosavi et al. (2020) they observed that lambda-cyhalothrin was recorded minimum locule damage by PBW among the treated insecticides.

#### **PBW** larval population

The plots sprayed with biorational insecticides recorded significantly lower larval population as compared to control. The least larval population of PBW was observed in the treatment sprayed with spinetoram 11.7 SC (3.45 larvae/10 bolls) and it was at par with the treatment sprayed with lambda-cyhalothrin 5 EC (3.56 larvae/10 bolls) and emamectin benzoate 5 SG (3.84 larvae/10 bolls) (Table 2). These treatments were superior over the remaining treatments viz., B. bassiana  $10^8$  + half dose of lambda-cyhalothrin, M. anisopliae  $10^8$  + half dose of lambda-cyhalothrin, B. bassiana

Table 7	table 2 : Effect of biorational insecticides on plink boilworm larval incidence, green boil and locule damage in cotion.	es on pink pouv	VOTIN LATVAL INCI	dence, greei	n Doll and locul	e uamage in coi	ton.			
ľ	Tr no Treatments	Green	en Boll damage(%)	(%)	Lo	Locule damage(%)	(	Larval inci	Larval incidence(Number / 10 bolls)	c/ 10 bolls)
		2 <sup>nd</sup> Spray	3rd Spray	Mean	2 <sup>nd</sup> Spray	3 <sup>rd</sup> Spray	Mean	2 <sup>nd</sup> Spray	3 <sup>rd</sup> Spray	Mean
$\mathbf{T}_{1}$	Spinetoram 11.7 SC	16.11(23.62) <sup>a</sup>	21.11(27.31) <sup>a</sup>	18.61	7.47(15.84) <sup>a</sup>	16.85(24.31)a	12.16	1.56(1.23) <sup>a</sup>	5.33(2.37) <sup>a</sup>	3.45
$\mathbf{T}_2$	Emamectin benzoate 5 SG	18.22(25.26)ª	18.22(25.26) <sup>a</sup> 22.22(28.07) <sup>a</sup>	20.22	8.6(17.05) <sup>ab</sup>	18.55(25.49) <sup>ab</sup>	13.58	$1.78(1.32)^{ab}$	5.89(2.42) <sup>a</sup>	3.84
$\mathbf{T}_{3}$	Lambda-cyhalothrin 5 EC	16.67(24.04) <sup>a</sup>	21.67(27.73) <sup>a</sup>	19.17	7.64(16.03) <sup>a</sup>	17(24.99) <sup>a</sup>	12.32	$1.44(1.19)^{a}$	5.67(2.30) <sup>a</sup>	3.56
$\mathbf{T}_{4}$	Beauveria bassiana 1x10 <sup>8</sup>	28.33(32.14)°	36.11(36.20) <sup>c</sup>	32.22	12.29(20.52) <sup>c</sup>	12.29(20.52) <sup>c</sup> 27.57(32.24) <sup>c</sup>	19.93	2.78(1.66) <sup>c</sup>	9.78(3.11)°	6.28
T,	Metarhizium anisopliae 1x10 <sup>8</sup>	30.56(33.54)°	37.67(37.26)°	34.11	12.48(20.68)°	12.48(20.68)° 28.18(32.02)°	20.33	3.00(1.73)°	10.00(3.15)°	6.50
Ľ	B. bassiana 10 <sup>8</sup> + Lambda- cyhalothrin 5 EC (half dose)	23.89(29.24) <sup>b</sup> 28	28.89(32.51) <sup>b</sup>	26.39	10.68(19.07) <sup>b</sup>	10.68(19.07) <sup>°</sup> 21.99(27.96) <sup>°</sup>	16.34	2.33(1.53) <sup>b</sup>	8.11(2.84) <sup>b</sup>	5.22
$\mathbf{T}_{7}$	<i>M. anisopliae</i> 10 <sup>8</sup> + Lambda- cyhalothrin 5 EC (half dose)	25(29.97) <sup>b</sup>	30.56(33.54) <sup>b</sup>	27.78	10.94(19.30)	10.94(19.30) <sup>b</sup> 22.89(28.58) <sup>b</sup>	16.92	2.56(1.60) <sup>b</sup>	8.22(2.86) <sup>b</sup>	5.39
$\mathbf{T}_{\mathbf{s}}$	Azadirachtin 0.15 EC (1500 ppm)	31.67(34.23)°	35.00(37.53)°	33.34	12.94(21.06) <sup>c</sup>	12.94(21.06)° 26.82(31.16)°	19.88	3.22(1.79) <sup>cd</sup>	9.44(3.05)°	6.33
T,	Control	44.44(41.79) <sup>d</sup>	53.33(46.91) <sup>d</sup>	48.89	21.2(27.41) <sup>d</sup>	42.65(40.73) <sup>d</sup>	31.93	5.00(2.23) <sup>d</sup>	13.44(3.65) <sup>d</sup>	9.22
	F test	Sig	Sig		Sig	Sig		Sig	Sig	
	S. Em (±)	2.10	2.56		0.70	2.17		0.39	0.18	
	CD at 5% (P=0.05)	6.32	7.69		3.28	6.52		0.30	0.53	
Figures	Figures in parenthesis are arcsine transformed values (except larval incidence, which is square root transformed)	ormed values (6	except larval inc	cidence. whi	ch is square roo	ot transformed)				

Means followed by same alphabet in columns did not differ significantly (p=0.05) by DMRT

 $10^8$ , azadirachtin 0.15 EC and M. anisopliae  $10^8$  recorded with 5.22, 5.39, 6.28, 6.33 and 6.50 larvae per 10 green bolls. The present findings on larval incidence of PBW on green bolls revealed that spinetoram 11.7 SC was highly potent in reducing larval population and it was at par with lambda-cyhalothrin 5 EC and emamectin benzoate 5 SG. The present findings are in accordance with the findings of Pathan et al. (2021), as they recorded lower number of PBW larvae in the treatments sprayed with chlorantraniliprole 18.5 SC followed by spinetoram 11.7 SC and lambda-cyhalothrin 5 EC. Cross reference with Visnupriya and Muthukrishnan (2017) found that plots treated with spinetoram 12 SC followed by emamectin benzoate 5 SG achieved great reduction of H. armigera larval population on Okra.

# Good opened bolls and Bad opened bolls

All the biorational insecticides are found to be significantly different and superior over the untreated control. The per cent good and bad opened bolls due to pink bollworm damage at harvest stage and found that the treatment sprayed with Spinetoram 11.7 SC recorded lowest percent bad opened boll (16.13%) and highest per cent good opened boll (83.87%), which was on par with the treatments, lambda-cyhalothrin and emamectin benzoate with 83.14 and 82.48% good opened boll, and 16.86 and 17.52% bad opened boll, respectively. Whereas, lowest per cent good opened boll (51.83%) was recorded in untreated control with highest per cent bad opened boll (48.17%) (Table 3).

#### Yield and cost economics

All the different treatments sprayed with biorational insecticides recorded significantly higher cotton yield as compared to control. The maximum cotton yield was recorded in the treatment sprayed with spinetoram (20.73 q/ha) followed by lambda-cyhalothrin (19.02 q/ha) and emamectin benzoate (18.52 q/ha). Whereas, EPF's combination with half dose

**Table 3 :** Evaluation of some biorational insecticides on percent infestation of pink bollworm on opened bolls at harvest stage after third spray and yield under field condition.

Tr. no.	Treatments	Dosage (g or ml a.i/ha)	Good opened boll (%)	Opened boll damage (%)	Yield (q/ha)	
T <sub>1</sub>	Spinetoram 11.7 SC	50	83.87(66.36) <sup>a</sup>	16.13(23.64) <sup>a</sup>	20.73	
T <sub>2</sub>	Emamectin benzoate 5 SG	11	82.48(65.29) <sup>a</sup>	17.52(24.71) <sup>a</sup>	18.42	
T <sub>3</sub>	Lambda-cyhalothrin 5 EC	20	83.14(65.79) <sup>a</sup>	16.86(24.21) <sup>a</sup>	19.02	
T <sub>4</sub>	Beauveria bassiana 1x10 <sup>8</sup>	-	69.15(56.27) <sup>c</sup>	30.85(33.73) <sup>c</sup>	13.72	
T <sub>5</sub>	Metarhizium anisopliae 1x10 <sup>8</sup>	-	68.23(55.70) <sup>c</sup>	31.77(34.30) <sup>c</sup>	12.89	
T <sub>6</sub>	<i>B. bassiana</i> 10 <sup>8</sup> + Lambda-cyhalothrin 5 EC (half dose)	-	75.65(60.46) <sup>b</sup>	24.35(29.54) <sup>b</sup>	16.04	
<b>T</b> <sub>7</sub>	<i>M. anisopliae</i> $10^8$ + Lambda-cyhalothrin 5 EC (half dose)	-	74.53(59.72) <sup>b</sup>	25.47(30.28) <sup>b</sup>	15.21	
T <sub>8</sub>	Azadirachtin 0.15 EC (1500 ppm)	-	67.95(55.54) <sup>c</sup>	32.05(34.47) <sup>c</sup>	13.35	
T <sub>9</sub>	Control	-	51.83(46.06) <sup>d</sup>	48.17(43.94) <sup>d</sup>	8.47	
	Ftest	Sig	Sig	Sig		
	S. Em (±)	0.60	0.85	0.54		
	CD at 5% (p=0.05)	1.80	1.79	1.63		

Figures in parenthesis are arcsine transformed values

Means followed by same alphabet in columns did not differ significantly (p=0.05) by DMRT.

Tr. no.	Biorational insecticides	Cotton yield (q/ha)	Cost of protect- ion (Rs.)	Cost of product- ion (Rs.)	Total cost of cultivat- ion (Rs.)	Gross returns (Rs./ha)	Net returns (Rs./ha)	Income Differ- ence	B: C Ratio	ICBR
<b>T</b> <sub>1</sub>	Spinetoram 11.7 SC	20.73	4850	58000	62850	124380	61530	68710	1.98	14.17
<b>T</b> <sub>2</sub>	Emamectin benzoate 5SG	18.42	376	58000	58376	110520	52144	59324	1.89	12.23
T <sub>3</sub>	Lambda-cyhalothrin 5EC	19.02	496	58000	58496	114120	55624	62804	1.95	12.95
<b>T</b> <sub>4</sub>	Beauveria bassiana 1x10 <sup>8</sup>	13.72	625	58000	58625	82320	23695	30875	1.40	6.37
<b>T</b> <sub>5</sub>	Metarhizium anisopliae 1x10 <sup>8</sup>	12.89	625	58000	58625	77340	18715	25895	1.32	5.34
T <sub>6</sub>	<i>B. bassiana</i> 10 <sup>8</sup> + Lambda-cyhalothrin 5EC (half dose)	16.04	870	58000	58870	96240	37370	44550	1.63	9.91
<b>T</b> <sub>7</sub>	<i>M. anisopliae</i> 10 <sup>8</sup> + Lambda-cyhalothrin 5EC (half dose)	15.21	870	58000	58870	91260	32390	39570	1.55	8.16
T <sub>8</sub>	Azadirachtin 0.15 EC (1500ppm)	13.35	2998	58000	60998	80100	19102	26282	1.31	5.42
<b>T</b> <sub>9</sub>	Untreated control	8.47	0	58000	58000	50820	-7180	-	0.88	-

 Table 4 : Economics of the different treatments.

Market value of cotton is 6000 Rs. per quintal

of lambda-cyhalothrin i.e. B. bassiana + lambdacyhalothrin and M. anisopliae + lambda-cyhalothrin recorded 16.04 and 15.21 g/ha, respectively (Table 3). On the basis of present costs of inputs and market selling price of cotton (Suraj Non-Bt @ Rs. 6000/q), the Incremental Cost Benefit Ratio (ICBR) was worked out to interpret the economics of different treatments. Among the different tested biorational insecticides, the highest BC and ICBR ratio was observed in spinetoram 11.7 SC (1.98 and 14.17, respectively) followed by lambdacyhalothrin 5 EC (1.95 and 12.95, respectively), emamectin benzoate 5 SG (1.89 and 12.23, respectively). However, EPF's combination with half dose of lambdacyhalothrin 5 EC such as *B. bassiana*  $10^8$  + lambdacyhalothrin and *M. anisopliae*  $10^8$  + lambda-cyhalothrin recorded BC ratio of 1.63 and 1.55, respectively. Lowest BC (0.88) was recorded in untreated control (Table 4). These findings are corroborated with Pathan et al. (2021), who observed higher ICBR ratio in spinetoram 11.7 SC (1: 19.25) followed by lambda-cyhalothrin 5 EC (1: 17.98).

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#### Author contribution statement

PT, VJT and VCBN designed and carried out the experiment; PT, VL and PRP recorded the data and interpreted the results; PT and SR analyzed the data; PR, RS, VCBN and VJT wrote the manuscript, and all the authors read and approved the final manuscript.

#### **Conflict** of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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