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Insecticide Efficacy against *Earias* Species Infestation of Okra and Residue Analysis of Chlorantraniliprole under Field Conditions in India

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Abstract: A field study was conducted to compare the efficacy of 10 recommended insecticides against *Earias* spp. on okra. Three okra plots (3 m × 5 m (15 m^2)) were subjected to each of the 10 insecticidal sprays applied twice at an interval of 10 days. On the third, seventh, and tenth day after each spray, percent shoot and fruit infestation and yield were calculated. All insecticides significantly reduced shoot and fruit infestation compared to the control. However, chlorantraniliprole 18.5%SC was the most effective across all time points and resulted in the highest yield. A second field study, to determine the time till the maximum residue limit (MRL—0.3 mg/kg) and the limit of quantification (LOQ—0.01 mg/kg) for both fruit and soil exposed to either the recommended (125 mL/ha) or a double strength dose (250 mL/ha) of chlorantraniliprole 18.5% SC was undertaken. MRL was reached on the same day following both doses. The LOQ was reached on the seventh and tenth day at recommended and a double strength dose, respectively. Chlorantraniliprole 18.5% SC provides effective control. However, *Earias* spp. resistance has been observed in other crops. Thus, constant monitoring in the field is needed to ensure its effectiveness.

Keywords: biological insecticide; botanical insecticide; *Earias vittela*; *Earias insulana*; pest control

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1. Introduction

During the last century, insecticides have become an important component of agricultural systems worldwide [1], allowing for a noticeable increase in crop yields and food production [1–4]. Insecticides represent a commonly used method for protecting vegetable crops [5,6], including okra, *Abelmoschus escalentus* (L) (Malvales: Malvaceae) [7,8].

In India in 2018, okra was cultivated over an area of 509,000 hectares with production and productivity of 6095 million metric tons and 119.74 quintal/hectares, respectively [9]. In India, okra is primarily cultivated during the *kharif* season (June–October), when temperatures are warm and humid. These conditions are also ideal for the okra shoot and fruit borer (*Earias* spp.), which is the most yield-limiting pest of okra [10–13]. The current practice is to limit infestation using a range of insecticides, including botanical products derived from plants and synthetic chemicals [14–17]. Therefore, it is necessary to determine the efficacy of insecticides under field conditions.

There are several synthetic insecticides used for the control of *Earias* spp., including organophosphates [18,19], pyrethroids [20,21], and neonicotinoids [22,23]. However, due to the frequent and often indiscriminate [24] use of insecticides, high levels of resistance have been shown in *Earias* populations exposed to certain organophosphate [25], carbamate [26],

and pyrethroid [25] insecticides in different countries [24,26]. In addition, synthetic insecticides have been shown to negatively impact the environment and animal health [27] through harmful chemical residue persistence [3,28], and have been lethal for beneficial insects, such as pollinators [29,30]. However, botanically derived insecticides have been demonstrated to be potentially more eco-friendly with generally a faster degradation time [31–33], leading to fewer negative impacts on beneficial insects [34,35]. Thus, the use of botanical insecticides, such as neem products and other biopesticides to control insect pests of various crops, may be an important alternative [36–38].

Okra is a fast-growing crop with fruits typically harvested every 2–3 days when pods are young. These pods are susceptible to *Earias* spp. infestation and thus may require insecticide application. Frequent picking allows very little time for the insecticide chemical residue on the surface of the fruits to dissipate before being sold to the public for consumption. Insecticide residue in fruits has been documented as being harmful to humans [39], while the residual effects of insecticides in soil have also been found to be harmful to non-target fauna living inside soil [40].

In India, the state of Haryana is one of the largest okra growing states with okra grown on 24.53 (000′ Ha), with a production of 233.96 (000′ MT) and productivity of 9.54 MT/ha [9]. The Central Insecticide Board and Registration Committee (CIB & RC) in India recommends more than 20 different insecticides, including a range of botanical and synthetic chemicals, to target okra shoot and fruit borer. From this list, the 10 most commonly used and recommended insecticides were selected, which were botanical, entomopathogenic or chemical in nature. Thus, the two major aims of this study were: (1) to compare the efficacy of selected insecticides in managing okra shoot and fruit borer; and (2) to determine the time until the maximum residue limit (the highest legal level of pesticide residue) and the limit of quantification (the level at which the residue cannot be detected) for both okra fruits and the cultivated soil.

2. Materials and Methods

2.1. Experimental Design to Determine the Effcacy of Insecticides

To compare the effectiveness of botanical and synthetic insecticides against *Earias vittella* and *Earias insulana* infestation in okra, 10 commonly used and recommended (CBI&RC) insecticides were selected. These included three botanicals (neem oil, Neem Seed Kernal Extract 5%, nimbicidine 300 ppm), one entomopathogenic fungi (*Beauveria bassiana* 1% WP), three synthetic pyrethroids (fenvalerate 20% EC, fenpropathrin 30% EC, lambda-cyhalothrin 4.9% CS), one organophosphate (malathion 50 EC), one anthranillic diamide (chlorantraniliprole 18.5% SC), and a combination of insect growth regulator (IGR) with synthetic pyrethroid (pyriproxifen 5% EC + fenpropathrin 15% EC). The viability of the entomopathogenic fungi (*B. bassiana*) culture was tested in a laboratory at CCS Haryana Agricultural University. After the culture was assessed and found to be viable, it was then applied to the treatment okra plants in the field.

For each of the 11 treatments (10 insecticides and one no-insecticide control), three replicate plots (3 m × 5 m (15 m^2)) were established using a randomized complete block design (RCBD) at CCS Haryana Agricultural University, Hisar field facility. Within each plot, 160 healthy (viable) seeds of the "Varsha uphar" variety of okra were sown on the 20 June 2019, with two viable seeds planted into each hole. Each hole was separated by 60 cm (inter-row space) and 30 cm (inter-plant spacing). Within each plot, plants were thinned by hand at the initial vegetative seedling stage to ensure that there were consistently 80 healthy plants within each plot. Each plot was comprised of well drained, sandy-loam soil, with the topography consistent among plots. Fertilizers were applied before sowing at a rate of 13 kg nitrogen and 24 kg phosphorous per acre, with two applications of nitrogen (at the rate of 13 kg/acre) every three weeks after sowing and at the initiation of flowering [41]. This experiment was conducted throughout the rainy season, and thus plots were only watered following a period of 5–6 days without rain, in which case each plot was watered for five minutes.

Each okra plant within a given insecticidal treatment plot was sprayed twice (using a knapsack sprayer) during the experiment. Spraying was initiated when the fruit infestation reached at least 2% of the economic threshold level (ETL) [42] within each replicate plot. The larval stage of *Earias* spp. in all treatment plots were found to reach the ETL at the same time; thus, all plots were sprayed on the same day. The second spray occurred 10 days after the first spray application. During both applications of the insecticides, the weather was sunny with low wind and no rain.

Shoots and the harvestable size fruits (5–8 cm long pods) were inspected on each sampling day for any signs of insect damage [43], including drooping, withering or drying of the shoots, and the presence of frass on the fruit. Within each plot, five okra plants were selected randomly from the central area of the plot, with plants located on the outer most edge of the plots excluded from the experiment to avoid any edge effects. To determine the level of *Earias* spp. infestation on each of the five selected plants, the number of infested shoots and fruits was counted for each of the treatment plots. From this, the proportion of infested shoots (number of infested shoots/total number of shoots), proportion of infested fruit by number (number of infested fruits/total number of fruits) and the proportion of infested fruit by weight (weight of infested fruits/total weight of fruits), was calculated on the third, seventh and tenth day after the first and second application of the insecticidal spray. In addition, the marketable fruit yield (fruits that were of commercial grade), expressed as metric tons per hectare (qha^{-1}), was recorded per plot and pooled across the three replicate plots for each insecticide, as well as the control.

2.2. Efficacy of Insecticides—Statistical Analysis

To determine the effectiveness of the 10 different insecticides in controlling okra infestation by *Earias* spp., an analysis of randomized complete block design (RCBD) using R-software [44] was performed. The "RCBD" function was used within the "doebioresearch" package. A two-way ANOVA was conducted for each day (third, seventh and tenth day following each spray) separately, comparing the dependent variables (number of infested fruits, infested fruits by weight and number of infested shoots) for each of the 10 insecticidal treatments (independent variables). Different treatment means were separated by Fisher's least significant difference (LSD) test at $\alpha = 0.05$.

2.3. Collection of Okra Fruit Samples for Residue Analysis

Following the efficacy study, an additional nine study plots were constructed and sown with okra in August of 2019. The same field preparation protocols were used, including fertilization rate, seed rate, and spacing, as outlined above to establish the experimental plots. Three replicate plots were sprayed with either the recommended (125 mL/ha) dose of chlorantraniliprole 18.5% SC, a double strength dose (250 mL/ha) or were left untreated (no insecticide applied). The insecticide spray was applied once 50% of the okra plants had reached the fruiting stage. Following the insecticidal spray, 500 g of okra fruit was collected from each of the experimental plots, with fruit pooled across the three replicate plots to give a total of 1.5 kg for each treatment. Fruits were harvested from the plots at 1 h (0 days), 1, 3, 5, 7, 10, and 15 days after the insecticide spray was applied. The collected fruit samples were collected in well labelled zip lock bags and brought to the laboratory for the residue analysis.

2.4. Preparation of Okra Fruit Samples for Residue Analysis

Following the methods used by [45], fresh okra fruits were chopped into small pieces and crushed in a warring blender to form a thick paste. From this paste, a 20-g sample was extracted, placed in a 250-mL conical flask along with 100 mL of acetone, and then shaken for one hour. The sample was then filtered through a Buchner funnel. Filtrate was transferred to 1000 mL separatory funnel partitioned twice with dichloromethane (100, 50 mL) followed by hexane (100, 50 mL). The organic phase of the sample was taken and then it was passed through a layer of anhydrous sodium sulphate. Cleaning of the sample was performed by adding 0.3 g activated charcoal to the organic phase which helped to

remove the color pigments and other impurities found within the sample. The sample was then passed through Whatman filter paper and dried using a rotary vacuum flash evaporator (Buchi R-210). The final volume (3 mL) of the sample was made in *n*-hexane and then injected into a Gas Chromatography Tandem Mass Spectrometer (GC-MS/MS) for further analysis.

2.5. Collecting Soil Samples for Residue Analysis

A total of 500 g of soil was collected along a zigzag transect throughout each experimental plot at the time the fruit was harvested. Soil samples were extracted from a depth of 1–15 cm throughout the plot. The collected samples were brought to the analytical laboratory where they were mixed together to make a sample of 1.5 kg per treatment, and then air dried. After drying, the samples were passed through a 2-mm sieve to remove any large debris.

2.6. Preparation of Soil Samples for Residue Analysis

Soil samples were processed following the method used by [46]. A 15-g representative sample was selected from the sample, and 0.3 g each of florisil and activated charcoal was added, mixed thoroughly. Ten grams of anhydrous sodium sulphate was added to a glass column (60 cm long × 22 mm interior diameter), the soil sample mixture was then added to the column and an additional 10 g of anyhydrous sodium sulphate was added on top of the sample. The sample in the column was then washed with 125 mL of hexane: acetone (9:1 *v*/*v*) solution. The eluate was concentrated to near dryness using a rotary vacuum flash evaporator and the final volume of 2 mL was made in *n*-hexane for GC-MS/MS analysis.

2.7. GCMS/MS Analysis of Chlorantraniliprole 18.5% Residue in Okra Fruit and Soil

To analyze the amount of chlorantraniliprole 18.5% SC within both the okra fruit and the surrounding soil, a gas chromatography-tandem mass spectrometry (GC-MS/MS) was used.

The gas chromatography mass spectrometer had a system split ratio of 1:10, emission current 150 µA and column Rtx-5 with a column length of 30 m, inside diameter 0.25 mm and film thickness of 0.25 µm. A 1-µL aliquot of 1 ppm concentration of each of the fruit and soil samples, subjected to either the recommended or double strength chlorantraniliprole, was injected into the injector port. For greater efficiency and better resolution, the temperature of injector port was maintained at 280 °C, with a detector temperature of 300 °C, ion source temperature 230 °C, and interface temperature of 250 °C. Multiple reaction monitoring (MRM) was used for the sensitive analysis of chlorantraniliprole 18.5% SC residues in okra fruits and soil samples, because this technique allows the detection and quantification of specific molecules in a complex mixture. Under such chromatographic conditions, the retention time (R_t) for chlorantraniliprole 18.5% SC was 20.6 min, with the mass divided by the charged number of ions (m/z) value 278 > 249 and 251. Analysis of each of the fruit and soil was performed in a well-ventilated and air-conditioned laboratory with the temperature maintained below 22 °C and a relative humidity below 60%.

The limit of quantification (LOQ) was found to be 0.01 mg kg^{-1}, calculated by using the sample concentration height, which was 1:10 signal to noise ratio, while the limit of detection (LOD) was 0.003 mg kg^{-1}, which was three times more than the level of base line noise.

3. Results

3.1. Earias Infestation before Application of Insecticide

The percent of damaged shoots and fruits before the initial spray of insecticides varied from 13.16 to 28.13% ($F_{(10)}$ = 1.24, p = 0.33, LSD = 16.61) (Table 1) and 20.71% to 34.54% ($F_{(10)}$ = 1.653, p = 0.163, LSD = 8.84) (Table 2) respectively, and thus was found to not significantly differ among the treatment plots. The percentage weight of infected fruits varied from 23.04% to 36.14%, and also did not significantly differ among the treatment plots ($F_{(10)}$ = 1.90, p = 0.11, LSD = 7.97) (Table 3).

Table 1. Efficacy of 10 recommended insecticides against okra fruit and shoot borer (*Earias* spp.) compared to a no insecticide control in regard to the percentage of shoot infestation following the application two sprays.

S. No.	Treatments	Dose	Percent Shoot Infestation after 1st Spray				Percent Shoot Infestation after 2nd Spray			
			Before Spray	3 DAS	7 DAS	10 DAS	Before Spray	3 DAS	7 DAS	10 DAS
T1	Neem oil	3–4 mL/L water	26.02 ^{a,*}	11.06 ^{f}	11.26 ^{f}	11.55 ^{f}	15.81 ^{bc}	5.18 ^{f}	5.54 ^{f}	5.00 ^{e}
T2	Neem Seed Kernal Extract 5%	50 mL/L water	14.31 ^{a}	8.48 ^{h}	8.26 ^{h}	8.62 ^{h}	13.54 ^{cd}	3.74 ^{h}	3.99 ^{gh}	3.62 ^{g}
T3	Nimbicidine 300 ppm	2.5 L/ha	28.10 ^{a}	10.21 ^{g}	10.22 ^{g}	10.68 ^{g}	7.70 ^{fg}	5.59 ^{f}	5.11 ^{f}	5.29 ^{e}
T4	*Beauveria bassiana* 1×10^9 cfu	4 Kg/ha	23.31 ^{a}	22.79 ^{b}	18.74 ^{b}	22.20 ^{b}	13.33 ^{a}	9.52 ^{b}	9.86 ^{b}	9.99 ^{b}
T5	Fenvalerate 20% EC	300–375 mL/ha	27.78 ^{a}	14.23 ^{d}	13.23 ^{e}	12.94 ^{e}	10.27 ^{ef}	6.41 ^{e}	6.18 ^{e}	6.78 ^{d}
T6	Chlorantraniliprole 18.5% SC	125 mL/ha	15.21 ^{a}	4.96 ^{j}	4.67 ^{i}	4.28 ^{j}	6.85 ^{g}	1.38 ^{j}	1.19 ^{i}	1.00 ^{h}
T7	Malathion 50% EC	1000–1250 mL/ha	13.16 ^{a}	12.18 ^{e}	12.54 ^{e}	12.96 ^{e}	11.82 ^{de}	4.38 ^{g}	4.42 ^{g}	4.00 ^{f}
T8	Fenpropathrin 30% EC	250–340 mL/ha	16.55 ^{a}	14.23 ^{d}	14.68 ^{d}	15.02 ^{d}	13.05 ^{d}	7.46 ^{d}	7.72 ^{d}	7.06 ^{d}
T9	Lambda-cyhalothrin 4.9% CS	300 mL/ha	23.00 ^{a}	19.45 ^{c}	18.57 ^{c}	18.78 ^{c}	16.75 ^{b}	8.32 ^{c}	8.65 ^{c}	9.09 ^{c}
T10	Pyriproxyfen 5% EC+ fenpropathrin 15% EC	500–750 mL/ha	13.86 ^{a}	7.61 ^{i}	7.90 ^{h}	7.96 ^{i}	11.59 ^{de}	3.25 ^{i}	3.60 ^{h}	3.75 ^{fg}
T11	Control	Untreated	28.13 ^{a}	30.99 ^{a}	30.15 ^{a}	31.83 ^{a}	22.09 ^{a}	20.75 ^{a}	20.51 ^{a}	21.18 ^{a}
	Mean		20.86	14.32	14.26	14.62	13.71	6.91	6.98	6.98
	t.value		2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08
	LSD		16.61	0.77	0.74	0.58	2.64	0.49	0.44	0.32
	p-value		0.33	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
	F-value		1.24	872.09	938.08	1718.99	30.71	971.18	1158.89	239.12

* Each value is the average of three replicates; *** *p*-value is in the range [0, 0.001]; DAS—Days After Spraying; LSD = Least Significant Difference; t = critical value of t; *p*-values shows the level of significance; values followed by the same letter are not significantly different from one another.

Table 2. Efficacy of 10 recommended insecticides against okra fruit and shoot borer (*Earias* spp.) compared to a no insecticidal control in regard to the percentage of fruit infestation on a number basis following the application of two insecticidal spray treatments.

S. No.	Treatments	Dose	Percent Fruit Infestation on Number Basis after 1st Spray				Percent Fruit Infestation on Number Basis after 2nd Spray				Yield (Metric Tons)
			Before 1st Spray	3 DAS	7 DAS	10 DAS	Before 2nd Spray	3 DAS	7 DAS	10 DAS	
T1	Neem oil	3–4 mL/L water	29.76 ^{ab,*}	16.76 ^{g}	16.24 ^{g}	15.94 ^{g}	15.67 ^{c,*}	14.90 ^{g}	13.36 ^{g}	12.39 ^{g}	4.08 ^{d}
T2	Neem Seed Kernal Extract 5%	50 mL/L water	24.92 ^{bc}	13.70 ^{h}	13.22 ^{h}	13.10 ^{h}	15.10 ^{c}	10.33 ^{j}	9.42 ^{i}	8.16 ^{j}	4.44 ^{b}
T3	Nimbicidine 300 ppm	2.5 L/ha	34.54 ^{a}	18.56 ^{f}	18.24 ^{f}	18.91 ^{f}	19.89 ^{bc}	15.60 ^{f}	14.18 ^{f}	13.79 ^{f}	3.96 ^{e}
T4	*Beauveria bassiana* 1×10^9 cfu	4 Kg/ha	26.69 ^{abc}	26.16 ^{b}	26.66 ^{b}	26.08 ^{b}	23.00 ^{b}	22.65 ^{b}	22.14 ^{b}	21.71 ^{b}	3.27 ^{i}
T5	Fenvalerate 20% EC	300–375 mL/ha	29.21 ^{abc}	24.19 ^{c}	23.09 ^{cd}	23.91 ^{c}	22.69 ^{b}	17.31 ^{e}	16.26 ^{e}	15.13 ^{e}	3.77 ^{f}
T6	Chlorantraniliprole 18.5% SC	125 mL/ha	20.71 ^{c}	10.53 ^{i}	9.26 ^{i}	9.10 ^{i}	7.93 ^{d}	7.33 ^{k}	6.10 ^{j}	6.03 ^{k}	4.59 ^{a}
T7	Malathion 50% EC	1000–1250 mL/ha	22.88 ^{bc}	21.56 ^{e}	20.76 ^{e}	20.00 ^{e}	19.76 ^{bc}	12.84 ^{h}	11.65 ^{h}	10.94 ^{h}	4.22 ^{c}
T8	Fenpropathrin 30% EC	250–340 mL/ha	25.36 ^{bc}	24.60 ^{c}	23.92 ^{c}	24.33 ^{c}	22.74 ^{b}	18.60 ^{d}	17.98 ^{d}	16.30 ^{d}	3.65 ^{g}
T9	Lambda- cyhalothrin 4.9% CS	300 mL/ha	31.00 ^{ab}	23.11 ^{d}	22.18 ^{d}	21.23 ^{d}	22.69 ^{b}	21.50 ^{c}	20.86 ^{c}	19.96 ^{c}	3.45 ^{h}
T10	Pyriproxyfen 5% EC+ fenpropathrin 15% EC	500–750 mL/ha	27.06 ^{abc}	13.24 ^{h}	13.10 ^{h}	13.03 ^{h}	14.83 ^{c}	11.22 ^{i}	9.98 ^{i}	8.75 ^{i}	4.39 ^{b}
T11	Control	Untreated	27.09 ^{abc}	33.76 ^{a}	39.02 ^{a}	40.54 ^{a}	37.30 ^{a}	32.93 ^{a}	28.50 ^{a}	25.30 ^{a}	3.12 ^{j}
	Mean		27.20	20.65	20.51	20.56	20.14	16.84	15.49	14.41	3.9
	t value		2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.07
	LSD		8.84	0.81	0.96	0.72	6.67	0.65	0.61	0.40	0.11
	p-value		0.16	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
	F-value		1.65	625.17	622.00	1201.55	10.64	1037.82	978.93	1950.78	170.3
	df		10	10	10	10	10	10	10	10	10

* Each value is the average of three replicates; *** *p*-value is in the range [0, 0.001]; DAS—Days After Spraying; LSD = Least Significant Difference; t = critical value of t; df = degree of freedom, *p*-values shows the level of significance; values followed by the same letter are not significantly different from one another.

Table 3. Efficacy of 10 recommended insecticides against okra fruit and shoot borer (*Earias* spp.) as compared to a no insecticidal control in regard to the percent fruit infestation on a weight basis following the application of two insecticidal spray treatments.

S. No.	Treatments	Dose	Percent Fruit Infestation on Weight Basis after 1st Spray				Percent Fruit Infestation on Weight Basis after 2nd Spray			
			Before Spray	3 DAS	7 DAS	10 DAS	Before Spray	3 DAS	7 DAS	10 DAS
T1	Neem oil	3–4 mL/L water	31.42 ^{abc,*}	18.76 ^{g}	18.24 ^{g}	17.94 ^{g}	18.00 ^{cd,*}	16.89 ^{g}	15.36 ^{g}	14.39 ^{g}
T2	Neem Seed Kernal Extract 5%	50 mL/L water	27.25 ^{bcd}	15.70 ^{h}	15.22 ^{h}	15.10 ^{h}	16.80 ^{de}	12.33 ^{j}	11.42 ^{i}	10.16 ^{j}
T3	Nimbicidine 300 ppm	2.5 L/ha	36.14 ^{a}	20.56 ^{f}	20.24 ^{f}	20.89 ^{f}	21.56 ^{bcd}	17.60 ^{f}	16.18 ^{f}	15.79 ^{f}
T4	*Beauveria bassiana* 1×10^{9} cfu	4 Kg/ha	28.68 ^{abcd}	28.16 ^{b}	28.66 ^{b}	28.08 ^{b}	25.00 ^{b}	24.65 ^{b}	24.14 ^{b}	23.71 ^{b}
T5	Fenvalerate 20% EC	300–375 mL/ha	31.74 ^{abc}	26.19 ^{c}	25.09 ^{cd}	25.91 ^{c}	24.47 ^{bc}	19.31 ^{e}	18.26 ^{e}	17.13 ^{e}
T6	Chlorantraniliprole 18.5% SC	125 mL/ha	23.04 ^{d}	12.53 ^{i}	11.26 ^{i}	11.10 ^{i}	10.76 ^{e}	9.33 ^{k}	8.10 ^{j}	8.03 ^{k}
T7	Malathion 50% EC	1000–1250 mL/ha	24.88 ^{cd}	23.56 ^{e}	22.42 ^{e}	22.00 ^{e}	21.76 ^{bcd}	14.84 ^{h}	13.65 ^{h}	12.94 ^{h}
T8	Fenpropathrin 30% EC	250–340 mL/ha	27.42 ^{bcd}	26.60 ^{c}	25.92 ^{c}	26.33 ^{c}	24.74 ^{b}	20.60 ^{d}	19.98 ^{d}	18.30 ^{d}
T9	Lambda-cyhalothrin 4.9% CS	300 mL/ha	33.00 ^{ab}	25.11 ^{d}	24.12 ^{d}	23.23 ^{d}	24.68 ^{b}	23.50 ^{c}	22.86 ^{c}	21.96 ^{c}
T10	Pyriproxyfen 5% EC+ fenpropathrin 15% EC	500–750 mL/ha	28.50 ^{abcd}	15.24 ^{h}	15.10 ^{h}	15.03 ^{h}	16.83 ^{de}	13.22 ^{i}	11.98 ^{i}	10.75 ^{i}
T11	Control	untreated	29.09 ^{abcd}	35.42 ^{a}	41.02 ^{a}	42.54 ^{a}	39.31 ^{a}	34.93 ^{a}	30.50 ^{a}	27.30 ^{a}
	Mean		29.20	22.62	22.48	22.55	22.17	18.84	17.49	16.41
	t value		2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08
	LSD		7.97	0.69	0.998	0.72	6.60	0.65	0.61	0.40
	p-value		0.11	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
	F-value		1.90	856.04	575.77	1201.66	10.48	1040.0	978.93	1950.78
	df		10	10	10	10	10	10	10	10

* Each value is the average of three replicates; *** *p*-value is in the range [0, 0.001]; DAS—Days After Spraying; LSD = Least Significant Difference; t = critical value of t; df = degree of freedom, *p*-values shows the level of significance; values followed by the same letter are not significantly different from one another.

3.2. Earias Infestation following the First Insecticidal Spray

All 10 insecticide treatments had a significantly lower percentage of shoot infestation compared to the no insecticide control after three ($F_{(10)} = 872.09$, $p < 0.001$; LSD = 0.77), seven ($F_{(10)} = 938.08$, $p < 0.001$, LSD = 0.74) and ten ($F_{(10)} = 1718.99$, $p < 0.001$, LSD = 0.58) days after the first insecticide application (Table 1). The number of infested fruits on all insecticide treated plots was found to be significantly lower compared to the no insecticide control after three ($F_{(10)} = 625.17$, $p < 0.001$; LSD = 0.81), seven ($F_{(10)} = 622.00$, $p < 0.001$, LSD = 0.96), and ten ($F_{(10)} = 1201.55$, $p < 0.001$, LSD = 0.72) days following the first insecticide application (Table 2). Likewise, all insecticide treatment plots were found to have a significantly lower percentage of infested fruit on a weight basis compared to the control across all three time points (three days—$F_{(10)} = 856.04$, $p < 0.001$; LSD = 0.69, seven days—$F_{(10)} = 575.77$, $p < 0.001$; LSD = 0.998, ten days—$F_{(10)} = 1201.66$, $p < 0.001$; LSD = 0.72).

Consistently, chlorantraniliprole 18.5% SC had the lowest, while *B. bassiana* 1% WP had the highest percentage of shoot infestation (Table 1), fruit infestation on a number (Table 2) and weight basis (Table 3) across all three time points. We found that shoot infestation differed significantly among most of the insecticide treatment plots. However, results for fenvalerate 20% EC were like fenpropathrin 30% EC on the third day, and fenvalerate 20% EC showed results similar to malathion 50% on seventh and tenth days after the 1st insecticidal application. In addition, the efficacy of most insecticides was significantly different in regard to the number (Table 1) and weight (Table 2) of infested fruit. However, Pyriproxyfen 5% EC + fenpropathrin 15% EC was found to not differ significantly to Neem Seed Kernal Extract 5%, while fenvalerate 20% EC did not differ to fenpropathrin 30% EC after the 1st spray.

3.3. Earias Infestation before the Second Insecticidal Spray

Following the initial spray but before the second application of insecticide (day 11), all treatment plots had significantly fewer infested shoots ($F_{(10)} = 30.71$, $p < 0.001$, LSD = 2.64),

and infested fruit on a number ($F_{(10)}$ = 10.64, $p < 0.001$, LSD = 6.68) and weight ($F_{(10)}$ = 10.48, $p < 0.001$, LSD = 6.60) basis, compared to the control.

3.4. Earias Infestation following the Second Insecticidal Spray

All insecticidal treatment plots had significantly lower numbers of infested shoots following the second insecticide application compared to the control, after three ($F_{(10)}$ = 971.18, $p < 0.001$; LSD = 0.49), seven ($F_{(10)}$ = 1158.89, $p < 0.001$, LSD = 0.44), and ten ($F_{(10)}$ = 2392.12, $p < 0.001$, LSD = 0.32) days. The percent of infested fruits, on a number basis, was also significantly lower compared to the control, following the second insecticide application, after three ($F_{(10)}$ = 1037.82, $p < 0.001$; LSD = 0.65), seven ($F_{(10)}$ = 978.93, $p < 0.001$, LSD = 0.61) and ten ($F_{(10)}$ = 1950.78, $p < 0.001$, LSD = 0.40) days. Likewise, the weight of infested fruits was significantly lower across all treatment plots compared to the control after three days ($F_{(10)}$ = 1040.0, $p < 0.001$; LSD = 0.65), seven days ($F_{(10)}$ = 978.93, $p < 0.001$; LSD = 0.61), and ten days ($F_{(10)}$ = 1950.78, $p < 0.001$; LSD = 0.40).

Consistently, chlorantraniliprole 18.5%SC had the lowest percentage of infested shoots (Table 1) and infested okra fruits on both a number (Table 2) and weight basis (Table 3) across all time points (three, seven, and ten days). Whilst *B. bassiana* 1% WP had the highest percentage of infested shoots and fruits compared to all other insecticides (Tables 1–3), most insecticidal treatments significantly differed from one another in terms of infested shoots, except neem oil and nimbicidine 300 ppm, which did not differ after the second application of the insecticide. In addition, significant differences were observed regarding the number and weight of infested fruits, among all of the insecticide treatments, except pyriproxyfen 5% EC + fenpropathrin 15% EC which did not differ to Neem Seed Kernal Extract 5% on seventh day following the second spray.

3.5. Effect of Insecticides on Okra Yield

Of the okra plants treated with an insecticide, chlorantraniliprole 18.5% SC had the highest cumulative yield (4.59 metric tons/ha) (Figure 1) whilst the fungi, *B. bassiana* 1% WP, had the lowest yield (3.27 metric tons/ha). However, this was still significantly higher than the no insecticidal control which yielded 3.12 metric tons/ha. Analysis of randomized complete block design revealed all other insecticidal treatments significantly differed from one another in terms of the total cumulative yield ($F_{(10)}$ = 170.3, $p < 0.001$, LSD = 0.11) (Table 1).

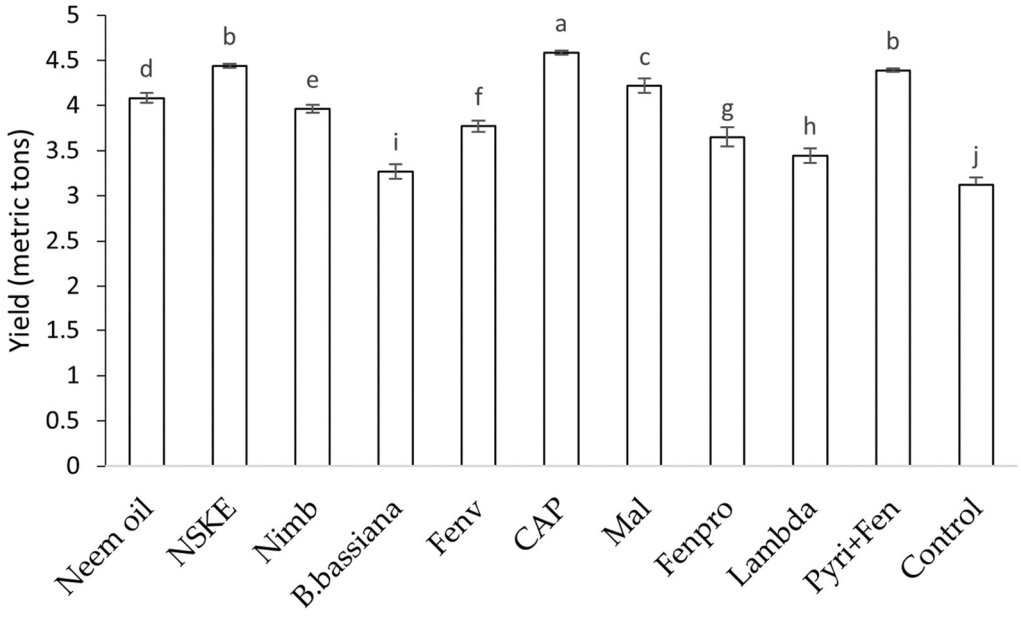

Figure 1. Yield, expressed as metric tons per hectare (q/ha), obtained from the 11 treatment plots (10 insecticides and a no insecticide control). The columns represent the mean values and error bars

denote the standard error (mean ± SE). Bars with a different letter indicates that the treatments are significantly different based on Fisher's LSD at $\alpha = 0.05$. Here, NSKE = Neem Seed Kernal Extract; Nimb = Nimbicidine; *B. bassiana* = *Beauveria bassiana*; Fenv = Fenvalerate; CAP = Chlorantraniliprole; Mal = Malathion; Fenpro = Fenpropathrin; Lambda = Lambda-cyhalothrin; Pyri+Fen = Pyriproxyfen 5% EC + fenpropathrin 15% EC.

3.6. GCMS/MS Analysis of Chlorantraniliprole 18.5% SC Residue in Okra Fruit and Soil

GCMS/MS revealed that on the same day that the recommended (125 mL/ha) and double strength chlorantraniliprole 18.5% SC (250 mL/ha) was applied, both the fruit and soil samples were below the MRL (0.3 mg/kg) within one hour following the spray application of the insecticide (Table 4). The LOQ (the level at which the residue cannot be detected in the sample) of 0.01 mg/kg, was reached seven days after the application of the recommended dose and 10 days after the double strength dose (Table 4). The LOQ within the soil collected from plots exposed to the recommended and double strength chlorantraniliprole 18.5% SC was reached on the same day as the application of the spray treatments (Table 4).

Table 4. Residues (mg kg^{-1}) * of chlorantraniliprole in okra fruits at single and double dose.

Days after Treatment	Chlorantraniliprole Residue (mg kg^{-1})				
	Recommended Dose of CAP (125 mL/ha)		Double Strength Dose of CAP (250 mL/ha)		Control
	Average Residues ± SD	% Dissipation	Average Residues ± SD	% Dissipation	Average Residues ± SD
0	0.083 ± 0.008	-	0.144 ± 0.006	-	ND
1	0.057 ± 0.006	31.33	0.101 ± 0.006	30.10	ND
3	0.025 ± 0.004	70.48	0.056 ± 0.004	60.90	ND
5	0.016 ± 0.001	81.33	0.019 ± 0.002	86.51	ND
7	<LOQ	-	0.015 ± 0.003	89.62	ND
10	-	-	<LOQ	-	ND
Soil at time of harvest	<LOQ		<LOQ		
Correlation Coefficient r = −0.984 Regression Equation y = −0.147x + 1.891 R^2 = 0.984 $t_{1/2}$ = 2.04 days			Correlation Coefficient r = −0.971 Regression Equation y = −0.148x + 2.149 R^2 = 0.971 $t_{1/2}$ = 2.06 days		

CAP = chlorantraniliprole 18.5% SC; ND = Not detected; $t_{1/2}$ = half-life; CD ($p = 0.05$) for days = 0.002; for dose = 0.002; for days × dose = 0.004; For regression equation [residues (mg kg^{-1}) × 10^3] is taken. * Average ± SD of three replicates; LOQ, Limit of Quantification (0.01 mg kg^{-1}).

4. Discussion

This study evaluated and compared the efficacy of ten insecticides that are chemical, biological, or botanical in nature, and which are endorsed by the Indian Government (CIB&RC) against okra shoot and fruit borer. We found that all of the 10 insecticides used in this study were highly effective in controlling *Earias* spp. within the trial period regardless of whether they were botanical, biological, or chemical in nature. Our experiment demonstrated that the chemical-based insecticide, chlorantraniliprole 18.5% SC, was the most effective against shoot and fruit borer (lowest shoot infestation, lowest fruit infestation and highest yield) when compared to all other insecticide treatments tested. We also found that chlorantraniliprole 18.5% SC significantly decreased infestation in okra fruits three days after the initial spray application. This indicates the fast action of chlorantraniliprole 18.5% SC against *Earias* spp. Our results confirm previous research, that chlorantraniliprole 18.5% SC was the most effective insecticide against *Earias* spp. on okra [47,48] and cotton [49] crops when compared to a range of other insecticides which differed in terms of both their modes of action and chemical nature.

Chlorantraniliprole 18.5% SC belongs to the anthranilic diamide group, which is a new group of chemicals that has emerged as one of the most effective groups of insecticides [50]. Chlorantraniliprole 18.5% SC has been found to control insects via activation

of the ryanodine receptors which leads to uncontrolled calcium release in the muscles of the target insect [50]. Chlorantraniliprole 18.5% SC has been found to be fast-acting [51] and highly effective in controlling a broad range of pests in the order Lepidoptera [50], including *Spodoptera frugiperda* [52], *Leucinodes orbonalis* [53], *Ostrinia nubilalis* [54], *Helicoverpa armigera* [55], and *Earias* spp. [56]. Whilst chlorantraniliprole 18.5% SC has been shown to have specific selectivity for the target pests [57], it has also been shown to have low toxicity for many beneficial organisms like pollinators [58,59], as well as natural enemies, including both predators and parasitoids [60–64]. However, a study which contrasted the activity levels of pollinating bees found that bees exposed to chlorantraniliprole 18.5% SC took longer to return to normal activity patterns compared to bees exposed to Neem Seed Kernal Extract [65]. Thus, there is trade-off between the most effective insecticide and those that have a lower level of toxicity towards non-target insects.

We found that the efficacy of the botanical insecticides; used in this study, Neem oil, Neem Seed Kernal Extract 5%, Nimbicidine 300 ppm, was significantly less efficient in controlling *Earias* spp. fruit infestation compared to chlorantraniliprole 18.5% SC, however, it was significantly more effective than the no insecticidal control. Likewise, some other studies have also found neem products to be highly effective against *Earias* spp. in okra in Bangladesh and southern part of India [66,67]. Neem based botanical insecticides are known to have low toxicity and residual effects [68], which indicates a lowered risk to natural enemies (predators and parasitoids) [68]. Furthermore, neem-based insecticides are cost effective and can be prepared by farmers at their home [69], making their use highly practical and economical when compared to chemical insecticides. The difference between the effectiveness of neem products may be due to the differences in the concentration of the active ingredients [70]. This is highlighted by previous studies which found that neem-based insecticides with a higher concentration of active ingredients (azadirachtin) were more effective against a range of pests, including *Plutella xylostella* [71] and *Coptotermes gestroi* [72].

Infestation of *Earias* spp. was also significantly less in the plots treated with IGR (pyriproxyfen) and synthetic pyrethroids (fenvelerate, fenpropathrin, and lambda-cyhalothrin) as compared to the control, a result which has also been found in other studies on okra crop [73]. Although synthetic pyrethroids were not as effective against *Earias* spp. infestation compared to chlorantraniliprole 18.5% SC, the multiple modes of action that these insecticides provide may allow better control over pests [74,75] due to their synergistic effects [76–78].

It took 10 days for the fungal insecticide *B. bassiana* 1% WP to reach the same reduced level of fruit and shoot infestation observed for all other insecticide treatments three days after the initial spray. The difference in activity against *Earias* spp. infestation observed between chemical insecticides and the fungal insecticides used in our study may, in part, be due to of the climatic field conditions experienced during our experiment [79]. The fungi (*B. bassiana*) depends on specific abiotic factors, including certain temperature, sunlight, moisture, and humidity levels [79] for inoculum buildup, storage, and development. Furthermore, for the *B. bassiana* to develop spores and reproduce, it depends on the microenvironment of the host body surfaces as well [80]. Hence, it is likely that climatic factors may have limited the effectiveness of *B. bassiana* against *Earias* spp. infestation as the region of Haryana where this experiment was conducted experiences high temperatures.

Despite the clear effectiveness of chlorantraniliprole 18.5% SC as an insecticide, there is the possibility that *Earias* spp. could become resistant when the chemical is used in excess or for an extended period of time [81]. Resistance to other anthranilic diamide insecticides has already been reported for lepidopteran pests, e.g., *Plutella xylostella* [81,82] and *Tuta absoluta* [83,84]. Thus, caution needs to be taken in relying solely upon one chemical, such as chlorantraniliprole 18.5% SC, as the primary method of control as this is likely to promote the development of insecticide resistance in *Earias* spp. if used continuously or inappropriately [84].

Analysis of the residue from both okra fruits and the surrounding cultivated soil exposed to the recommended and a double strength dose of chlorantraniliprole 18.5% SC showed that MRL is achieved on the same day as the insecticide application, which is ideal,

given fruits are continually harvested throughout the fruiting season. Our study found that LOQ for fruits exposed to the recommended dose of chlorantraniliprole 18.5% SC was reached seven days after the spray application under field conditions in Haryana. A similar study which used the recommended dose of chlorantraniliprole 18.5% SC on okra, conducted in Tamil Nadu, India, found that the LOQ was reached 10 days after the insecticide application [85]. The difference between these studies could be attributed to the environmental conditions under which each experiment was conducted [86,87], with Tamil Nadu being a coastal region which experiences greater humidity than Hisar, Haryana, which is located in a semi-arid region.

Similarly, studies which have also examined the chemical residual nature of chlorantraniliprole 18.5% SC in vegetable crops exposed to the recommended dose, found that the insecticide reached the LOQ in seven days for eggplant [88], seven days in cabbage [89], 21 days in tomato [90], and seven days in capsicum [91]. Differences in the time to reach the LOQ are primarily driven by the dissipation behavior of chlorantraniliprole 18.5% SC which is dependent on the environmental conditions, dosage of insecticide applied, and the plant species being treated [92,93].

5. Conclusions

All insecticides used in this experiment were effective against *Earias* spp. infestation and as a result we recommend consistent monitoring of pests with the use of target specific insecticides that balance the effectiveness of the insecticide against the toxicity of the chemical towards non-target insects. Residue of okra fruit following the recommend and a double dose of chlorantraniliprole 18.5% SC reached the safe level, that is MRL, on the same day as the insecticide application and reached LOQ within one week and 10 days, respectively. Additionally, chlorantraniliprole 18.5% SC was not detected in the soil on the same day as the insecticide was applied. The insecticide that was consistently the least effective, the botanical fungal insecticide *B. bassiana*, may have been limited by the climatic field conditions experienced during the experiment.

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