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Investigating the efficacy of a Diamide insecticide over some time against *Spodoptera frugiperda* Smith, 1797 (Lepidoptera: Noctuidae)

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Abstract
Spodoptera frugiperda Smith, 1797 (Lepidoptera: Noctuidae) is commonly known as fall armyworm or FAW. It affects more than 300 crops including maize, sorghum, sugarcane, paddy, spinach, potato, castor, and chickpea where maize is being attacked the most (Montezano *et al*., 2018) [7]. This insect pest has been causing severe loss to crops since its first invasion in India (Deshmukh *et al*., 2018) [3]. Various classes of Insecticides like organophosphates carbamates and synthetic pyrethroids are being used by the farmers in the agricultural fields to control the pest but in vain. Hence, a new insecticide, a diamide: chlorantraniliprole was chosen for laboratory testing against *S. frugiperda*. Insect pest was brought from fields and reared in the lab for multiple generations to create a pure breed and study the pesticide-resistance development in insects if any. Laboratory rearing of *S. frugiperda* was done on a chickpea-based artificial diet. The LC50 was achieved at a concentration of 0.05 ppm in the first three generations of testing on larval instars whereas, in the fourth generation, LC50 was achieved at 0.5 ppm. The effectiveness however reduced after three generations. The present work highlights that; the diamide Chlorantraniliprole is effectively controlling the population of *S. frugiperda* from generations 1 to 3 and can be recommended to the farmers. At the same time, overuse of Chlorantraniliprole cannot be advocated as, in Generation 4 perishability decreases leading to the development of resistance against Chlorantraniliprole in *S. frugiperda*.

Keywords: Chlorantraniliprole, fall armyworm, lepidoptera, insect-pest

Introduction

One of the troublesome agricultural pests is the insect *Spodoptera frugiperda* Smith, 1797 (Lepidoptera: Noctuidae). The *Spodoptera frugiperda* originated in America (Sparks, 1979) [12]. An unexpected breakout of the fall armyworm population in Africa was reported by Goergen *et al*., 2016) [4]. The first time infestation of *Spodoptera frugiperda* in India was reported by Deshmukh *et al*., in 2018) [3] in the state of Karnataka. The problem worsened as *S. frugiperda* became resistant to many commonly used older classes of insecticides like organophosphates, carbamates, and synthetic pyrethroids and caused crop failure in Florida where broad spectrum insecticide resistance was observed in field strain (Yu, 1991) [13]. In Gujarat, the first *S. frugiperda* invasion reports came from the maize fields of Anklav village of Anand by Sisodiya *et al*, 2018 [11]. The first report of FAW was from Southern parts of Rajasthan like Banswara (Babu *et al*., 2019) [1]. In Maharashtra, *Spodoptera frugiperda* infestation was detected on sugarcane in Sangli district (Chormule *et al*., 2019) [2].

The insect pest FAW is already known to be resistant to older classes of insecticides including organophosphates, carbamates, and pyrethroids (Yu *et al*., 1991) [13]. Therefore, chlorantraniliprole, a new-age insecticide against lepidopteran pests was selected for laboratory studies. Generation studies were done to test the efficacy of the insecticide over multiple generations. The main purpose of the study was to examine the capacity of the insecticide chlorantraniliprole to fight *S. frugiperda* keeping in mind the possibility of resistance development in future generations.

Methodology

Spodoptera frugiperda was collected from Vadodara's agricultural fields from the crop of maize in the third-fourth instar stage (Figure 1). The pest was collected after a complete inspection. Various life stages of the insect pest, including eggs, larvae, pupae, and adults, were reared in different boxes in the lab. The ideal temperature, humidity, and photoperiod were tuned for artificial rearing (25±2 °C temperature and 70% humidity, 12:12 D: L). An artificial diet was used to raise the larvae (Table 1). The diet used was first standardized and provided to the adults (Table 2). To avoid cannibalism, trays were designed with different cells so that every cell could be used to inhabit a single larva. Sticker lids were made to cover the tray, which prevented the escape of insects. Diets were changed regularly until pupal formation. Pupae were reared into adults and given a diet based on honey.

Testing of Chlorantraniliprole on larvae: Technical-grade chlorantraniliprole was bought from Sigma Aldrich. After creating stock solutions of the insecticides serial dilutions were prepared to make doses in ppm from stock solutions of the insecticide. According to the recommendations of the Insecticide Resistance Action Committee (IRAC), the pesticide was added to the standard diet. The concentration range of insecticide was investigated for each generation. The start of resistance was defined as the generation that had the least impact on the population or the point at which the concentrations appeared to be ineffective in managing the population in the needed numbers. The pest was believed to have perhaps developed resistance by this generation.

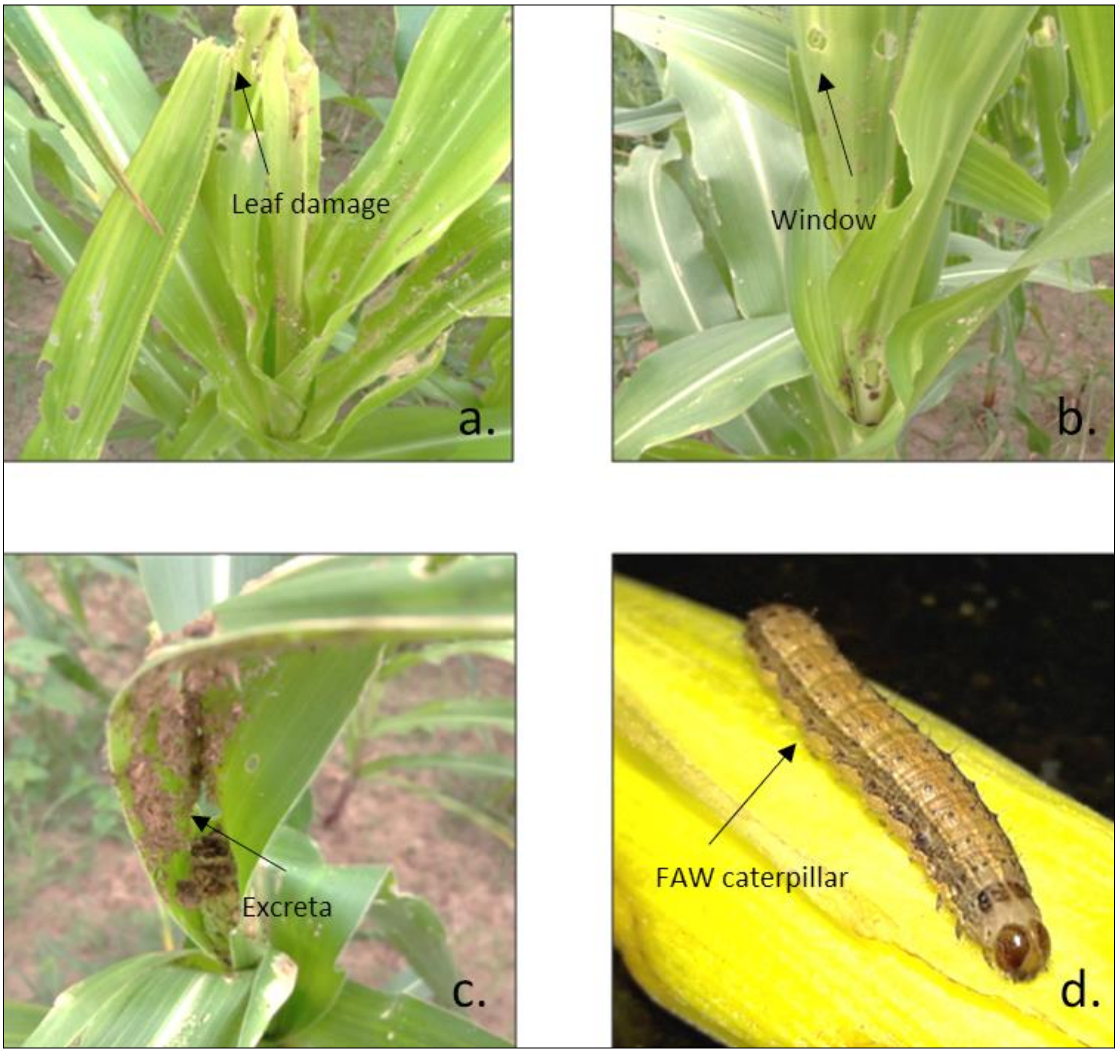


Fig 1: a. *S. frugiperda* damaging the leaves of maize fields b. Transparent patches called "windows" Created by the larval instar c. Larval excreta on leaves d. FAW caterpillar on maize cob

Table 1: Artificial diet for larval stages

S.No.	Ingredients	Amount
1.	Agar-agar powder	20g
2.	Ascorbic acid	5.3 g
3.	Becosule	12 ml
4.	Formaldehyde (10%)	13.5 ml
5.	Chickpea powder	160 g
6	Wheat germ	60 g
7.	Methyl-paraben	3.3 g
8.	Propionic acid	2 ml
9.	Sorbic acid	1.7 g
10.	Yeast powder	53 g
11.	Sucrose	36 g
12.	Water	1000ml

Table 2: Artificial diet for adults

S.No.	Ingredients	Amount
1.	Honey	100 g
2.	Sucrose	100 g
3.	Becosule	4 g
4.	Methyl paraben	4 g
5.	Ascorbic acid	40 g
6.	Water	1000 ml

Results

The insecticide selected was chlorantraniliprole against S*podoptera frugiperda.* To test the efficacy, mother culture was multiplied through lab rearing on a suitable artificial diet.

Any kind of bacterial or fungal infections were examined. If a larva didn't move after coming in touch with the brush it was thought to be dead. If a larva moved less erratically than an untreated larva; it was deemed to be moribund. A larva was deemed to be alive if, following exposure to a stimulus from the brush, it moved normally in comparison to an untreated larva (or any physical stimulus).

Testing the insecticide Chlorantraniliprole on the First generation (G1) of larva:

The pest *Spodoptera frugiperda* was treated with Chlorantraniliprole at different concentrations. The concentrations, i.e., 10, 5, 1, 0.5, 0.1, 0.05, 0.02 and 0.01 ppm, gave % mortalities as 100.00, 100.00, 100.00, 93.33, 60.00, 53.33, 46.66, and 20.00, respectively. LC50 value came at 0.05 ppm. No mortality in control was observed (Table 3).

The surviving insects from the various dose ranges were taken to further generations. Regular treatments on the third instar larvae were given until a decline in expected mortality was observed.

Testing the insecticide Chlorantraniliprole on the Second generation (G2) of larva:

The concentrations, i.e., 10, 5, 1, 0.5, 0.1, 0.05, 0.02 and 0.01 ppm, gave % mortalities as 100.00, 100.00, 100.00, 93.33, 60.00, 50.00, 46.66, and 16.67, respectively. LC50 came at 0.05 ppm. No mortality in control was observed (Table 4).

Testing the insecticide Chlorantraniliprole on the Third generation (G3) of larva:

The concentrations, i.e., 10, 5, 1, 0.5, 0.1, 0.05, 0.02 and 0.01 ppm, gave % mortalities as 100.00, 93.33, 86.66, 60.00, 50.00, 46.66, 20.00, 10.00 in the third generation, Respectively. LC50 came at 0.05 ppm. No mortality in control was observed (Table 5).

Testing the insecticide Chlorantraniliprole on the Fourth generation (G4) of larva:

The concentrations 10, 5, 1, 0.5, 0.1, 0.05, 0.02 and 0.01 ppm gave % mortalities of 100.00, 90.00, 60.00, 50.00, 46.66, 20.00, 0.00, 0.00 in the fourth generation. LC50 came at 0.5 ppm. No mortality in control was observed (Table 6).

Mortality values over the generation are noted and a significant change in the fourth generation was seen (Table 7).

Table 3: Larval (%) mortality obtained in *S. frugiperda* against Chlorantraniliprole (G1)

Sets of conc.	Dose (ppm)	No. of treated larvae	No. of larvae succumbed	Defunct larvae	Total (Succumbed+ Defunct)	Larval per cent mortality
1	10	30	30	0	30	100.00
2	5	30	30	0	30	100.00
3	1	30	30	0	30	100.00
4	0.5	30	26	2	28	93.33
5	0.1	30	15	3	18	60.00
6	0.05	30	14	2	16	53.33
7	0.02	30	13	1	14	46.66
8	0.01	30	4	2	6	20.00
9	Control	30	0	0	0	0.00

Table 4: Larval (%) mortality obtained in *S. frugiperda* against Chlorantraniliprole (G2)

Sets of conc.	Dose (ppm)	No. of treated larvae	No. of larvae succumbed	Defunct larvae	Total (Succumbed+ Defunct)	Larval per cent mortality
1	10	30	30	0	30	100
2	5	30	30	0	30	100
3	1	30	28	2	30	100
4	0.5	30	24	4	28	93.33
5	0.1	30	16	2	18	60.00
6	0.05	30	14	1	15	50.00
7	0.02	30	13	1	14	46.66
8	0.01	30	3	2	5	16.67
9	Control	30	0	0	0	0.00

Table 5: Larval (%) mortality obtained in *S. frugiperda* against Chlorantraniliprole (G3)

Sets of conc.	Dose (ppm)	No. of treated larvae	No. of larvae succumbed	Defunct larvae	Total (Succumbed+ Defunct)	Larval per cent mortality
1	10	30	30	0	30	100.00
2	5	30	27	1	28	93.33
3	1	30	23	2	25	86.66
4	0.5	30	14	4	18	60.00
5	0.1	30	14	1	15	50.00
6	0.05	30	13	1	14	46.66
7	0.02	30	5	1	6	20.00
8	0.01	30	2	1	3	10.00
9	Control	30	0	0	0	0.00

Table 6: Larval (%) mortality obtained in *S. frugiperda* against Chlorantraniliprole (G4)

Sets of conc.	Dose (ppm)	No. of treated larvae	No. of larvae succumbed	Defunct larvae	Total (Succumbed+ Defunct)	Larval per cent mortality
1	10	30	30	0	30	100.00
2	5	30	23	4	27	90.00
3	1	30	15	3	18	60.00
4	0.5	30	13	2	15	50.00
5	0.1	30	13	1	14	46.66
6	0.05	30	4	2	6	20.00
7	0.02	30	0	0	0	0.00
8	0.01	30	0	0	0	0.00
9	Control	30	0	0	0	0.00

Table 7: Mortality of *S. frugiperda* larvae over generations (G1-4)

Concentration (ppm)	G-1	G-2	G-3	G-4
10	100.00	100	100.00	100.00
5	100.00	100	93.33	90.00
1	100.00	100	86.66	60.00
0.5	93.33	93.33	60.00	50.00
0.1	60.00	60.00	50.00	46.66
0.05	53.33	50.00	46.66	20.00
0.02	46.66	46.66	20.00	0.00
0.01	20.00	16.67	10.00	0.00
Control (or Untreated)	0.00	0.00	0.00	0.00

Generation 1^{st}- 4^{th} (G1-4)

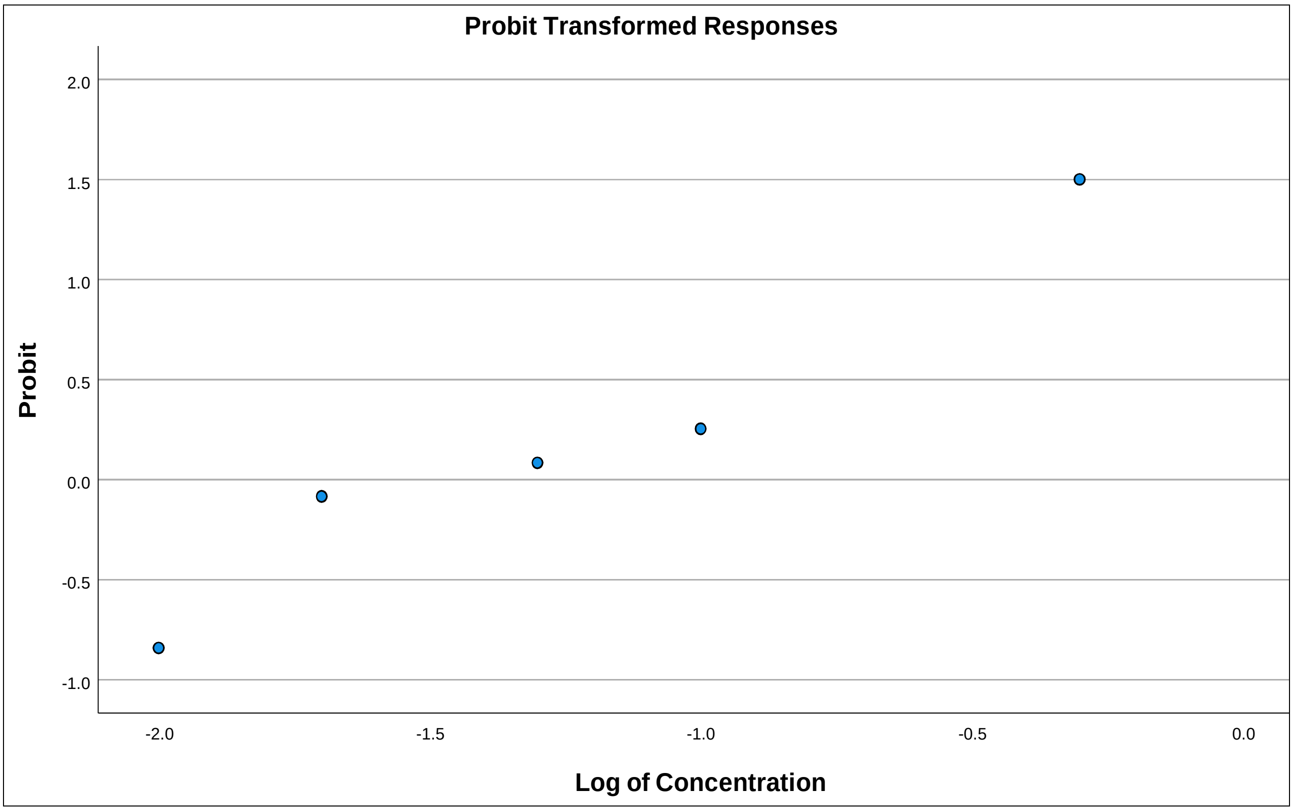
Mortality in the first three generations at different doses of insecticide Chlorantraniliprole remains almost the same (Tables 3, 4, and 5) whereas in the fourth generation, there was a decline of mortality of larval instar when subjected to the insecticide (Table 6)

Parameter estimates and probit-transformed responses were noted. Estimates for Chlorantraniliprole generation 1^{st} (G1) to Chlorantraniliprole generation 4^{th} (G4) is shown (Table 8-11**)**. Probit-transformed responses in every generation to both insecticides are shown graphically (Graph 1-4**)**.

Table 8: Probit analysis using software SPSS for chlorantraniliprole- G1

Parameter Estimate			Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBITa	Concentration	1.340	.182	7.370	<.001	.984	1.696
	Intercept	1.900	.256	7.422	<.001	1.644	2.156

a. PROBIT model: PROBIT (p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithms.)

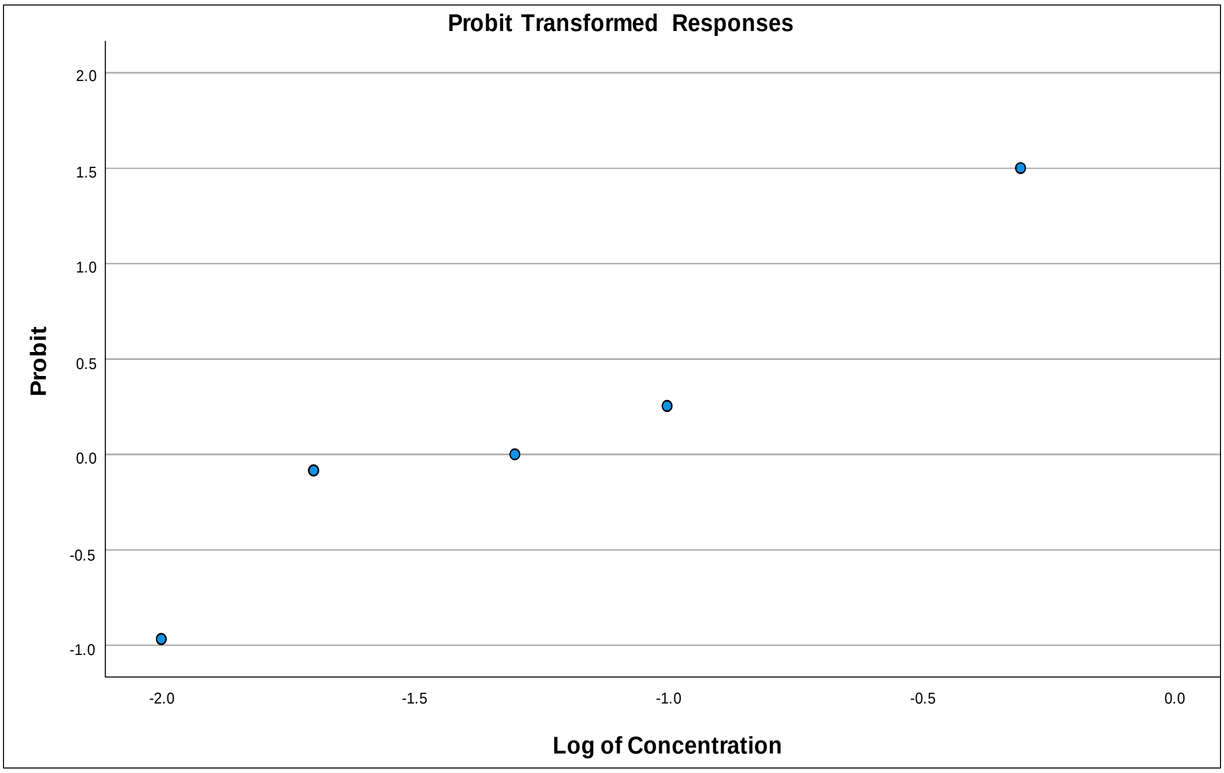


Graph 1: Probit response for Chlorantraniliprole (G1)

Table 9: Probit analysis using software SPSS for chlorantraniliprole- G2

Parameter	Estimate		Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBITa	Concentration	1.384	.185	7.479	<.001	1.022	1.747
	Intercept	1.916	.259	7.386	<.001	1.656	2.175

a. PROBIT model: PROBIT (p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithms.)

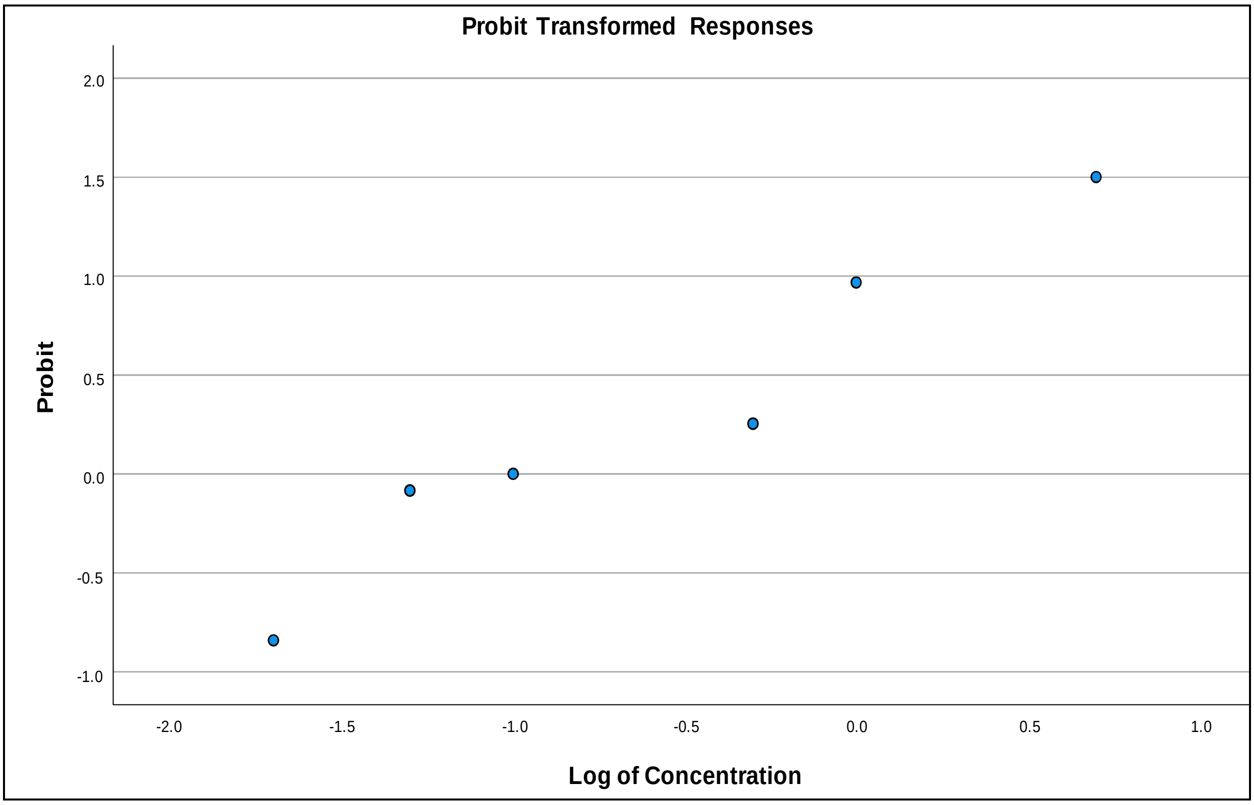


Graph 2: Probit response for Chlorantraniliprole (G2)

Table 10: Probit analysis using software SPSS for chlorantraniliprole- G3

Parameter	Estimate		Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBITa	Concentration	1.088	.121	9.030	<.001	.852	1.324
	Intercept	.929	.139	6.700	<.001	.790	1.068

PROBIT model: PROBIT (p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithms.)

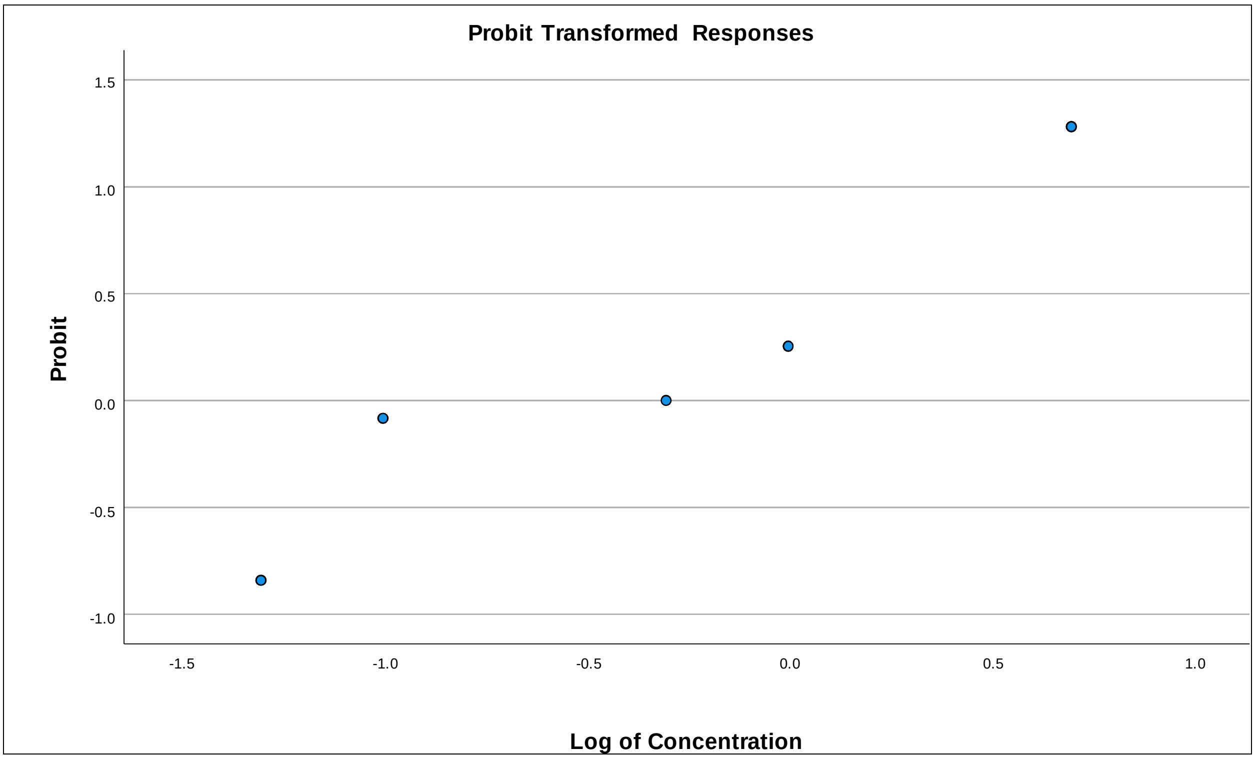


Graph 3: Probit response for Chlorantraniliprole (G3)

Table 11: Probit analysis using software SPSS for chlorantraniliprole- G4

Parameter	Estimate		Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBITa	Concentration	1.209	.127	9.527	<.001	.960	1.457
	Intercept	.545	.123	4.428	<.001	.422	.668

a. PROBIT model: PROBIT (p) = Intercept + BX (Covariates X are transformed using the base 10.000 logarithms.)



Graph 4: Probit response for Chlorantraniliprole (G4)

Probit analysis using SPSS was done, and mortality values over the generations were found (Graphs 1-4). In Graph, the shift in the probit value can be seen as compared to Graphs 1-3. This shows the change in the concentration (increment) for causing the mortality of the insect pest.

Discussion

A prevalent pest in recent times is *Spodoptera frugiperda Smith*, with maize as the most preferred host crop. Padhee & Prasanna in 2019 [8] analysed the instance of fall armyworm infestation in India, highlighting the countrywide spread. The spread started from nine districts of Karnataka to many other Indian states. Fall armyworm has been infamous in the country in recent times due to its severe invasion in India and its' not responding to the commonly available insecticides. The severity of the pest arises due to the resistance present in the insect to the majority of the older classes of insecticides. This calls for the need to test newer insecticides against insect pests. Newer insecticides are known to be efficient in eliminating lepidopteran pests. Singh *et al*., 2022 [10], examined the effectiveness of Spinosad and Emamectin Benzoate against *Helicoverpa armigera* on tomatoes in Varanasi. Both insecticides were found to be effective in reducing the loss caused by the fruit borer and increasing the yield of the tomato crop.

In our study low quantity of chlorantraniliprole is found to be sufficient in controlling the insect pest *S. frugiperda*. Paramasivam *et al*., 2022 [9] in Tamil Nadu, evaluated the risk assessment of Chlorantraniliprole in chilli crops. They found that harvested chilli can be safely consumed, provided the insecticide is applied at the recommended dose. Such studies suggested that testing the new-age diamide-chlorantraniliprole (Chemical formula-C18H14BrCl2N5O2) against the pest *Spodoptera frugiperda* is advisable and safe. It is a novel insecticide belonging to the diamide class, discovered by Dupont, and used to control lepidopteran pests. A control population was maintained throughout the study and was not subjected to any testing. Results showed that 0.01 ppm, gave % a mortality of 20% in G1, 16.67% in G2, and 10.00 % in G3 but, no mortality in G4 (Table 7). We observed that a low quantity of the insecticide Chlorantraniliprole is sufficient to cause mortality to the insect pest with low risk to the environment. However, efficacy was reduced in the fourth generation. Such a decrease in the expected mortality of the pest insect raises the possibility of resistance development being initiated in the pest against the insecticides. Kumar *et al*., 2022 [6], checked the susceptibility of test pesticides in different state populations of FAW. From the five test populations, the Bihar test population had the least susceptibility to insecticides including avermectins and diamides, whereas Karnataka and Tamil Nadu were found most susceptible. Continuous application of the same insecticides in higher dosages can be a reason for the resistance. This calls for sustainable usage of the insecticide along with other methods of control. It is hence recommended that a good IPM should be practised to avoid or reduce the problem of resistance.

Conclusion

Spodoptera frugiperda Smith is a major agricultural pest all over India. It affects more than 300 crops including maize, sorghum, sugarcane, paddy, spinach, potato, castor, and chickpea (Montezano *et al*., 2018) [7]. This insect pest has been causing severe loss to crops since its first invasion in India (Deshmukh *et al*., 2018) [3]. Most affected by *S. frugiperda* was the Maize crop. Its presence was observed in various Maize fields of Vadodara, causing heavy damage to the crop. The fall armyworm starts attacking the crop once the maize crop is 2 to 3 weeks old. The 3rd & and 4th instar larvae of *S. frugiperda* damage the tassel, the ear of corn, and the leaves and also damage silk, husk and fruit. Farmers are using various insecticides such as organophosphates (malathion, chlorpyriphos), carbamates (aldicarb, carbaryl), pyrethroids, (deltamethrin, cypermethrine), etc to control *S. frugiperda* in the agricultural fields. Even synergistic insecticides such as Hamla (a combination of organophosphorus and synthetic pyrethroid) sprayed in agricultural fields are not able to bring down the population of fall armyworms. Hence, a new insecticide, a diamide: chlorantraniliprole was chosen for laboratory testing against *S. frugiperda*. Chlorantraniliprole is a green label insecticide recommended against lepidopterans, causing less harm to the non-target insects and only eliminating the target pests. *S. frugiperda* was brought from fields and reared in the lab for multiple generations to study the pesticide-resistance development of the insect if any. Laboratory rearing of *S. frugiperda* was done on a chickpea-based artificial diet (Table 1). The studies involve exposing successive generations of insects to sublethal doses of a chlorantraniliprole and monitoring changes in their susceptibility to this insecticide. Diamide chlorantraniliprole was found to be very effective against the pest *S. frugiperda* under laboratory conditions. The LC50 was achieved at a concentration of 0.05 ppm in the first three generations of testing whereas LC50 achieved was at 0.5 ppm in the fourth generation (Tables 3, 4, 5 & 6). The dosage was repeated over generations on the third instar larval stage. The effectiveness however reduced after three generations. This shows that overuse of the diamide insecticide can lead to the development of resistance in the insect over some time. The present work highlights that the diamide Chlorantraniliprole is effectively controlling the population of *S. frugiperda* from generations 1 to 3. However, generation 4 does not show the right mortality as it leads to the development of resistance against Chlorantraniliprole in *S. frugiperda*.

Present research recommends that; the judicious use of Chlorantraniliprole can be tried against fall armyworms in the agricultural fields after extensive testing.

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