RESEARCH ARTICLE

Synergistic Effect of Sesame Oil on Chlorantraniliprole Toxicity and Detoxifying Enzymes Activity against Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith)

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ABSTRACT

The present study aimed to investigate the toxicity and synergistic effect of binary mixtures containing chlorantraniliprole and sesame oil at different ratios against second and third instar larvae of fall armyworm (FAW), *Spodoptera frugiperda*. The toxicity of different concentrations (LC_{25} and LC_{50}) of chlorantraniliprole with or without sesame oil (1, 2.5 and 5 per cent) on second and third instar larvae of FAW was determined using the leaf discdip bioassay method. A combination of chlorantraniliprole LC_{50} plus sesame oil 2.5 per cent has resulted in 74.42 and 81.81 per cent larval mortality on the second and third instars larvae of FAW, respectively. The mortality percentage was 1.52 and 1.57 fold higher than the single effect for the second and third instars of FAW, respectively. These results showed that this combination exhibited high potentiating synergism in both the instars of FAW larvae under laboratory conditions. The activity of Carboxyl Esterase (CarE), Mixed Function Oxidase (MFO) and Glutathione-S-Transferase (GST) were found to be lesser in chlorantraniliprole LC_{50} plus sesame oil 2.5 per cent combinations than in single toxicity treatments. Therefore, sesame oil showed good synergism with chlorantraniliprole at the ratio of chlorantraniliprole LC_{50} plus sesame oil 2.5 per cent on fall armyworm under laboratory conditions.

Keywords:*Chlorantraniliprole; Detoxifying enzymes; Fall armyworm; Maize; Sesame oil; Synergist*

INTRODUCTION

Fall armyworm (FAW), *Spodoptera frugiperda*(J.E. Smith) is a cosmopolitan pest of maize native to the tropical and subtropical regions of America (Wiseman *et al.,* 1966; Gowtham *et al*., 2022). Host plants of FAW include more than 353 plant species (Montezano *et al.,* 2018). It can attack all the growth stages of maize and cause more than 70 per cent of crop damage. In India, fall armyworm incidence was first reported in Chikkaballapur, Karnataka, in 2018 (Sharanabasappa *et al.,* 2018). It has become one of the major pests in maize-growing regions of Karnataka, Tamil Nadu, Andhra Pradesh, Telangana and Maharashtra. In recent days, chemical-based control measures have dominated other management practices. Even though the chemical insecticides are more effective, the residual and environmental effects cause long-term imbalance in the cropping ecosystem (Karunaratne and Hemingway, 2000; Selvi*et al.,* 2010). Currently, botanical-based insecticides are regarded as an eco-friendly alternative to chemical insecticides (Borah *et al.,* 2010; Jeeshna *et al.,* 2010). But they are time-consuming and requires large quantity (Israel *et al.,* 2008). To enhance their action and reduce the requirements, an integrated approach is required for efficient, eco-friendly, and cost-effective management. In this regard, synergistic activity between synthetic insecticide and botanicals has been recommended as a powerful and effective tool as it is the less hazardous and effective strategy (Bernard and Philogene, 1993). The importance of selecting botanicals as synergists in mixed formulations with different synthetic insecticides is increasingly recognized in FAW management. The mixture has economic benefits and could be more effective than the individual components of the mixture. Sesame oil has synergistic activity with insecticides such as pyrethrums and rotenone. The synergistic activity of sesame oil has been attributed to the presence of sesamol, sesamolin (Peter, 2012), sesamin (Soe *et al.,* 2019), piperonylbutoxide, sesamex, safrole, sesamin and episesamin (Gokbulut, 2010). Furthermore, the chemical structure of the sesame oil is similar to that of the insecticide synergist, piperonylbutoxide, which has played a significant role in reducing the population of pyrethroid resistance insects (Collins, 1990). With this background, the present study was designed with the objective to investigate the synergistic effects of sesame oil with chlorantraniliprole on the survival of FAW larvae and study the levels of esterase, glutathione-S-transferase, and mixed-function oxidase activity after adding these synergists and to improve the toxicity of chlorantraniliprole by adding sesame oil in combination.

MATERIAL AND METHODS

Study insect

Fall armyworm culture was maintained at the FAW lab, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore. First, adults were provided with potted maize seedlings for oviposition. Then the plants were taken out from the oviposition cage and placed in an insect-proof cage to allow the eggs to hatch and larvae to develop. Semi-synthetic artificial diet was used to ensure a constant supply of FAW culture (Ashok *et al*., 2021). The bioassays were performed on second and third instar larvae.

Bioassay

Laboratory bioassays were conducted to estimate the toxicity level of chlorantraniliprole. The toxicity of various concentrations (LC_{25} and LC_{50}) of chlorantraniliprole with or without sesame oil (1, 2.5 and 5 per cent) on second and third instar larvae of FAW was determined using the leaf-disc dip bioassay method (Ahmad *et al.,* 2009). The maize leaf disc of 4.5 cm diameter was cut and dipped in different concentrations of selected insecticide alone and in combinations (chlorantraniliprole with sesame oil). Each disc was allowed to air dry under shade for one hour. After complete evaporation, the leaves were transferred to clean bioassay containers poured with 1 per cent agar. Newly moulted second and third instar larvae of *S. frugiperda* were pre-starved for four hours before bioassay. Pre-starved larvae were individually placed into the six-well culture plates, with three replicates per treatment. Larval mortality was recorded at 24, 48 and 72 hours after treatment (HAT). All the experiments were carried out at 28±1 ° C and 12:12 hour of light:dark.

Potentiating synergism

A system of a component "A" causing the effect M_A (Insecticide mortality) and a synergist ("S") which alone causes no effect (M_S=0), but which in combination produce an effect that is significantly greater than M_A. This type of synergism may be found when non-lethal concentrations of an insecticide are combined with a synergist (sesame oil)(Burges and Hussey, 1971).

Protein quantification

The total protein content of all enzyme sources was determined using the Bradford method at 595 nm, with bovine serum albumin used as a standard curve (Bradford, 1976). Using the standard graph, the quantity of protein in the enzyme extract was calculated.

Determination of detoxification enzymes activities

The experiment was performed to study the level of detoxifying enzymes such as carboxyl esterase (CE), mixed-function oxidase (MFO) and Glutathione-S-transferase (GST) in FAW larvae after treating with different ratios of insecticide plus synergist. Untreated larvae were maintained as control. The experiment was replicated three times, each with ten larvae. The surviving larvae were used for assay to examine the activities of ESTs, GSTs and MFOs activities.

Enzyme homogenate preparation

Five surviving larvae treated with chlorantraniliprole along with sesame oil were homogenized in five mL homogenization buffer (ice-cold 20 mM phosphate buffer) as follows to measure the activities of three enzymes: esterases (larvae were homogenized in 1.0 mL phosphate buffer containing 0.1 per cent Triton X-100, pH 7.0), mixed-function oxidases (homogenized in 1.0 mL phosphate buffer containing 1 mM EDTA and 1 mM DTT, pH 7.3), and glutathione S-transferases (homogenized in 1.0 mL phosphate buffer, pH 7.5). The homogenate was centrifuged for 10 min at 15,000 rpm at 4 ºC and the supernatant was used as an enzyme solution and stored at 4 ºC for further use. The storage time of the enzyme should be lesser than 12 hours. This supernatant was used to analyze the activity of detoxification enzymes and protein concentrations.

Carboxylesterases (CarEs) activity

The CarEs activity was assayed using the methodology described by Devonshire (1977) with some modifications. Sample enzyme homogenate (1 mL) was mixed with 5 mL of substrate solution (10 mM α-naphthyl acetate solution) and was incubated at room temperature. After 30 minutes of incubation 1 mL of chromogenic reagent (l per cent fast blue B salt: 5 per cent sodiumlauryl sulphate = 2: 5, v/v) was added. A red color was developed immediately, followed by changing to fairly stable blue color, measured at 600 nm. Specific activity (SA) of the enzyme was calculated using the formula, which was expressed as n moles of α-naphthol released minute^{-1}mg of protein^{-1}.

$$SA = \frac{\mu g \text{ of } \alpha - \text{naphthol released}}{\text{Molecular weight of } \alpha - \text{naphthol}} \text{ X } \frac{1}{30} \text{ X } \frac{1000}{\mu g \text{ of protein}} \text{ X } 1000$$

Glutathione S-transferases (GSTs) activity

To determine the total GST activity CDNB (1-chloro 2, 4-dinitrobenzene) and glutathione were reduced as substrates. GST activity was analyzed by adding 0.1 mL of glutathione reduced, 0.1 mL CDNB and 0.1 mL gut homogenate enzymatic solution in a 3 mL final volume. The enzymatic reaction was monitored for optical absorbance increase at the wavelength of 340 nm at 37 °C for 10 min at 1 min interval in the spectrophotometer. Specific GST activity was calculated and articulated in n moles min^{-1} mg protein^{-1}.

Mixed-function oxidases (MFOs) activity.

MFOs activity was assayed using a methodology described by Hansen and Hodgson (1971) with slight modification. Sample enzyme homogenate (500 µL) was added to centrifuge tube containing 500 µL of tris buffer (pH 7.8) and 20 µL of p-nitroanisole. Then, 50 µL of NADPH was added and kept in dark condition at room temperature and allowedfor 30 minutes. The reaction was stopped by adding 0.5 mL of sodium hydroxide. The reaction mixture was centrifuged at 10,000 rpm for 30 minutes. The absorbance of the supernatant was determined at 400 nm. The specific activity (SA) of the enzyme was calculated using the formula and expressed as n moles of p-nitrophenol released minute^{-1} mg of protein^{-1}.

$$SA = \frac{\mu g \text{ of } \alpha - \text{nitrophenol released}}{\text{Molecular weight of } \alpha - \text{nitrophenol}} \text{ X } \frac{1}{30} \text{ X } \frac{1000}{\mu g \text{ of protein}} \text{ X } 1000$$

Data analysis

Probit analysis was done to calculate LC_{25} and LC_{50} using SPSS. The log concentration probit (LCP) lines were drawn by plotting log concentrations on X-axis and probits on Y-axis. The response of tested insect populations was studied at different concentrations of the chlorantraniliprole. The combined toxicity of chlorantraniliprole with FAW was studied by combining the different lethal concentrations (LC_{25} and

LC_{50}) at different proportions (Finney, 1971). Mortality was corrected by Abbott's formula (Abbott, 1925) for each Probit regression analysis.

RESULTS AND DISCUSSION

Toxicity and synergist effects of chlorantraniliprole along with sesame oil

A study was made to test the toxicity of chlorantraniliprole against the second and third instar larvae of FAW (Table 1). The LC_{25} of chlorantraniliprole against the second and third instar larvae of FAW was 0.87 ppm and 1.52 ppm, respectively. Similarly, LC_{50} of chlorantraniliprole for the second and third instar larvae of FAW was 4.08 ppm and 6.50 ppm, respectively. A combination of chlorantraniliprole and sesame oil exhibited potentiating and antagonistic synergism against the second and third instar larvae of FAW. Combination of chlorantraniliprole LC_{25} plus sesame oil 2.5 per cent showed mortality of 32.56 per cent and 36.36 per cent on second and third instar larvae of FAW, respectively. Similarly, chlorantraniliprole LC_{50} plus sesame oil 2.5 per cent has resulted in 74.42 and 81.81 per cent larval mortality against second and third instars larvae of FAW, respectively. It was found that the mortality percentage was 1.52 fold higher than the single effect. It was evident that the combination of chlorantraniliprole LC_{50} plus sesame oil 2.5 per cent has produced potentiating synergism.

Meanwhile, chlorantraniliprole LC_{25} plus sesame oil 5 per cent has antagonistic synergism for both second and third instar larvae of FAW. On the other hand, LC_{50} of chlorantraniliprole plus sesame oil 5 per cent has antagonistic synergism for the second instar but showed potentiating synergism for the third instar of FAW larvae. Chlorantraniliprole LC_{50} plus sesameoil 2.5 per cent combination exhibited high potentiating synergism in both second and third instar larvae of FAW under laboratory conditions.

The results were in accordance with other studies reporting sesame oil synergized fluvalinate up to 48 fold at LC_{90} against citrus thrips, *Scirtothripscitri* (Mout.) (Immaraju *et al.,* 1990). The synergistic effect of sesame oil with different insecticides was reported in *Helicoverpa armigera* (Sundramoorthy and Chitra, 1992; Manoharan and Uthamasamy, 1993; Gowda, 1996; Rajesekhar *et al.,* 1996). A combination of fenvalerate and sesame oil caused 69.26 per cent mortality in eggs of *Plutella xylostella* (Vastrad *et al.,* 2004). Similarly, pyridalyl plus sesame oil (99:1), abamectin plus sesame oil (90:10), spinosad plus sesame oil (90:10) and malathion plus sesame oil (95:5) were the different combinations tested earlier for the synergistic action of sesame oil (El-Razik *et al.,* 2014). Karso and Al-Mallah (2015) found sesame oil plus acetamiprid (2:1) showed synergistic action against larvae of *Trogoderma granarium*. In addition, Visetson *et al.* (2003) found 10 per cent sesame oil showed good synergism with cypermethrin yielding synergistic ratios (SR) ranging from 1.54-6.33 in contact testing method and 2.04-5.88 in the no-choice leaf dipping method against *P. xylostella.* Raghavendra *et al.* (2017) observed that sesame oil exhibited a high level of malathion resistance suppression in *Sitophilus oryzae* with SR of 74.48 in *Tribolium castaneum*.

Effect of chlorantraniliprole and sesame oil on detoxifying enzymes activities

Comparing the single and insecticide combination treatments CarE activity was found to be lesser in chlorantraniliprole LC_{50} plus sesame oil 2.5 per cent (3.64 fold) in treated insects followed by chlorantraniliprole LC_{25} plus sesame oil 2.5 per cent (4.79 fold) than the chlorantraniliprole LC_{50} (6.81 fold) and chlorantraniliprole LC_{25}(5.83 fold), respectively. The CarE activity of chlorantraniliprole alone in second instar larvae was lesser than those with combinations (chlorantraniliprole along with sesame oil) as shown in Fig. 1. A similar trend was also obtained in third instar larvae (Fig. 2). This results revealed that the combination of chlorantraniliprole and sesame oil inhibits the detoxification process. The MFO activity was also found be lesser in insecticide combination treatments chlorantraniliprole LC_{50} plus sesame oil 2.5 per cent (1.34 fold) followed by chlorantraniliprole LC_{25} plus sesame oil 2.5 per cent (1.56 fold) than single action treatments chlorantraniliprole LC_{50} (1.58 fold), chlorantraniliprole LC_{25} (1.44 fold) in the second instar larvae. GST activity was also lower in insecticide combination treatment in chlorantraniliprole LC_{50} + sesame oil 2.5% (1.34 fold) followed by chlorantraniliprole LC_{25} + sesame oil 2.5% (1.56 fold) than the chlorantraniliprole LC_{50} (2.01 fold), chlorantraniliprole LC_{25} (1.79 fold) in relation to control in second instar larvae.

These data confirmed the findings reported for both PBO and sesame oil played the same role in increasing cypermethrin efficacy in *P. xylostella.* Both PBO and sesame oil mixed with cypermethrin inhibited monooxygenase activity by approximately two-thirds but induced glutathione-S-transferases ca. 2.3 fold in both methods. The synergists had no effect on esterase activity (Visetson *et al.,* 2003).

Raghavendra *et al.* (2017) found that with the use of sesame oil with malathion against *S. oryzae* resistance level reduced from 98.00 to 25.00 per cent with an SR of 74.48 per cent. It is concluded that, among the enzymes, the MFO and carboxyl esterase play an important role in the resistance development in *S. oryzae* to malathion. Sesame oil suppressed insecticide resistance to higher level by inhibition effect of these enzymes.

CONCLUSION

Sesame oil showed good synergism when combined with chlorantraniliprole. Combination of chlorantraniliprole LC_{25} plus sesame oil 2.5 per cent and chlorantraniliprole LC_{50} plus sesame oil 2.5 per cent was proved to have potentiation synergism against second and third instar larvae of FAW. The synergistic action is due to the suppression of detoxifying enzymes like CarE, GST and MFO. Use of natural synergist along with insecticide proved to be effective in managing the FAW. This combination also minimise the chance of resistance development in the course of time.

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Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

Consent for publication

All the authors agreed to publish the content.

Competing interests

There were no conflict of interest in the publication of this content

Author contributions

Idea conceptualization- MM, NS, Experiments- GV ,Guidance –MM, NS, SG, SV, DU, Writing original draft - GV, MM, NS, Writing- reviewing &editing - MM, NS, SG, SV, DU.

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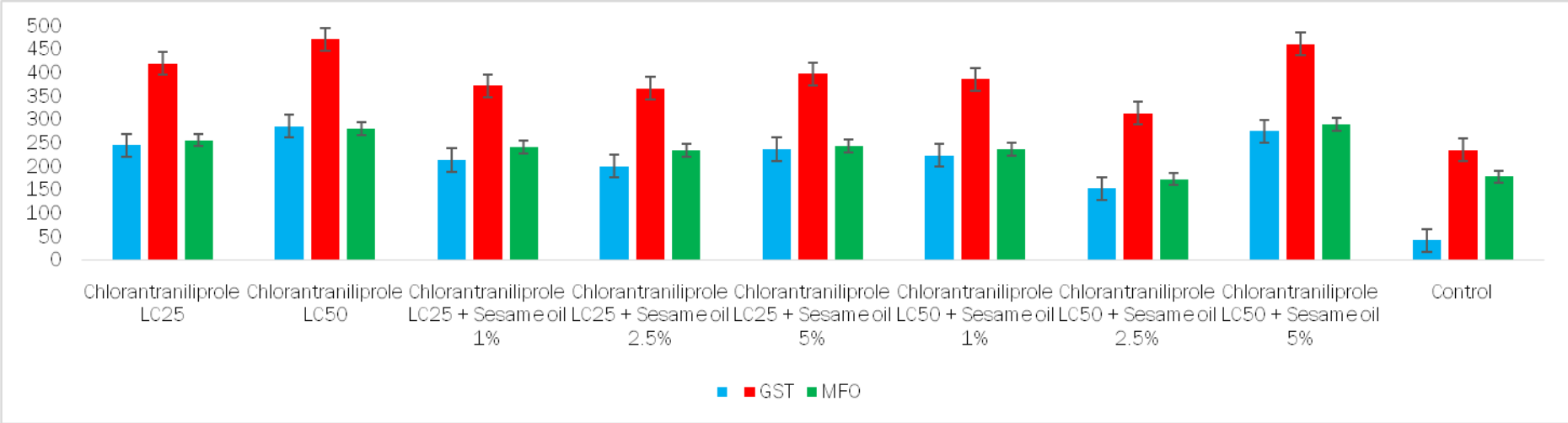

Fig. 1. Levels of detoxifying enzymes in chlorantraniliprole plus sesame oil treated second instar larva of *S. frugiperda*

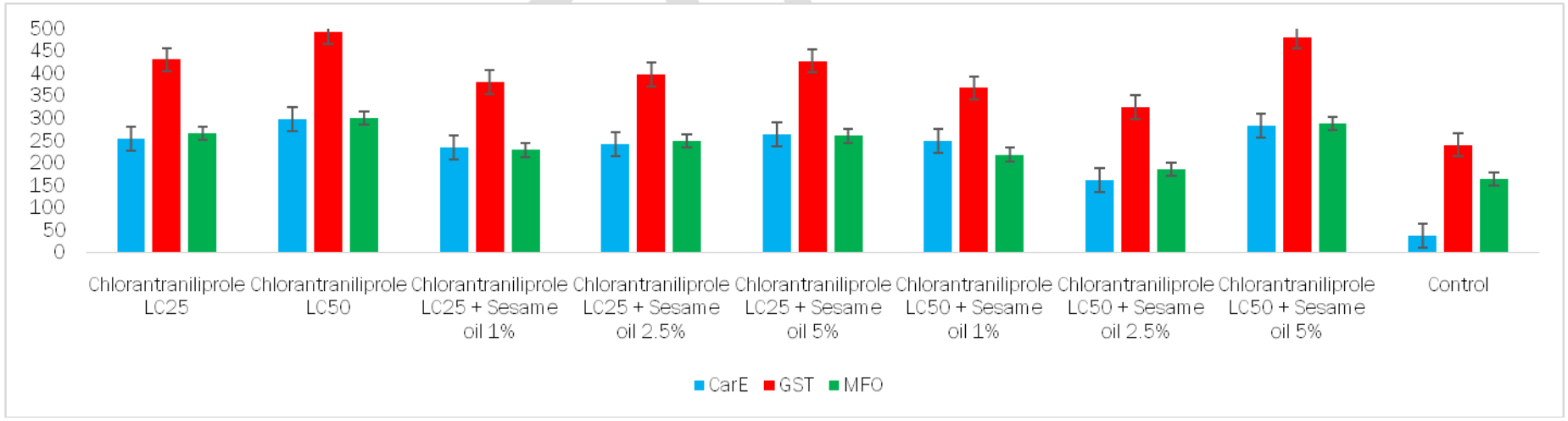

Fig. 2. Levels of detoxifying enzymes in chlorantraniliprole plus sesame oil treated third instar larva of *S. frugiperda*

Table 1. Toxicity of tested insecticides against 2nd and 3 rd instar of *S. frugiperda*

Instar	Insecticide	LC 25 ppm	LC 50 ppm	Slope	χ2
2 nd instar	Chlorantraniliprole	0.87 (0.373 – 2.007)	4.08 (2.582 – 6.432)	1.0017	0.1261
3 rd instar	Chlorantraniliprole	1.52 (0.79 – 2.91)	6.50 (4.28 – 9.89)	1.0748	0.8848

Table 2. Interactive effects of Chlorantraniliprole + Sesame oil on 2nd and 3rd instar of *S. frugiperda*

Treatments	2nd instar			3rd instar		
	Mortality (%)	Fold increase over chlorantraniliprole alone	Synergism	Mortality (%)	Fold increase over chlorantraniliprole alone	Synergism
Chlorantraniliprole LC_{25}	25.58	-	-	25.00	-	-
Chlorantraniliprole LC_{25} + Sesame oil 1%	27.91	1.09	Potentiating synergism	34.09	1.36	Potentiating synergism
Chlorantraniliprole LC_{25} + Sesame oil 2.5%	32.56	1.27	Potentiating synergism	36.36	1.45	Potentiating synergism
Chlorantraniliprole LC_{25} + Sesame oil 5%	18.60	0.73	Antagonistic synergism	20.45	0.82	Antagonistic synergism
Chlorantraniliprole LC_{50}	48.84	-	-	52.27	-	-
Chlorantraniliprole LC_{50} + Sesame oil 1%	53.49	1.10	Potentiating synergism	56.82	1.09	Potentiating synergism
Chlorantraniliprole LC_{50} + Sesame oil 2.5%	74.42	1.52	Potentiating synergism	81.82	1.57	Potentiating synergism
Chlorantraniliprole LC_{50} + Sesame oil 5%	46.51	0.95	Antagonistic synergism	54.55	1.04	Potentiating synergism
Control	0.00	-	-	0.00	-	-