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Efficacy of gallic oil extract (*Allium sativum*) and its nano emulsion on mortality and physiology of cotton leaf worm *Spodoptra littoralis* (Lepidoptera: Noctuidae)

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Abstract

The cotton leaf worm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is a major insect pest attacks a large-scale economic crop, there are many different ways to control this pest using chemicals and essential oils. Chemical pesticides are most effective, but they cause pollution to humans, plants and the environment. One of these essential oils is garlic oil and its nano emulsion that is used in the presented study to evaluate the mortality of second instar larvae. Also, physiological tests were conducted on the fourth instar larvae of cotton leaf worm. The results of the mortality test showed the effectiveness of nano emulsion of garlic oil more than the bulk garlic oil with LC_{50} 56 ppm however LC_{50} for garlic oil was 7633.58 ppm. While the physiological test is done for estimating acid phosphates, alkaline phosphates and digestive enzymes (Lipase), and the results showed that the control experiments were 176 u/L, 185 u/L and 49.7u/L, respectively. on the other hands, garlic oil extract showed 97.5U/L,133 U/L and 38 U/L respectively and garlic oil nano emulsion showed 150 U/L,164U/L and 39 U/L, respectively.

Introduction

The Egyptian cotton leaf worm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is one of the most important insect pests which feed on the crops (Azab *et al*., 2001), the application of conventional pesticides lad to insecticide resistance (Saleem *et al*., 2008). Currently, insecticides are used to control cotton leaf worm infestation which is harmful to humans and animals.

Presently insecticides are used to control insect pest infestation that caused hazardous to animals and humans (Ismail and Shaker, 2014). Many plants have natural substances that can be used to control insect pests and these plants have a deleterious effect in several manners like toxicity, mortality, antifeedants growth inhibitor, suppression of reproductive behavior, and reduction of fecundity and fertility (Jbilou *et al.*, 2006 and Hamada *et al.*, 2018).

Garlic oil contains some Sulphur-containing compounds (Aliin, ajoene, allicin, dially l trisulphide, dithin, sallylcysteine), enzymes, minerals (Mg, Zn, Se and Ge), vitamins (C, A, B complex), amino acids, proteins, saponins and flavonoids (Block, 1992 and Johnson *et al.*, 2013) therefore, garlic essential oil was

reported to have insecticidal effects on pests of stored products (Ho *et al*., 1996 and Moawad and Sadek, 2018). Trying to use garlic oil as nano emulsion, could have a beneficial effect on pest management.

The aim of this study is to compare the impact of garlic oil and its nano emulsion on cotton leaf worm under laboratory conditions and to determine the effect of these substances on physiology analysis of cotton leaf worm.

Materials and methods

1. Tested cotton leaf worm:

A laboratory strain of *S. littoralis* was reared under constant conditions of 27±2°C, photoperiod of 14 hrs. light and 10 hrs. dark and 65±5% R.H. Larvae were fed on castor leaves that were used at second instar for laboratory experiments.

1.1. Tested oils:

1.1.1. Garlic oil (Essential oil)

1.1.2. Garlic oil (Nano emulsion)

1.2. Preparation and isolation of essential garlic plant oil:

Essential garlic oil was extracted from the clover of the garlic plant and extracted by steam distillation apparatus that was found in Plant Protection Institute, Mansoura, Egypt. Separated oil was dried over anhydrous sodium sulfate and stored in dark glass bottles at 4° C in a refrigerator until used.

1.3. Preparation of nano emulsions of garlic oil:

The method of preparation of was described by Jerobin *et al*. (2012) garlic oil was diluted with distilled water in a ratio of 1:1 (Oil to water), and Tween 80 was added as an emulsifier. Then, the emulsion was sonicated for 30 minutes using the ultrasonic cleaner set (Model WUC-DO3H 290W) at 60 Hz. Also, it was resonicated for 1 minute using high energy the ultra-sonication probe (model VCX750) set to 750W and 20 kHz, and then it was resonated again by the ultrasonic cleaner set under cooling conditions for 30 minutes (Youssef *et al*., 2018).

1.4. Preparation of the stock solution of the tested materials:

Concentrations of garlic oil and the Nano-emulsion of garlic oil were prepared on basis of the tested material weight and the volume of the distilled water (w/v). Tween 80 (0.1%) was used as an emulsifier. The stock concentrations were kept in glass stoppered bottles and stored under refrigeration. Such stock solutions were prepared periodically. Five diluted concentrations were used to draw the LC-P Lines for each material and four replicates were used for each concentration.

1.5. Larvicidal activity of tested oil (Essential and nano):

To determine the toxicity action of the tested materials, 2nd larval instar was used. The castor bean leaf discs were dipped into the treatments for 20 seconds and then left for air dryness. Ten larvae for each replicate were released to each leaf disc placed. The used concentrations were 500, 1000, 5000, 7500 and 10000 ppm (for garlic oil), however, the concentrations were 40, 80, 100, 250 and 500 ppm for garlic oil nano emulsion. The same number of leaf discs was dipped into dis. water as an untreated check. The percentage of mortality was recorded after one day, three days, five days and seven days. Data were corrected relatively to control mortality (Abbott, 1925). LC_{50} and LC_{90} values were determined using the probit analysis statistical method of (Finney, 1971 and Sun, 1950 equation) (Used to determine LC_{50} index).

$$\text{Toxicity Index for } LC_{50} = \frac{LC_{50} \text{ of the most effective compound}}{LC_{50} \text{ of the least effective compound}} \times 100$$

2. Physiological analysis:

In this presented study, 4th instar larvae of cotton leaf worm were used for this purpose. Acid phosphates (ACP) and alkaline phosphates (ALT) were determined according to the method described by Powell and Smith (1954), on the other hand, aspirated amino transaminase was determined according to the method of Reitman and Frankle (1957).

Results and discussion

1. Efficiency of garlic oil and nano emulsion against leave of *Spodoptra littoralis*:

Data in Table (1) demonstrated that, the concentration 500 ppm of nano emulsion was the highest concentration that caused the highest mortality rate 90%, moreover the highest concentration of garlic oil 10000 ppm caused 66.67% mortality rate. The rest of nano emulsion concentrations were 40, 80, 100 and 250 ppm caused mortality rate 40%, 53.33%, 73.33%, 80%, respectively. Otherwise, garlic oil concentrations were 500, 1000,5000, 7500 ppm notated 10%, 16.67%, 30%, 46.67 and 66.67%, respectively. The obtained results agreed with Hashem *et al.* (2020) who evaluated the toxic effect of aniseed (*Pimpinella anisum* L.) essential oil and its nano emulsion against the red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and proved that the nano emulsion has the highest effect that the essential oil. In addition, Marouf *et al.* (2021) tested the efficacy of camphor oil and its nano emulsion against *S. littoralis and* proved that the nano emulsion has the highest effect than the essential oil these results agreed with the presented data. Furthermore, data in Table (2) and Figure (1) indicated that, LC_{50} of nano emulsion of garlic oil is lower than LC_{50} of bulk garlic oil which recorded 56 ppm for nano emulsion but the garlic oil recorded 7633.58 ppm. This means that, nano emulsion of garlic oil is more efficient than garlic oil. These results were in agreement with Dimetry *et al*. (2019) and Ebeid (2020) who indicated the efficiency of nano emulsions of some essential oils against *S. littoralis* and *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) than the bulk essential oils. At the same time, LC_{90} showed the efficiency of nano emulsion than the essential garlic oil and recorded 469.91 and 98082.88 ppm for nano emulsion of garlic oil and bulk garlic oil, respectively. The toxicity index was 100% for the nano emulsion while it was 0.73for bulk garlic oil. Moreover, Table (2) and Figure (1) showed that, slope values were 1.39& 1.16 for nano emulsion and the essential garlic oil, respectively. Also, LC_{90}/ LC_{50} values were 12.8 and 08.4 for garlic essential oil and nano emulsion, respectively. This means that, the highest slope value or the lowest LC_{90}/ LC_{50} means the steepest line of toxicity (El-Shewy, 2018) and Hashem *et al.* (2020) proved the effectiveness of nano emulsion of jojoba oil than its bulk essential oil against *A. ipsilon.*

Table (1): Mortality % of 2nd instar larvae of the cotton leaf worm, *Spodoptera littoralis* treated with garlic oil and its nano emulsion under laboratory conditions.

Treatments	Conc. (ppm)	Mortality after treatments %				Total Mortality %
		One day	Three days	Five days	Seven days	
Garlic oil	500	----	-----	3.33	6.67	10
	1000	-----	3.33	6.67	6.67	16.67
	5000	3.33	10	10	6.67	30
	7500	6.67	10	20	10	46.67
	10000	6.67	20	26.67	13.33	66.67
Garlic oil Nano emulsion	40	-----	10	13.33	16.67	40
	80	3.33	16.67	20	13.33	53.33
	100	6.67	23.33	26.67	16.67	73.33
	250	10	30	20	20	80
	500	10	30	36.67	13.33	90

Table (2): Efficiency of some plant active ingredients against 2nd instar larvae of the cotton leaf worm *Spodoptera littoralis.*

Treatments	Conc.	Corrected mortality%	LC_{50}	LC_{90}	Slope± S.D.	Toxicity index LC_{50}	LC_{90}/LC_{50}
Garlic oil	500	10	7633.58	98082.88	1.16± 0.13	0.73	12.8
	1000	16.67					
	5000	30					
	7500	46.67					
	10000	66.67					
Garlic oil Nano-emulsion	40	40	56	469.91	1.39± 0.17	100	8.4
	80	53.33					
	100	73.33					
	250	80					
	500	90					

R: Regression **P: Probability**

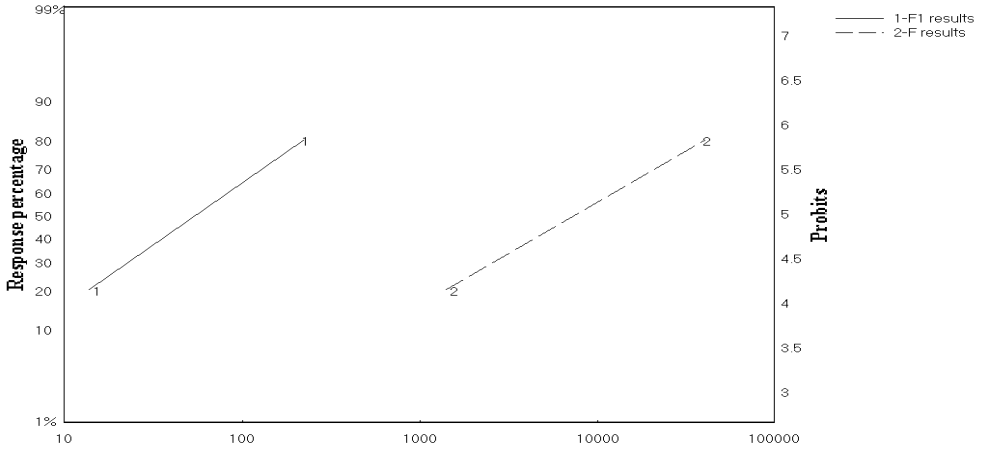

Figure (1): Slope values for nano emulsion and essential oil.

2. Efficacy of garlic oil and its nano emulsion on physiology of cotton leaf worm, *Spodoptra littoralis* :

Data in Table (3) evaluated the efficacy of garlic oil and its nano emulsion on physiological changes of *S. littoralis.* The obtained data was compared between control, and garlic oil and nano emulsion. Acid phosphates was recorded at 176U/L in control while it was recorded 150U/L in nano emulsion. Alkaline phosphates were recorded at 185 u/L in control despite of nano emulsion and garlic oil recorded at 164U/L, and 133 U/L, respectively. Lipase was recorded at 49.7U/L in control even though recorded at 39 U/L in nano emulsion and 38U/L in garlic oil. Hamadah *et al*. (2016) revealed a depressed activity in the haemolymph of only newly moulted larvae and changed acid and alkaline phosphates. Anwar and Abd El-Mageed (2005) proved inhibition of ACP when *S. littoralis* was treated with some IGRs such as chlorfluazuron and flufenoxuron.

Table (3): Efficacy of garlic oil and its nano emulsion physiology of cotton leaf worm *Spodoptra littoralis :*

Analysis	Control	Garlic oil	Nano emulasion
Acid phosphates	176 u/L	97.5U/L	150 U/L
Alkaline phosphates	185 u/L	133 U/L	164U/L
Lipase	49.7u/L	38 U/L	39 U/L

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