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Efficacy of Camphor Oil and Its Nano Emulsion on The Cotton Leafworm, *Spodoptera Littoralis*

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ABSTRACT

Laboratory experiments were tested to detect the effect of crude camphor oil and its emulsion against 2nd instar larvae of the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Because of the problems of chemical pesticides to the environment and all organisms, the natural control replaced pesticides. In the recent years, many formulations as Nanoemulsions have been used for pest toxicity target. The results indicated that the Nano emulsion of camphor oil is the most effective material than the camphor essential oil with LC_{50} 88.67 ppm and 1699.85 ppm for the camphor Nano emulsion and camphor oil, respectively. So, the Nano emulsion formulation may represent a new category of natural and bio-pesticides and this should be considered in IPM programs.

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INTRODUCTION

The *Spodoptera littoralis* (Boisd) adult (Lepidoptera: Noctuidae) lay between 300 and 500 eggs. It is the most serious pest of various crops such as cotton, tomato, and potato (Senrung *et al*., 2014). This pest developed resistance to many pesticides (Niranjankumar and Regupathy 2001).

Chemical control is the most common method of *S. littoralis* control due to its ease of use and reliability (Zhou *et al*., 2011). The natural plant products showed a strong development against a variety of insect pests (Tanzubil and Mccaffery, 1990). Essential oils may have repellent or attractive effects and had insecticidal action against pests (Rodriguez and Levin, 1975). Using Nano oils in the pest management sector could be of both ecological and economic benefit (Bixby, 2011). In recent years, some studies have shown that essential oils were toxic to different pests (Dugassa *et al.* 2009, Kumar *et al.* 2012). The camphor tree, *Cinnamomum camphora* (L.) J. Presl (Lauraceae), rich in camphor, occurs naturally in Asian countries (Chen *et al*. 2013). It has great commercial value to the cosmetic industry. Camphor exhibits a number of biological properties such as antimicrobial, anticoccidial, antiviral, antinociceptive, anticancer and insecticidal activities. In addition, it's used as a skin penetration enhancer (Chen *et al.* 2013). The present study aimed to find out the effect of camphor essential oil comparing with its Nano emulsion against the cotton leafworm, *Spodoptera littoralis* under laboratory.

MATERIALS AND METHODS

Rearing of *S. littoralis*:

Laboratory strain of *S. littoralis*, (maintained on above 30 generations), that was initiated from freshly collected egg mass supplied from the division of cotton leafworm of Plant Protection Research Institute (PPRI), Dokki, Egypt. The larval stages were reared on castor leaves that were provided daily under constant conditions of 27±2°C, photoperiod of 14 h light and 10 h dark and 65±5% R.H. The adult were kept separately and mated on the third day of emergence in clean jars (4 lb.) then adults were fed on 10% honey solution and fresh green leaves of tafla, *Nerium oleander* (L.) were provided for egg-laying.

Preparation and Isolation of Essential Camphor Plant Oil:

Essential oil (volatile oil) of camphor was extracted from the leaves of the camphor 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 the refrigerator until used.

Preparation of Nano-Emulsions of Camphor Oil:

The method of preparation was described by Jerobin *et al.* (2012). Camphor oil was diluted with distilled water to 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 ultrasonication probe (model VCX750) set to 750W and 20 kHz, and then it was resonicated again by the ultrasonic cleaner set under cooling conditions for 30 minutes (Youssef *et al.,* 2018).

Preparation of the Stock Solution of the Tested Materials:

Concentrations of camphor oil and the Nanoemulsion of camphor oil were prepared on basis of the tested material weight and the volume of the distilled water (w/v). Tween 80 (0.1%) used as 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.

Method of Application (Leaf dipping method):

To determine the toxicity action of the tested materials, 2nd instar larvae were 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 camphor oil), however, the concentrations were 40, 80, 100, 250 and 500 ppm for camphor oil Nanoemulsion. 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. Sun equation, 1950 (used to determine LC_{50} index).

Toxicity Index for LC_{50} =

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

RESULTS AND DISCUSSION

The Efficiency of Camphor Oil and Camphor Oil Nanoemulsion Against Larvae of *S. littoralis*:

Data in Table (1) showed that the highest tested concentration 500 ppm of nanoemulsion caused the highest mortality rate 80%, while, the same concentration 500 ppm of camphor oil caused the lowest mortality percent 33.33%. The other concentrations of Nanoemulsion were 40, 80, 100 and 250 ppm recorded 36.67, 43.33, 56.67 and 66.66 ppm, respectively. However, the other concentrations of camphor oil were 1000, 5000, 7500 and 10000 ppm recorded 43.33, 56.67, 66.67 and 86.67 %, respectively. Ahmed *et al.* (2020) evaluated the toxic effect of aniseed (*Pimpinella anisum* L.) essential oil (EO) and its Nano emulsion (NE) against the red flour beetle *Tribolium castaneum* and proved that the Nano emulsion had the highest effect than the essential oil. This result agreed with the obtained results.

Table 1: Mortality % of 2nd instar larvae of *S. littoralis* treated with camphor oil and camphor oil nanoemulsion under laboratory conditions.

Treatments	Conc. (ppm)	Mortality after treatments %				Total Mortality %
		One day	Three days	Five days	Seven days	
Camphor oil	500	3.33	10	10	10	33.33
	1000	10	10	10	13.33	43.33
	5000	13.33	13.33	16.67	13.33	56.67
	7500	20	13.33	13.33	20	66.67
	10000	30	20	20	16.67	86.67
Camphor oil nano emulsion	40	6.67	13.33	10	6.67	36.67
	80	13.33	10	10	10	43.33
	100	16.67	20	10	10	56.67
	250	20	23.33	13.33	10	66.66
	500	23.33	30	26.67	10	80

Our results also agreed with Nadia *et al.* (2019) who tested the toxicity effects of four essential oils peppermint, thyme, camphor and sage oils against the fourth instar larvae of *Agrotis ipsilon* and their nano emulsions, they indicated the highest toxicity of nano emulstions than the essential oils.

Efficiency and Toxicity Index of The Tested Materials Against 2nd Instar Larvae larvae of *S. littoralis:*

The results in Table (2) and Fig. (1) indicated that LC_{50} for Nano- emulsion of camphor oil was 88.67 ppm. While LC_{50} of the camphor oil was 1699.85 ppm. Also, LC_{90} was 1389.67 and 46370.88 ppm for the Nanoemulsion of camphor oil and bulk camphor oil, respectively. The slope value was 1.072 and 0.893 for the Nano- emulsion of camphor oil and the camphor oil, respectively. The value of LC_{90} / LC_{50} was 15.67 and 27.28 for the nano-emulsion of camphor oil and bulk camphor oil, respectively. The highest slope value or the lowest LC_{90} / LC_{50} means the steepest toxicity line. Borie *et al.,* (2014) reported that *S. littoralis* treatment with Nano-silica gave the highest toxic action at all concentrations after 15 days caused also; reduction in the biological parameters as larval and pupal duration, adult longevity and also female's fecundity. Derbalah *et al.,* (2014) declared that Nano oils had a slight mortality rate against the newly hatched larvae of *Pectionophora gossypiella*. Also, Adel *et al.,* (2014) showed that geranium essential oil Nanoparticles was more effective on both larval and pupal development, longevity, the fecundity of female and percentage of hatchability. And also, El-Shewy (2018) confirmed that Nanoemulsion of

Jojoba oil was more effective than crude Jojoba oil when applied on 4th instar larvae of the black cutworm, *Agrotis ipsilon*.

Table 2: Efficiency of camphor oil and its Nano-emulsion against 2nd instar larvae of *S. littoralis.*

Treatments	Conc.	Corrected mortality %	LC_{50}	LC_{90}	Slope± S.D.	Toxicity index LC_{50}	LC_{90}/LC_{50}
Camphor oil	500	33.33	1699.85	46370.88	0.893± 0.11	5.22	27.28
	1000	43.33					
	5000	56.67					
	7500	66.67					
	10000	86.67					
Camphor oil nano emulsion	40	36.67	88.67	1389.67	1.072± 0.156	100	15.67
	80	43.33					
	100	56.67					
	250	66.66					
	500	80					

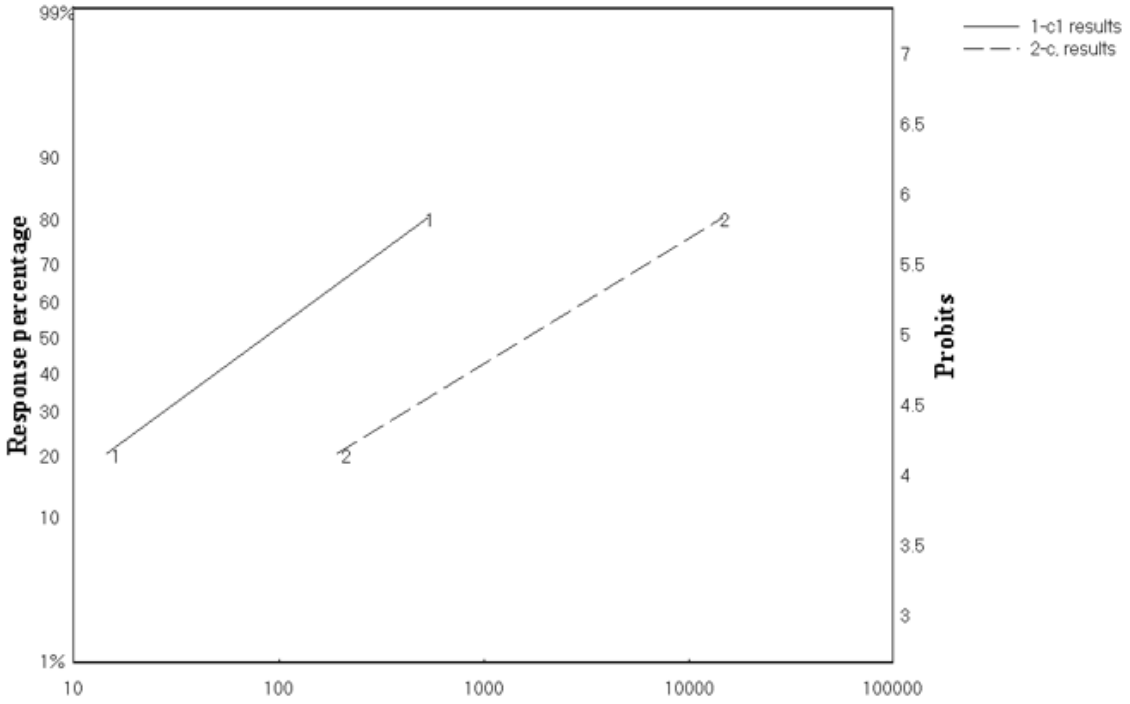

Fig. 1: LC-P line for camphor oil and its Nanoemulsion against 2nd instar larvae of *S. littoralis*

Conclusion

The toxicity of camphor oil and its Nanoemulsion was examined against 2nd instar larvae of *S. littoralis* and the results proved the effectiveness of the Nanoemulsion than the crude oil.

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