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Research Article
Insecticidal Efficiency of Some Green-based Formulations on *Spodoptera littoralis* and their Side Effects on Albino Rats

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Abstract

Background and Objective: Insecticides were broadly utilized to suppress particular species of insects; it contaminates crop products, groundwater, soil and the environment, health risks to humans. This research intended to study the physico-chemical properties of some prepared green-based formulations namely, Laury, Orego and Rosa compared with a commercial formulation (Pestban), as well as, their efficiency in both laboratory and field against *Spodoptera littoralis*. The biochemical and histopathological alterations after treating with these formulations were also examined. **Materials and Methods:** Three types of formulations were naturally prepared using mineral and vegetable oils. Physical and chemical characteristics of the tested formulations were determined. The insecticidal efficiency under laboratory and semi-field conditions was evaluated, the toxicological studies were also investigated and statistical analysis using one-way analysis of variance, the LC_{50} and LC_{90} values were calculated using SPSS version 21.0. **Results:** Rosa was the most potent with LC_{90} value of 2.54%, followed by Laury and Orego with 3.78 and 6.18%, respectively. All tested natural based formulations were less toxic to the larvae than the reference insecticide, Pestban. Concerning the semi-field evaluation, the effectiveness of the tested formulations was similar to the laboratory studies, where they ranked descending as, Rosa, Laury and Orego. The side effects of the green-based formulations on liver and kidney of male rats were safe, whether, chemically or histopathologically, while, Pestban revealed adverse effects. **Conclusion:** The prepared green-based formulations have potential effects against *S. littoralis*. The toxicological studies demonstrated no evidence of toxicity and no biochemical changes in both liver and kidney biomarkers.

Key words: Pestban, essential oils, green-based formulations, *Spodoptera littoralis*, biochemical, histopathological alterations, insecticidal efficiency

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cotton is the most utilized natural fiber worldwide, hence considered as an important item of the textile industry. In 2017, the worldwide production of cotton totaled 120.86 million bales. India, China and the United States produce more than half of the global yield[1].

The Egyptian cotton leafworm, *S. littoralis* (Boisd.), is a fertile and highly polyphagous insect. It is regarded as a major pest of great economic prominence in several countries where it attacks a wide spectrum of host plants. It is one of the most devastating insects invading cotton plants. This insect causes an intense decrease in cotton yield and quality[2-4].

It has been recently clarified that pest species could easily evolve resistance to many synthetic pesticides presently marketed[5-6]. In addition, the injurious impacts of synthetic pesticides on both the environment and human health have been displayed[7-10]. Therefore, there is an increasing interest toward replacement tools for eco-friendly pest control[11-13].

A pesticide formulation is a composition of active and inert substances, which represents an end-use pesticide product. Pesticides are formulated for making them safer and easier to handle, where most pesticide active ingredients, in pure (technical grade) model are not appropriate for the application. In their concentrated form, some are excessively toxic, most do not mingle well with water, some are unstable and the others are difficult (or unsafe) to handle, convey or store. To assure the quality of the formulations, the physico-chemical exams on formulations must be implemented.

The attempts to discover new natural products with potential for pest control, this study led to examine the insecticidal efficiency of some natural based formulations against *S. littoralis*, as well as their side effects on the experimental animals.

MATERIALS AND METHODS

All experiments were accomplished for 10 months of the years 2017 and 2018 under semi-field conditions and The Laboratories of Pests and Plant Protection, Therapeutic Chemistry, Pathology Departments of National Research Centre (NRC), Dokki-Cairo, Egypt.

Insect: A laboratory strain of *S. littoralis* (Boisd.) (Lepidoptera: Noctuidae) was reared on castor leaves according to Eldefrawi *et al.*[14] under controlled conditions (25±2°C and 65±5% R.H.) in the laboratory.

Preparation of the tested essential oils: The air parts of bay laurel (*Laurus nobilis*), sweet marjoram (*Origanum majorana*) and rosemary (*Rosmarinus officinalis*) were dried and pulverized. The essential oils were isolated after hydro-distillation for 4 h in a steam distillation using a Clevenger apparatus.

Preparation of the tested formulated compounds: Three types of formulations were naturally prepared and namely as follow:

- **Laury (20% EC):** The formulation was prepared by mixing bay laurel oil (20%) in appropriate amounts of emulsifier (10%) and natural solvent (mineral (20%) and vegetable (50%) oils)
- **Orego (35% W/O/W):** The compound was prepared by mixing oregano oil (35%) with appropriate amounts of sodium salicylate (6.5%) and two different emulsifiers (6%) in water (52.5%)
- **Rosa (15% EW):** The formulation was prepared by mixing rosemary essential oil (15%) with methyl salicylate (wintergreen oil) (6.5%) appropriate amounts of emulsifier (4%) and mineral oil (6.5%) in water (68%)

Physico-chemical properties of the tested formulations

Emulsion stability test: To prepare standard hard water, anhydrous calcium chloride (0.304 g) and magnesium chloride hexahydrate (0.139 g) were dissolved in distilled water and completed[15] to 1 L.

The emulsion stability test was carried out according to WHO specifications[16]. Into a 250 mL beaker having an internal diameter of 6-6.5 cm, 75-80 mL of hard water were poured. The beaker contents were stirred with a glass rod and then completed to 100 mL by addition of the tested water. The beaker contents are poured immediately into a clean, dry, graduated 100 mL cylinder. The cylinder was kept at 30-31°C for 1 h and examined for any free oil or creaming separation. The volume of free oil, cream or solid matter, if any, should not transcend 2 mL.

Foam test: The emulsion stability test was carried out also to measure the foam amounts formed on the emulsion surface in the cylinder after 5 min. The foam layer should not exceed 5 mL for passing the test.

pH test: The test was carried out according to CIPAC specifications[15]. About 1 g of the tested formulation was weighed and transferred to measuring cylinder (100 mL)

containing about 50 mL distilled water. The cylinder was made up to 100 mL and shook vigorously for 1 min and then it was allowed to settle. The pH of the supernatant liquid was measured.

Insecticidal activity of the tested formulations against *Spodoptera littoralis*

Laboratory experiments: Dipping technique was carried out as described by Shepard[17]. Leaves of castor bean were soaked for 5 sec in a series of concentrations of each formulation. The leaves were placed in Petri-dishes with 10 larvae of 4th instar larvae. Four replicates were carried out for each treatment. Larvae in control treatment were fed on leaves treated only with water. The mortality percentages were recorded after 24 h of treatment. The mortality data were subjected to Probit analysis to obtain the LC_{50} and LC_{90} values[18].

Semi-field experiments: The planting of cotton plant was done using pots (20 cm diam.) under field conditions. The three formulations, Laury, Orego and Rosa, as well as a reference commercial insecticide (Pestban 48% EC) were sprayed with LC_{90} concentrations multiplied five times. Ten replicates of pots were used for each treatment. Control was treated with water only. The sprayed cotton leaves were randomly selected among the various replicates of the treatments after zero, 1, 3, 5 and 10 days of the treatment. The collected samples were transferred to the laboratory, where the larvae of cotton leafworm were subjected to the treated leaves. Four replicates were used for each treatment. After treatments, 10 larvae were placed in Petri dishes for 2 days. The survivor larvae were fed on untreated castor leaves until pupation. Cumulative mortalities were calculated at the end of each testing time and corrected according to Abbott[19].

Toxicological studies

Animals: Male albino rats (*Rattus norvegicus*) weighing 100±5 g were obtained from the Animal Breeding House of the National Research Centre (NRC). Rats were kept in polypropylene cages, with free access to standard pellet diet and water, 12 h light/dark cycle, 22±2°C temperature and 48% relative humidity in the laboratory. The rats were acclimatized for 1 week before the start of the experiment. All the rats were kept according to the guidelines and welfare regarding animal protection in Animal Breeding House of NRC which approved by NRC Local Ethical Review Committee and were conducted in accordance with "the Guide for the Care and Use of Laboratory Animals"[20].

Experimental design: Rats were divided into five-groups, five rats of each. Group 1 was received distilled water (1 mL/rat) and served as a control. The remaining four groups (2, 3, 4 and 5) received LC_{90} concentrations of the tested formulations for 15 consecutive days.

Biochemical effects: At the end of the experiment, rats were fasted overnight and blood samples were collected by puncturing the retero-orbital venous plexus of the animals with a fine sterilized glass capillary. The collected blood was left to clot in clean dry tubes and centrifuged at 3000 rpm for 10 min at 4°C using Heraeus Labofuge 400R (Kendro Laboratory Products GmbH, Germany) to obtain the sera. Serum samples were stored at -20°C in the deep freezer until analysis. The serum was used to determine some biochemical measurements such as the aminotransaminase enzymes (AST, ALT) activities[21] and total bilirubin (TB)[22] for liver functions. The methods employed in the assay of kidney functions of urea[23] and creatinine levels[24].

Histopathological examination: The treated rats were sacrificed at the end of the experiment, samples from liver and kidney fixed in alcoholic Bouin's solution for 24 h and washed, few drops of lithium carbonate were used to wash out the picric acid from the material. The samples were dehydrated in standard alcoholic series and cleared in xylol before embedding in paraffin wax, sectioned at the thickness of 2-3 microns and stained according to the technique of Conn[25], using Delafield's Haematoxylin and Eosin. The tissues were examined under light microscopy for histological evolution.

Statistical analysis: Statistical analysis were carried out to determine the differences between treatments and days after spraying by using one-way analysis of variance (ANOVA) Costat[26] and Duncan's multiple range test[27] was applied at 5% probability level. The concentration-mortality data were subjected to Probit analysis to calculate the LC_{50} and LC_{90} values using the Statistical Package for the Social Sciences SPSS[28] software program. The values of LC_{50} were considered significantly different if the 95% confidence limits did not overlap.

RESULTS

Physico-chemical properties of the tested formulations: The results of emulsion stability, foam formation and pH of Laury, Orego and Rosa natural prepared formulations, as well as

Pestban commercial formulation were exhibited in Table 1. All the tested formulations traversed the emulsion stability. Rosa formulation did not record any separation layers. In the same trend, Pestban traversed the emulsion stability, as there were not any observed separation layers. Each of the prepared and commercial formulations passed the foam formation. Orego did not record any foam layers. The pH of Laury, Orego and Rosa, natural prepared formulations were around 7, while the pH of Pestban was 3.8.

Laboratory evaluation of the tested formulations: The LC_{50} and LC_{90} (%) values of the tested natural based formulations along with Pestban commercial formulation against the 4th instar larvae of *S. littoralis* were given in Table 2. Rosa was the most effective with LC_{50} value of 1.28%, followed by Laury and Orego with 2.17 and 2.5%, respectively. The corresponding values of LC_{90} were 2.54, 3.78 and 6.18%, respectively. Pestban was more efficient than the examined essential oil formulations against the larvae.

Semi-field evaluation of the tested formulations: Semi-field application was determined to study initial, residual effect and persistence of Laury, Orego and Rosa compared with Pestban as a reference insecticide against 4th larval instar of *S. littoralis*. Data were tabulated in Table 3. As for the natural formulations, Rosa caused the highest mortality at the initial kill (zero time), followed by Laury, while, Orego was the least in this respect. There was not any natural based formulation recorded 100% mortality at zero time.

With regard to the aforementioned results, the efficiency of all tested compounds decreased gradually after spray. The activity of all essential oil formulations against *S. littoralis* was lower than Pestban after zero and one day from application, while the efficiency after 3 and 5 days were not significantly different with Rosa. The mortality of Rosa continued until 5 days after treatment as Pestban, while the mortality of Laury and Orego lasted for 3 and 1 days after application, respectively.

Side effects of the tested compounds on rats

Effects on liver and kidney functions: The biochemical analysis of serum of male rats exposed to green-based formulations showed insignificant changes of all the tested liver (AST, ALT, TB) and kidney (Creatinine, Urea) dysfunction biomarkers compared with the control. While Pestban induced hepatotoxicity reflected by elevating the previous serum parameter levels (Table 4).

Histopathological changes

Liver: As exhibited in Fig. 1a, liver sections from control rats showed a normal structure of the hepatic lobules which formed the structural units of the liver; each was consisted of cords of hepatocytes and blood sinusoids in between, with a well-preserved cytoplasm and well-defined nucleus and nucleoli. Rats that treated with Laury, Orego and Rosa essential oil formulations showed a normal structure of the hepatic lobules and hepatocytes (Fig. 1b-d), respectively. While, rats daily given an oral dose of Pestban showed necrosis of liver cells and dilated blood sinusoids (arrow) (Fig. 1e), focal necrosis associated with inflammatory infiltration (Fig. 1f) and temperate lymphocyte infiltration in the portal and periportal areas (green arrow) (Fig. 1g) associated with dilated and congested veins (asterisk), some pyknotic nuclei were observed (red arrow).

Kidney: Histological examination of control of rat kidney showed the normal structure of renal corpuscles (asterisk) and renal tubules, proximal convoluted tubules (red arrow) and distal convoluted tubules (green arrow) (Fig. 2a). Animals treated with Laury, Orego and Rosa showed normal renal corpuscle and renal tubules, proximal convoluted tubules and distal convoluted tubules (Fig. 2b-d),

Table 1: Physico-chemical properties of the tested formulations

Formulations	Emulsion stability (separation mL)	Foam formation (mL)	pH
Laury	1.9	1.5	7.0
Orego	2.0	0.0	7.8
Rosa	0.0	2.5	7.2
Pestban	0.0	2.0	3.8

Table 2: Toxicity of the tested essential oil formulations against 4th instar larvae of *Spodoptera littoralis*

		95% confidence limits (%)			95% confidence limits (%)				
Formulations	LC_{50}[a] (%)	Lower limit	Upper limit	LC_{90} (%)	Lower limit	Upper limit	Slop±(SE)[b]	Intercept±(SE)[c]	(χ^2)[d]
Laury	2.17	2.07	2.26	3.78	3.45	4.29	5.31±0.31	-23.02±1.34	36.21
Orego	2.50	2.14	2.88	6.18	4.95	8.89	3.26±0.16	-14.33±0.72	86.53
Rosa	1.28	1.18	1.37	2.54	2.24	3.05	4.31±0.21	-17.70±0.86	87.92
Pestban	12×10^{-5}	93×10^{-6}	15×10^{-5}	29×10^{-5}	21×10^{-5}	55×10^{-5}	3.45±0.3	-0.31±0.08	7.68

[a]Concentrations trigger 50% mortality after 24 h of treatment, [b]Slope of concentration mortality regression line, [c]Intercept of regression line and [d]Chi-square value

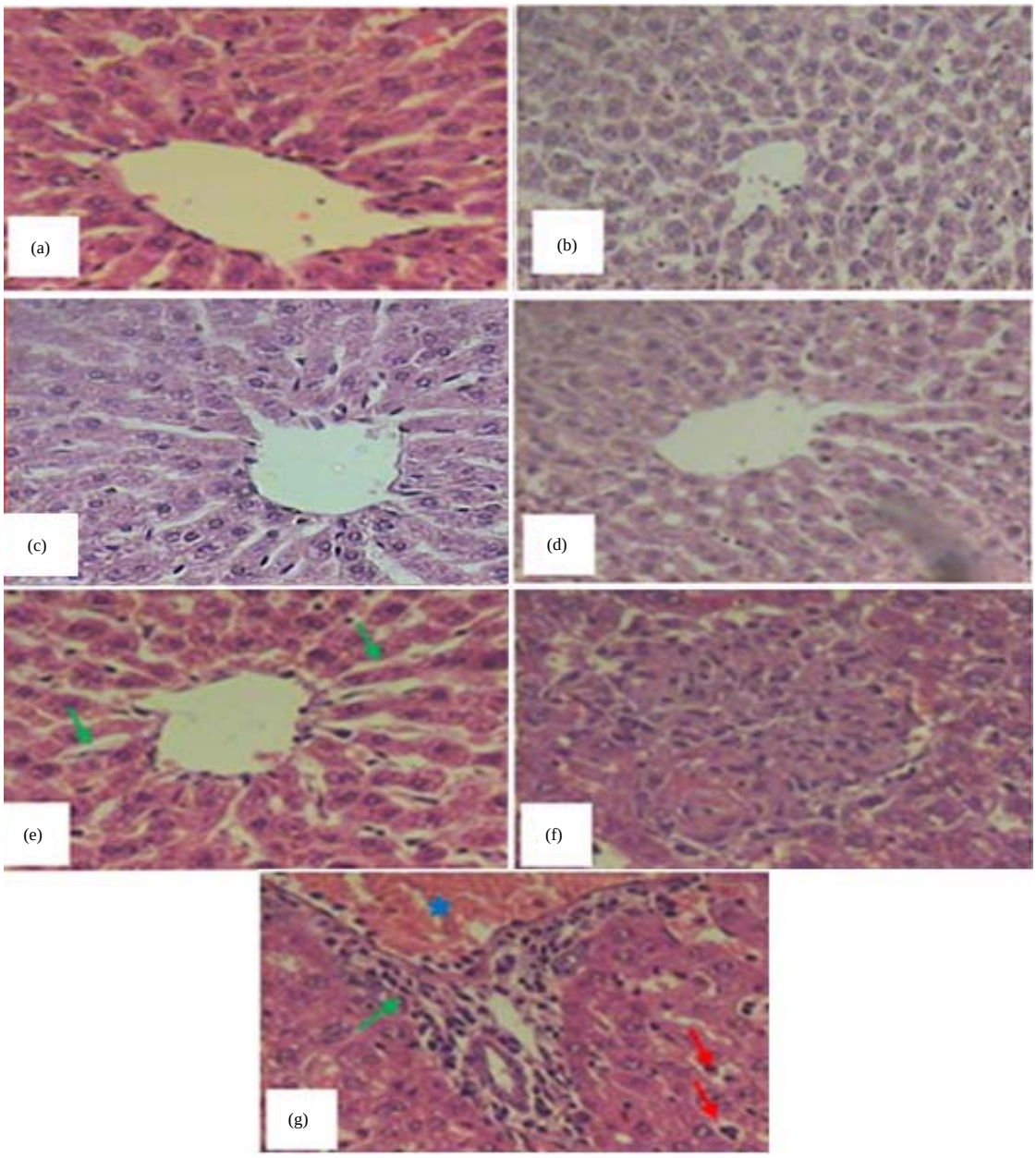

Fig. 1(a-g): Sections of liver from (a) Control, (b) Laury, (c) Orego, (d) Rosa and (e-g) Pestban formulations (H and E stain-X150)

Table 3: Mortality percentages of 4th instar larvae of *Spodoptera littoralis* treated with essential oil formulations

	Mortality after days of treatment (%)			
Formulations	0	1	3	5
Laury	63.33^{c}	43.33^{bc}	23.33^{b}	0.00^{b}
Orego	56.67^{c}	33.33^{c}	0.00^{c}	0.00^{b}
Rosa	80.00^{b}	53.33^{b}	40.00^{a}	23.33^{a}
Pestban	100.00^{a}	86.67^{a}	50.00^{a}	20.00^{a}
Control	3.33^{d}	0.00^{d}	0.00^{c}	3.33^{b}
$LSD_{0.05}$	6.85	13.53	10.59	7.28

$LSD_{0.05}$ least significant difference at 0.05 level of probability. Averages accompanied by the same letter in a column are not significantly different at $p<0.05$

respectively. On the other hand, rats treated with Pestban showed some cellular debris in the dilated interstitial space (blue arrow) and the urinary spaces appeared with dilatation (green arrow) (Fig. 2e), inflammatory infiltration in the interstitial distances (Fig. 2f), moreover, the renal corpuscles exhibited congestion, hyper-cellularity (asterisk) and wide urinary spaces (green arrow) and the cells of the renal tubules demonstrated many degenerative changes with pyknotic nuclei (red arrow).

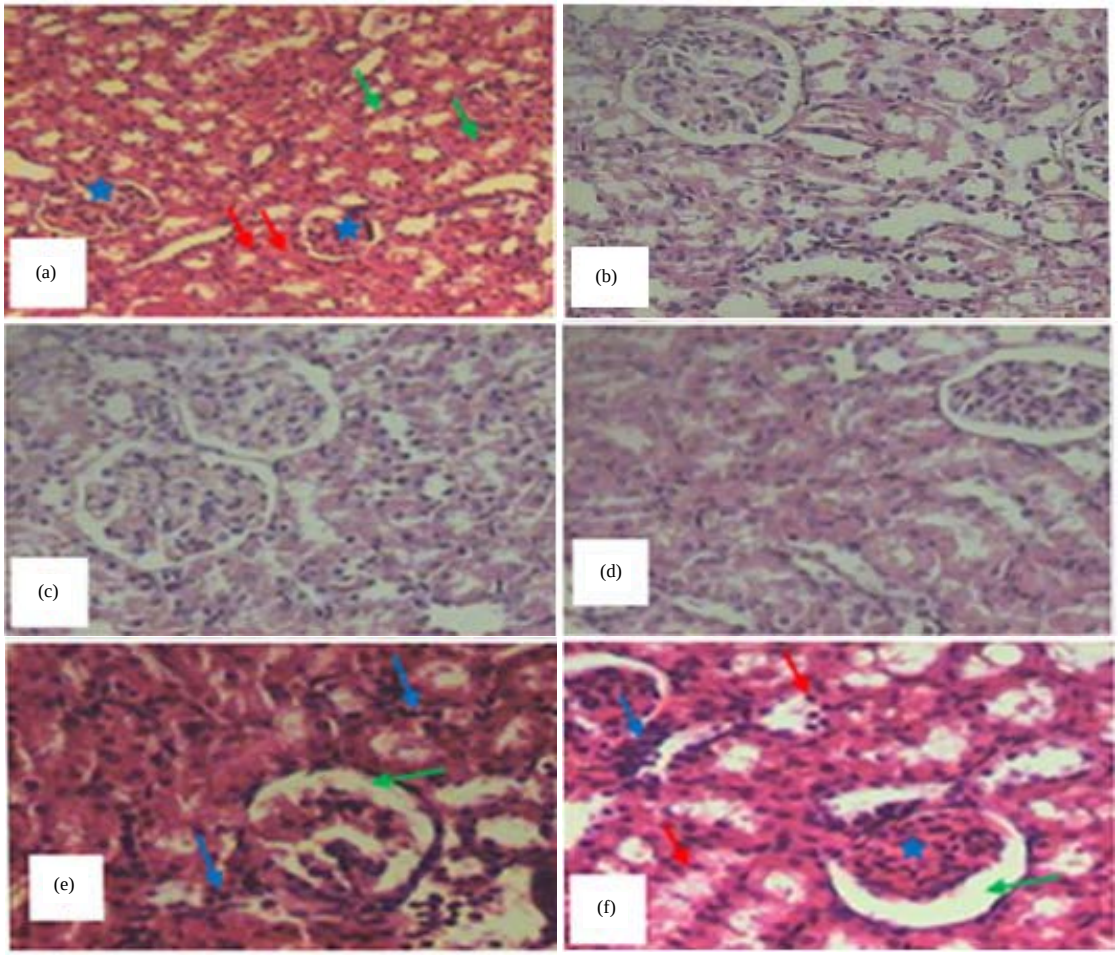

Fig. 2(a-f): Sections of kidney from (a) Control, (b) Laury, (c) Orego, (d) Rosa and (e-f) Pestban formulations (H and E stain-X150)

Table 4: Levels of some markers of hepatic-renal functions in serum of rats

	Liver functions			Kidney functions (mg dL^{-1})	
Treatments	AST (U L^{-1})	ALT (U L^{-1})	TB (mg dL^{-1})	Creatinine	Urea
Laury	26.85±1.53^{a}	25.13±1.28^{a}	0.48±0.13^{a}	0.92±0.09^{a}	33.15±1.72^{a}
Orego	27.92±1.14^{a}	23.86±1.25^{a}	0.57±0.09^{a}	0.97±0.21^{a}	31.23±0.59^{a}
Rosa	27.95±1.63^{a}	22.79±1.81^{a}	0.54±0.17^{a}	0.87±0.06^{a}	32.05±2.73^{a}
Pestban	132.75±5.86^{b}	182.60±9.43^{b}	1.40±0.18^{b}	2.45±0.16^{b}	67.70±2.51^{b}
Control	28.79±1.17^{a}	25.53±2.10^{a}	0.54±0.10^{a}	0.88±0.11^{a}	33.73±1.01^{a}
$LSD_{0.05}$	4.06	5.95	0.12	0.16	2.63

AST: Aspartate transaminase, ALT: Alanine transaminase, TB: Total bilirubin, $LSD_{0.05}$ least significant difference at 0.05 level of probability. Each value is a mean of five rats±SD, values superscripted with same alphabets in the same column are not significantly different at $p<0.05$

DISCUSSION

The effectiveness of different types of green-based formulations was assessed on *Spodoptera littoralis.* The results of the physical and chemical characteristics of the tested formulations revealed that the volume of cream layer did not traverse 2 mL, the volume of cream layer, if any, should not exceed[16] 2 mL. In addition, the foam layers formed did not exceed 5 mL, the limit of foam layer volume should not surpass[16] 5 mL, therefor all the green formulations passed each of the emulsion stability and foam formation. This implied that the examined formulations could be applied in the field without any separation or foam problems. The pH of the natural prepared formulations were near seven. Our results matched with Halcomb[29], who reported that a safe pH of spray solution is within range of 4.5-7.0. A chemical reaction

occurs for many utilized pesticides in the existence of water that own pH value higher than 7 (alkaline hydrolysis), thus it lowers the potency of the pesticide's active ingredient[30]. The pH affects also on each of the penetration of spray solutions via the cuticle and the leaf surface through the phytotoxicity.

The current study demonstrated that all the tested formulations exhibited a toxic effect to *Spodoptera littoralis.* Pavela[31] evaluated the efficiency of various essential oils against *S. littoralis* larvae. Twenty essential oils were highly effective to the 3rd instar larvae. Our results were in agreement with Priyanka and Srivastava[32], who evaluated some essential oils against 3rd instar larvae of *Spodoptera litura.* Essential oils were evaluated at 1 and 2% concentrations. The mortality was between 6.66-100%.

The evolution of eco-friendly biopesticides in order to control agricultural pests is an important defiance currently. Essential oils were considered as a valuable alternative for insect control agents[33-34]. The mortality of the green based formulations showed that there was not any formulation investigated 100% mortality at zero time; so the obtained mortalities might be optimized by increasing the fold of LC_{90} applied concentrations. Abbassy *et al.*[35] revealed the toxicity effect of *Majorana hortensis* against *S. littoralis* with LC_{50} value of 3.14 g L^{-1} in a residual film technique. Souguir *et al.*[36] evaluated the toxicity of some essential oils against 3rd larval instar of *S. littoralis*. All essential oils showed a toxic effect on the larvae. Data obtained by Ali and Ibrahim[37] exhibited the insecticidal activity of camphor oil against fourth instar larvae of *S. littoralis* with LC_{50} value of 163.1 mg mL^{-1}. In addition, Abdelgaleil and El-Sabrout[38] studied the insecticidal potency of *Artemisia monosperma*, *Callistemon viminals*, *Citrus aurantifolia* Swingle and *Cupressus macrocarpa* essential oils contra 4th instar larvae of *S. littoralis*. Data revealed that *A. monosperma* and *C. macrocarpa* were the most efficient.

The noxious effects of insecticides on human and their ecosystem are a critical problem worldwide, so several researchers try to find out new compounds, particularly from natural sources such as essential oils as green insecticides.

The results of the green formulations on the biochemical parameters revealed insignificant changes in all tested liver and kidney biochemical parameters, while Pestban caused perturbation of these biomarkers. The liver is the mean organ in the body has a crucial function in xenobiotic detoxification. It is the primary target of toxic xenobiotic and their metabolites. Therefore, alterations in liver function biomarkers are typically used as biomarkers for liver toxicity and damage[39-40]. It has been mentioned that the increase in the activity of liver enzymes can be attributed to cell injury[41], hepatotoxicity and change in proteins biosynthesis. ALT, AST and TB are important indicators of liver damage in clinical findings. These enzymes are secreted into the blood in hepatocellular injury and their levels increase. The remarked increase in the levels of aminotransferase (ALT and AST) as well as TB, is the major diagnostic symptoms of liver diseases[40]. Because of the kidney's high blood flow, its capability to concentrate chemicals and the existence of renal xenobiotic metabolizing enzymes, the kidney may also be a site of toxicity of xenobiotics[40].

Previous studies demonstrated that essential oils had no significant changes in activities of liver and kidney functions[42-44].

Histopathological studies have been broadly utilized as biomarkers for the toxicological inquiries, in addition, pesticide toxicities[45-46]. It had been reported that organophosphate insecticides were known to occur several histopathological alterations in the liver tissues[47-48].

CONCLUSION

The efficiency of the tested green-based formulations had promising effects against *S. littoralis* in each laboratory and semi-field evaluation. In addition, their side effects revealed that they are harmless on tested animals, hence on humans chemically and histopathologically. Further studies of these formulations should be investigated under field conditions.

SIGNIFICANCE STATEMENT

The present research was investigated to prepare some green-based formulations to combat Egyptian cotton leafworm, *S. littoralis*. The tested natural formulations revealed a promising insecticidal efficiency. The green-based formulations also revealed no adverse effects on experimental animals. The prepared formulations could be deemed as a good alternative and safe method for controlling *S. littoralis* than the conventional insecticides.

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