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Original article

The larvicidal effect of neemazal T/S, clove oil and ginger oil on tomato leafminer, *Tuta absoluta* compared to coragen

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ABSTRACT

The present study aimed to evaluate the toxicity and biochemical changes of *Tuta absoluta* 3rd instar larvae affected by neemazal T/S, clove oil and ginger oil. These compounds were evaluated compared to the recommended pesticide, Coragen 20% SC. by means of sublethal concentrations, LC_{25} and LC_{50} under constant laboratory conditions. Results showed that neemazal T/S is more toxic than detected oils compared with higher toxicity of coragen with LC_{50} values of 57.52, 159.94, 633.38 and 930.71 μg mL^{-1} for coragen, neemazal, ginger oil and clove oil, respectively. There were highly significant differences between all treatments and untreated larvae. Neemazal possessed the greatest effect on activity level of most physiological parameters than selected oils. Larval content of digestive enzymes was decreased significantly 48 h after all treatments except for lipase, α-esterase and β-esterase (in case of coragen and clove oil). Also, total proteins, total carbohydrates, total lipids and total free amino acids take the same trend. Based on this study, these sublethal doses caused a significantly dose-dependent perturbation in determined components.

1. Introduction

Tomato (*Lycopersicon esculentum* Mill) is considered one of the most important economic vegetables in Egypt and hosted by 200 species of arthropods. During the second half of 2009, the tomato cultivations in Egypt invaded with a newly dangerous insect pest namely *Tuta absoluta* which may reduce the yield productivity up to 100%. The damage is mainly caused by the larvae stage, which feed and grow on soft tissues such as leaves, shoots and fruits from the aerial part of the plant at any stage of tomato growth.

Insecticides used indiscriminately have caused serious problems such as direct toxicity to parasites, predators, pollinators, fish, and humans (Munakata, 1977), pesticide resistance (Georghiou and Taylor, 1977; Schmutterer, 1981), and crop plant susceptibility to insect pests (Pimentel, 1977), as well as increased environmental and social costs (Pimentel et al., 1980). Essential oils (EO) and plant extracts represent alternatives to pesticide for pest control (Fouad et al., 2014; Machial et al., 2010) with the advantage of high diffusion rate in the environment because of their low molecular weight and high vapor pressure (Bakkali et al., 2008). Essential oils are cost effective and have lower risk on the environment and beneficial organisms (Matos Neto et al., 2002; Alagawany et al., 2021; El-Tarabily et al., 2021). Beside interfering with the biology, physiology, and nervous system of the insect according to (Mikhaiel, 2011; Mann and Kaufman, 2012). Consequently, use of essential oils may allow a more sustainable agricultural practice (Isman, 2006).

The azadirachtin is nowadays one of the most important natural insecticide due to a secondary metabolite produced by the neem tree (*Azadirachta indica* A. Juss.). Many formulations of neem seed oil exhibit insecticidal properties like, antifeedant, ovicidal, larvicidal, insect growth regulatory, and repellent activity in a wide variety of insect taxa, including Lepidoptera (Chaudhary et al., 2017).

Ginger *(Zingiber officinale)* is a well-known spice used either fresh or dried in the daily diet in many countries (Gao and Zhang, 2010). The rhizomes is discovered to have various pharmacological activities such as anti-inflammation, antitumor, and antioxidant (Masuda et al., 2004; Zhou et al., 2006), with a domi-

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nant component of α-zingiberene (Nampoothiri et al., 2012; Mahdavi et al., 2018). While Eugenol with natural abundance in clove essential oil (Cruz et al., 2014; Jairoce et al., 2016) belongs to monoterpenes, a large group of volatile and lipophilic compounds which are capable of rapid penetration inside insects and interfere with their physiological functions (Chaieb et al., 2007; Saad et al., 2018). Little studies were availible on the larvicidal effect of these oils on *Tuta absluta*.

The toxicity and the activity of sublethal concentrations of some plant extracts on physiological status have been studied on different insect species, likewise Khosravi and Sendi (2013) who investigated elm leaf beetle *Xanthogaleruca luteola* larvae enzymes. Yazdani et al. (2014) determined the physiological aspects on the lesser mulberry pyralid *Glyphodes pyloalis* Walker. Nair et al. (2017) detected *Sitophilus oryzae* Linn biochemical parameters at different sublethal doses. Abdel-Razi (2018) studied the mixtures of leaves extracts, plants oils and the pesticide, coragen 20% SC against housefly *Musca domestica* adults.

The present work was carried out to determine the toxicity of neemazal formulation, ginger oil and clove oil compared to the recommended pesticide (Coragen 20% SC) on 3rd larval instar of tomato leaf miner. In addition to, the effect of their sublethal concentrations (LC_{25} and LC_{50}) on the larval physiological aspects.

2. Material and methods

This study was carried out in Plant Protection Department, Faculty of Agriculture, Zagazig University, Egypt under controlled laboratory conditions.

2.1. Insect rearing

The populations of *T. absoluta* were established using neonate larvae (which served as the initial culture) collected from untreated tomato fields, in Dyarb Negm - Sharkia Governorate. The stock culture was maintained in the laboratory in plant protection department, Faculty of Agriculture, Zagazig University. Fresh tomato leaves were provided to the larvae until pupation. After pupation, the pupae were kept in transparent cylindrical cups (3.5 × 2 cm) till adult emergence. The newly emerged moths were enclosed in transparent ribbed cups (11.5 × 6 cm) covered with black muslin for oviposition and fixed with a rubber band. Two droplets of 10% honey solution were added in the cup as food. The insects were left for two days to copulate and lay eggs. Musilin cloths with deposited eggs were collected and kept in Petri dishes (9 cm) containing a moistened disc of filter paper. Hatched larvae were introduced individually to fresh tomato leaflets using a moistened soft hair brush in transparent cups (5.5 × 9 cm). Larvae investigated daily until the second molting to obtain the desired third instar larvae based on observing head exuvia and width of the head capsule (Rasheed et al., 2018).

2.2. Tested prepared botanical extracts

Three tested components were used as shown in Table 1 compared to the recommended pesticide, Coragen 20% SC (Chlorantraniliprole). Samples of clove flowers buds and ginger rhizomes were bought from a herbs store in Zagazig city, Egypt. Flowers buds and ginger rhizomes were extracted according to (Salem et al., 2013).

2.3. Toxicity bioassays

The toxicity of neemazal T/S and tested oils on the 3rd larval instar of *T. absoluta* was determined by using standard leaf dipping (Sparks and Nauen, 2015). Prior to the experimental phase, larvae were starved for 4 h. Preparatory tests were initially performed to find the effective dose ranges. Several concentrations of each compound were prepared in water with 0.1% Tween 80 as an emulsifier (Amizadeh et al., 2015).

The leaves for bioassays were taken from tomato plant, 50 days after seedlings have been transplanted. Tomato leaflets and leaves with their petiole were cut from the 4th leaf from the stem apex. The leaves were dipped in each concentration for 20 s after which leaves were left to air-dry. The experiment had three replicates. Each replicate consisted of 20 larvae. By using a fine soft brush, the larvae were kept in petri dish covered with moistened filter paper and were allowed to feed on a pair of treated leaves for 48 h. Numbers of dead insects were cumulatively counted till 48 h of treatment and mortality percentages were estimated. Leaves treated with water only used as a control with the same number of larvae and replicates. Then LC_{25}, LC_{50} and LC_{90} were determined (Saad et al, 2021a).

2.4. Analytical methods of larval supernatant components

In subsequent experiment, newly moulted 3rd instar larvae were fed on tomato leaves treated with sublethal concentrations (LC_{25} and LC_{50}) of both plant extracts and the recommended insecticide to determine their effects on haemolymph components. The required samples obtained from 48 h old 3rd instar treated larvae.

All supernatant components of homogenated larvae were determined in the Department of Pest Physiology, Plant Protection Research Institute, Dokki, Giza, Egypt.

The insects were prepared as illustrated by Amin (1998). Larvae were homogenized in distilled water (50 mg/ ml). Homogenates were centrifuged at 8000 rpm for 15 min at 2 °C in a refrigerated centrifuge. Then, the deposits were discarded and the supernatant, which is indicated as enzyme extract, can be stored at least one week without perceivable loss of activity when stored at 5 °C.

2.4.1. Hydrolyzing enzymes

2.4.1.1. Carbohydrate hydrolyzing enzymes. Digestive enzymes (trehalase and amylase) were determined according to the modifications of Amin (1998) to the method described by Ishaaya and Swirski (1976). Amylase kit was obtained from Egyptian Company for Biotechnology (SAE), Al-Obour City, Industrial area, block 20008, piece 19A, Cairo, Egypt. While β-glucosidase activity was measured as described by Lindroth (1988).

2.4.1.2. Protein hydrolyzing enzymes. Protease activity was measured as described by Hamdy (1977), with some modifications, by measuring the increase in free amino acids split from substrate protein (albumin), during one hour incubation at 30°C. Glutamic

Table 1
List of the tested formulation and essential oils.

Common name	Scientific name	Family	Used part	Source
Neemazal T/S formulation (1% azadirachtin)	*Azadirachta indica* (A. Juss.)	Meliaceae	Seeds (Formulation)	Trifolio-M GmbH-Germany
Ginger oil	*Zingiber officinale Roscoe*	Zingiberaceae	Rhizomes	Extracted
Clove oil	*Syzygium aromaticum L.*	Myrtaceae	Flowers buds	Extracted

pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) were determined colorimetrically according to the method of Reitman and Frankel (1957).

2.4.1.3. Lipids hydrolyzing enzymes. Lipase activity was determined by a slight modification of the procedure of Talhoun and Abdel-Ghaffar (1986). The method was based on the determination of the decrease in ester content of triolein as substrate. Non-specific esterases (α-esterases and β-esterases) were determined according to van Asperen (1962) using α-naphthyl acetate or β- naphthyl acetate as substrates, respectively.

2.4.1.4. Determination of total carbohydrates, total lipids, total proteins and total free amino acids. Total carbohydrates were estimated in acid extract of the sample by the phenol–sulphuric acid reaction (Nielsen, 2017; Saad et al, 2021b). Total lipids were estimated by the method of Knight et al., (1972) by a kit (Biodiagnostic 29 Tahreer St., Dokki, Giza, Egypt) that was purchased from High Lab Company. Total proteins was determined by the method of Ernst and Zor (2010). Total free amino acids was colorimetrically assayed by ninhydrin reagent according to the method described by Lee and Takahashi (1966); El-Sobki et al. (2021).

2.5. Statistical analysis

Corrected percentage mortality of Larvae after 48 h was calculated according to Abbott (1987) formula. Probit analysis as described by Finney (1952) was performed to estimate toxicity values and slope of regression line for each tested substance (probit regressions by Polo-PC software.)

Statistical analysis of biochemical changes was performed using Statistix 9 software. An ANOVA model was used for individual treatment comparisons at $P < 0.05$ and means were separated by the Least Significant Difference (LSD). Results were recoded as mean ± standard error (SE).

3. Results

3.1. Effect of neemazal T/S, ginger oil and clove oil compared to coragen pesticide on some biochemical parameters in the supernatant of T. absoluta3rd instar homogenated larvae

The LC_{50} of both plant extracts and coragen on *Tuta absoluta* larvae were introduced in Table 2. The LC_{50} values of neemazal formulation, ginger oil and clove oil were 159.94, 633.38 and 930.71 μg mL^{-1}, respectively compared with 57.52 μg mL^{-1} of coragen.

The effects of exposure to LC_{25} and LC_{50} of neemazal T/S, ginger oil, clove oil and coragen for 48 h on some biochemical parameters in 3rd instar larvae of tomato leaf miner are presented in Tables 3-7 as follows:

3.1.1. Carbohydrate hydrolyzing enzymes

Treatments with LC_{25} and LC_{50} concentrations of tested compounds reduced the studied hydrolyzing enzymes (trehalase, amylase and β-glucosidase) in treated larvae after 48 h as follows:

3.1.1.1. Trehalase enzyme. Results given in Table 3 clearly indicated that the trehalase enzyme activity significantly differed between neemazal, plant oils and coragen as well as between the used concentrations ($F = 34.90$, df = 4, $p = 0.0000$). The highest enzyme activity and the lowest one (258.07 μg/ml and 122.43 μg/ml) were recorded in case of ginger oil at LC_{25} and LC_{50} for coragen, respectively compared with 270.61 μg/ml in control. Generally, the tested compounds can be arranged descendingly according to their effects as follows: ginger oil (245.16 μg/ml), clove oil (216.83 μg/ml), neemazal formulation (181.84 μg/ml) and coragen (171.21 μg/ml).

3.1.1.2. Amylase enzyme. The results of this study indicated that amylase specific activity in the treated homogenated larvae was significantly reduced by all tested compounds than in control (Tab. 3). At LC_{25}, ginger oil significantly introduced the highest reduction of amylase enzyme (114.51 μg/ml) after 48 h, followed by coragen (133.25 μg/ml). By increasing the concentration to LC_{50}, coragen obviously came in the first in reducing amylase enzyme (62.13 μg/ml), followed by ginger oil (69.00 μg/ml) without significant differences. While clove oil occupied the last category in reducing amylase amount in both concentrations, this opposed to control recording (182.53 μg/ml). Analysis of variance showed a significant difference between tested materials and control ($F = 372.65$, df = 4, $p = 0.0000$) and between concentrations ($F = 288.52$, df = 1, $p = 0.0000$).

3.1.1.3. β-glucosidase enzyme. In case of β-glucosidase enzyme, neemazal, both oils and coragen treatments reduced the activity of β-glucosidase than control as indicated in Table 3 ($F = 89.01$, df = 4, $p = 0.0000$). The amount of enzyme decreased by increasing treatments concentration ($F = 103.41$, df = 1, $p = 0.0000$). Neemazal formulation was the most effective one and occupied the first category from the side of reducing β-glucosidase at both concentrations (LC_{25} and LC_{50}) representing by 170.15 μg/ml and 132.28 μg/ml, respectively. Ginger oil introduced the least category in reducing β-glucosidase (204.21 μg/ml) at LC_{25} concentration while clove oil recorded the lowest one (185.17 μg/ml) at LC_{50}. Treatment with higher concentration of coragen did not differ significantly with neemazal and ginger oil.

3.1.2. Protein hydrolyzing enzymes

Activities of all determined enzymes decreased after 48 h of treatment with all tested compounds except for ALT in case of ginger oil (Table 4).

Table 2
Toxicity of neemazal, ginger oil, clove oil and coragen on tomato leafminer, *Tuta absoluta* 3rd instar larvae after 48 h.

Treatments	N.	LC_{25} (95% CI)	LC_{50} (95% CI)	LC_{90} (95% CI)	Slope ± S.E.
Azadirachta indica	360	95.97 (76.68–110.09)	159.94 (144.32–179.61)	422.39 (327.20–668.49)	3.0391 ± 0.46
Zingiber officinale	360	241.58 (172.52–312.14)	633.38 (510.87–781.43)	3958 (2801–6426)	1.61 ± 0.16
Syzygium aromaticum	360	283.79 (190.73–380.40)	930.71 (726.39–1217)	8902 (5395–18989)	1.31 ± 0.15
Coragen (Chlorantraniliprole)	360	27.91 (20.13–34.48)	57.52 (49.08–66.70)	227.54 (168.74–370.46)	2.15 ± 0.28

LC_{25}, LC_{50} and LC_{90} values based on % and CI 95% Confidence intervals, tested compounds activity is considered significantly different when the 95% CI fail to overlap. N is the number of insects that is used in bioassay.

Table 3
Effect of neemazal T/S, ginger oil and clove oil sublethal doses compared to coragen pesticide on carbohydrate hydrolyzing enzymes activity (μg/ml) in the supernatant of *Tuta absoluta* 3rd instar homogenated larvae under laboratory conditions.

	Enzymes								
	Trehalase		Mean	Amylase		Mean	β-glucosidase		Mean
Treatments	LC_{25}	LC_{50}		LC_{25}	LC_{50}		LC_{25}	LC_{50}	
Control	270.61 ± 6.25a			182.53 ± 3.12a			225.3 ± 3.26a		
Neemazal formulation	225.31 ± 4.38c	138.37 ± 3.57e	181.84	159.11 ± 6.73c	125.30 ± 4.28d	142.20	170.15 ± 2.81d	132.28 ± 2.79f	151.22
Ginger oil	258.07 ± 8.15ab	232.26 ± 2.21c	245.16	114.51 ± 3.39e	69.00 ± 2.22f	91.76	204.21 ± 4.25b	151.35 ± 7.28e	177.78
Clove oil	249.15 ± 5.93b	184.50 ± 5.59d	216.83	173.60 ± 3.60b	163.24 ± 3.74c	168.42	200 ± 6.79b	185.17 ± 2.12c	192.59
Coragen 20% SC	220 ± 7.69c	122.43 ± 4.69f	171.21	133.25 ± 2.88d	62.13 ± 2.75f	97.69	177.42 ± 4.07cd	141.03 ± 2.96ef	159.22
LSD ≤ 0.05 Treat.	10.37			6.29			9.27		
Conc.	6.56			3.98			5.87		
Treat. × Conc.	14.67			8.89			13.12		

Table 4
Effect of neemazal T/S, ginger oil and clove oil sublethal doses compared to coragen pesticide on protein hydrolyzing and transaminase enzymes activity (μg/ml) in the supernatant of *T. absoluta* 3rd instar homogenated larvae under laboratory conditions.

	Enzymes								
	Protease		Mean	AST (GOT)		Mean	ALT (GPT)		Mean
Treatments	LC_{25}	LC_{50}		LC_{25}	LC_{50}		LC_{25}	LC_{50}	
Control	127.19 ± 3.91a			14.72 ± 0.58a			166.42 ± 4.92b		
Neemazal formulation	95.57 ± 3.49c	85.48 ± 3.96de	90.52	10.29 ± 0.37c	8.67 ± 0.35de	142.20	124.05 ± 2.53c	67.42 ± 2.61e	95.74
Ginger oil	98.35 ± 3.43c	78.55 ± 2.29e	88.45	12.17 ± 0.56b	7.88 ± 0.23ef	91.76	169.32 ± 2.13b	196.27 ± 3.24a	182.80
Clove oil	116.15 ± 3.30b	94.62 ± 4.41c	105.38	12.51 ± 0.78b	10 ± 0.32c	168.42	77.45 ± 2.37d	72.18 ± 2.39de	74.82
Coragen 20% SC	86.28 ± 2.47d	67.29 ± 2.10f	76.79	9.53 ± 0.32cd	7.18 ± 0.29f	97.69	71.81 ± 2.44de	70.22 ± 2.16e	71.02
LSD ≤ 0.05 Treat.	5.30			0.89			4.23		
Conc.	3.35			0.57			2.68		
Treat. × Conc.	7.50			1.26			5.99		

3.1.2.1. Protease enzyme

The results showed that the highest value of the protease activity was found in the untreated larvae 127.19 (μg/ml) and the activity was sharply decreased after all used substances at both doses (F = 6.41, df = 4, p = 0.0022). It is noticeable that activity of this enzyme was concentration dependent (F = 77.87, df = 1, p = 0.0000). Obviously, the activity declined to the lowest values after 48 h in coragen treatment at LC_{25} and LC_{50} (86.28, and 67.29 (μg/ml), respectively. On contrary, clove oil showed the highest values of studied enzyme at both concentrations (116.15 and 94.62 μg/ml, respectively). Neemazal effect was significantly on a par with ginger oil at LC_{25} as well as at LC_{50}.

3.1.2.2. AST (GOT) enzyme activity

Data in Table 4 showed that the tested compounds caused a significant decrease in the activity of GOT in the treated larvae than the untreated larvae (F = 6.66, df = 4, p = 0.0018). The activity of this enzyme markedly decreased by concentration increase (F = 63.99, df = 1, p = 0.0000). Coragen caused the highest reduction at both concentrations after 48 h of treatment, meanwhile clove oil appeared the least effect on studied enzyme among the tested compounds at distinct concentrations. The general mean values of GOT enzyme activities in the supernatant of the homogenated larvae reached to 8.35, 9.48, 10.03 and 11.26 μg/ml in case of coragen, neemazal, ginger oil and clove oil, respectively, compared with 14.72 μg/ml in control.

3.1.2.3. ALT (GPT) enzyme activity

The results indicated that the activity of GPT significantly decreased in all investigated substances at LC_{25} compared to control, followed by drastic decline at LC_{50} (F = 32.86, df = 1, p = 0.0000) except for ginger oil. Ginger oil caused no significant increase (169.32 μg/ml) in studied enzyme at LC_{25}, then followed by significant raise at LC_{50} (196.27 μg/ml). The difference between coragen, clove oil and neemazal treatments at LC_{50} was not significant.

3.1.3. Lipid hydrolyzing enzymes

As shown in Table 5, lipase and α- esterase enzyme level activities increased while, β-estarase revealed different attitudes in the larvae fed on the tested substances at both concentrations, LC_{25} and at LC_{50}.

3.1.3.1. Lipase enzyme. Lipases are enzymes that specially hydrolyze the outer links of fat molecules. In our investigation, data revealed the significant increasing effect of sublethal doses of neemazal, plant oils and coragen on lipase activity of the treated 3rd instar larvae than control (F = 23.50, df = 4, p = 0.0000). The tested compounds were significantly more effective at higher concentrations (F = 187.81, df = 1, P = 0.0000). Clearly, coragen was the most effective one from side of increasing lipase at each concentration, representing by 117.47 μg/ml as a general mean, followed by neemazal (98.18 μg/ml) and finally clove oil (87.39 μg/ml) in comparison to control (78.14 μg/ml).

3.1.3.2. Non-specific esterases determination. The activity of alpha-esterase in larval content after 48 h of treatment with investigated materials considerably increased than control and the increase was dose-dependent. The enzyme activity reached to the maximum value in coragen (445.35 μg/ml) at LC_{50} compared with 247.33 μg/ml of control. On the contrary, the least activity occurred in ginger oil at LC_{25} with 250 μg/ml. Statistical analysis of data indicate that all tested treatments gave a significant increase in enzyme activity with control (F = 52.85, df = 4, p = 0.0000), with no significant increase between neemazal and clove oil at LC_{50}.

Table 5
Effect of neemazal T/S, ginger oil and clove oil sublethal doses compared to coragen pesticide on lipid hydrolyzing enzymes activity (μg/ml) in the supernatant of *T. absoluta* 3rd instar homogenated larvae under laboratory conditions.

	Enzymes								
	Lipase		Mean	α –Esterase		Mean	β –Esterase		Mean
Treatments	LC_{25}	LC_{50}		LC_{25}	LC_{50}		LC_{25}	LC_{50}	
Control	78.14 ± 2.54e			247.33 ± 2.78g			34.57 ± 1.07b		
Neemazal formulation	88.14 ± 1.22cd	108.22 ± 3.25b	98.18	306.44 ± 8.92e	426.05 ± 4.57b	366.25	27.73 ± 1.11cd	22.87 ± 1.44e	25.30
Ginger oil	82.06 ± 1.33de	110.73 ± 4.82b	96.40	250 ± 5.80fg	374.60 ± 3.74c	312.30	30.24 ± 1.24c	24.18 ± 1.07de	27.21
Clove oil	79.77 ± 2.30e	95.00 ± 1.50c	87.39	264.51 ± 4.02f	409.63 ± 8.63b	337.07	34.83 ± 1.35b	36.33 ± 1.43b	35.58
Coragen 20% SC	93.43 ± 1.24c	141.51 ± 2.78a	117.47	357.28 ± 4.05d	445.35 ± 4.72a	401.32	37.31 ± 1.16ab	40.24 ± 1.41a	38.78
LSD ≤ 0.05 Treat.	10.37			6.29			9.27		
Conc.	6.56			3.98			5.87		
Treat. × Conc.	14.67			8.89			13.12		

Regarding to beta-esterase activity, data in Table 5 indicated that neemazal and ginger caused significant decrease (25.30 and 27.21 μg/ml, respectively) after 48 h of exposure to both determined concentrations. While coragen caused significant increase (38.78 μg/ml) in studied enzyme activity. On the other hand, clove oil caused no significant increase of the enzyme in 3rd instar larvae at both concentrations compared with 34.57 μg/ml of the control. Analysis of variance showed that all treatments differed significantly with control (F = 4.79, df = 4, p = 0.0083), but the difference between concentrations were not significant (F = 2.57, df = 1, p = 0.1264).

3.2. Effects of neemazal T/S, ginger oil, and clove oil on energy reserves and total amino acid concentration in homogenated larvae when compared to coragen

3.2.1. Total proteins

As clearly shown from the results compiled in Table 6, the protein content was markedly reduced in larvae treated with both concentrations of neemazal, selected oils and coragen after 48 as compared with control. This organic component greatly influenced by increasing dose (F = 189.44, df = 1, p = 0.0000). Larvae treated with neemazal showed lower protein content (20.12 μg/ml) than tested oils. While coragen owned the first arrange in reducing total protein among the tested compounds recording 16.43 μg/ml compared with 33.45 μg/ml of control.

3.2.2. Total carbohydrates

Tabulated results in Table 6 showed that total carbohydrates decreased gradually from 48.6 μg/ml in control to 41.20 μg/ml in ginger as the highest value among tested materials, then terminated by coragen that significantly gave the least content of total carbohydrates (30.26 μg/ml) in larvae supernatant. Statistical analysis gave evidence of significant differences in total carbohydrates in both concentration of all treatments (F = 19.14, df = 4, p = 0.0000).

3.2.3. Total lipids

As for total lipids, treatment of 3rd instar larvae with LC_{25} and LC_{50} concentrations of tested materials caused reduction in lipid content after 48 h comparing to control (Table 7) (Tab. 5) (F = 15.38, df = 4, p = 0.0000). Statistical analysis of data showed no significant differences between most of tested compounds and control (7.67 μg/ml) at LC_{25} concentration except for neemazal which recorded significant decrease in lipid content (6.88 μg/ml). Meanwhile, significant decrease was observed between the investigated compounds and control at LC_{50} concentration. This reduction at LC_{50} was more severe in the case of neemazal (3.50 μg/ml) with no significant difference with coragen treatment (4.13 μg/ml).

3.2.4. Total amino acids concentration

Results illustrated markedly decrease in larval content of total free amino acids after 48 h of exposure to LC_{25} and LC_{50} of all tested substances compared to control (Table 7) (F = 33.86, df = 4, p = 0.0000). At LC_{25} concentration, all tested compounds significantly reduced total free amino acids compared with 417.67 μg/ml of the control, without significant difference between neemazal and coragen treatments. At LC_{50} concentration, all investigated compounds represented significant reduction with control, but no significant differences between them except for ginger oil (309.47 μg/ml) occupying the last category in reducing the amount of studied parameter.

4. Discussion

The present study revealed that neemazal formulation produced higher insecticidal activity than the tested plant oils (ginger and clove). The mortality difference observed between tested

Table 6
Effect of neemazal T/S, ginger oil and clove oil sublethal doses compared to coragen pesticide on the total proteins, total carbohydrates and total lipids concentration (μg/ml) in the supernatant of *T. absoluta* 3rd instar homogenated larvae under laboratory conditions.

	Enzymes								
	Total proteins		Mean	**Total carbohydrates**		Mean	**Total lipids**		Mean
Treatments	LC_{25}	LC_{50}		LC_{25}	LC_{50}		LC_{25}	LC_{50}	
Control	33.45 ± 0.91a			48.60 ± 0.26a			7.67 ± 0.23a		
Neemazal formulation	25.51 ± 0.90d	14.73 ± 0.92f	20.12	38.24 ± 1.14c	29.45 ± 0.95e	33.85	6.88 ± 0.32bc	3.50 ± 0.29e	5.19
Ginger oil	30.30 ± 1.25b	21.07 ± 0.33e	25.69	42.30 ± 0.98b	40.10 ± 1.14bc	41.20	7.54 ± 0.20ab	5.34 ± 0.26d	6.44
Clove oil	29.13 ± 0.56bc	27.60 ± 0.76cd	28.37	38.57 ± 0.97c	25.67 ± 0.38f	32.12	7.75 ± 0.29a	6.32 ± 0.21c	7.04
Coragen 20% SC	22.03 ± 1.31e	10.82 ± 0.82g	16.43	35.41 ± 1.39d	25.10 ± 0.98f	30.26	7.07 ± 0.20ab	4.13 ± 0.20e	5.60
LSD ≤ 0.05 Treat.	1.58			1.87			0.51		
Conc.	1.00			1.18			0.32		
Treat. × Conc.	2.24			2.64			0.72		

Table 7
Effect of neemazal T/S, ginger oil and clove oil sublethal doses compared to coragen pesticide on the total amino acids concentration (µg/ml) in the supernatant of *T. absoluta* 3rd instar homogenated larvae under laboratory conditions.

Treatments		Organic components		
		Total free amino acid		Mean
		LC_{25}	LC_{50}	
Control		417.67 ± 7.31a		
Neemazal formulation		261.03 ± 8.10d	193.55 ± 4.58e	227.29
Ginger oil		368.21 ± 7.69b	309.47 ± 6.19c	338.84
Clove oil		320.33 ± 6.49b	187.66 ± 4.56c	254.00
Coragen 20% SC		254.11 ± 4.32d	183.25 ± 5.14e	218.68
LSD ≤ 0.05	**Treat.**	12.03		
	Conc.	7.61		
	Treat. × Conc.	17.01		

botanical compounds could be due to azadirachtin (a tetranortriterpenoid) as an active ingredient in neemazal. While volatiles, mostly monoterpenes, in oils caused the insecticidal activity (Huang et al., 2000).

Most biochemical components in treated larvae were decreased significantly 48 h after all treatments. Carbohydrate hydrolyzing enzymes (trehalase, amylase and β-glucosidase) were reduced in treated larvae. Determined decrease in trehalase was also recorded by Gaaboub et al. (2012); Tatun et al. (2014b) in *Tribolium castaneum* and (Oladipo (Nee Ajayi) et al., 2019) in regard to *n*-hexane extract of *Senna occidentalis* on *Callosobruchus chinensis* (L.). Selem and El-Sheikh (2015) represented normal trehalase activity in applying neemazal T/S, willow or chasteberry on *musca domestica* as control.

α-amylase is the most important digestive enzymes of many insects that feed exclusively on plants during larval and/or adult stage. When the activity of the amylases is inhibited, energy is shorten as a result of impaired nutrition of the organism (Mehrabadi et al., 2010). The present decrease in amylase is consistent with other reports Tatun et al. (2014a); Yazdani et al. (2014); Mojarab-Mahboubkar et al. (2015); Bezzar-Bendjazia et al. (2017).

In insects, digestive β-glucosidases are important for the hydrolysis of di- and oligo-β-saccharides derived from hemicelluloses and cellulose and are involved in insect–plant interactions (Terra and Ferreira, 1994). Resulted decrease in this enzyme coincided with those reported by Bigham et al. (2010); Zibaee and Bandani (2010); Zibaee et al. (2010); Khosravi and Sendi (2013).

Protein hydrolyzing enzymes (Protease, AST and ALT) were also reduced in treated larvae after 48 h of treatment. Proteases play an important role in the food digestion in insects by converting protein to amino acids needed for the body (Terra and Ferreira, 2005). The role of plant extracts in suppressing protease activity could be due to the plant defense compounds that act on digestive enzymes (Ryan, 1990; Franco et al., 2005). Our finding is generally agree with Khosravi and Sendi (2013); Mojarab-Mahboubkar et al. (2015); Bezzar-Bendjazia et al. (2017) who inferred that botanical insecticides may inhibit the production of certain types of proteases and disable them to digest ingested proteins. Also, the decline of this enzyme activity could be due to a cytotoxic effect of different extracts on the midgut epithelial cells, that synthesize amylase (Jbilou and Sayah, 2007). The amino transferases, AST and ALT are important compounds of amino acid catabolism; which is involved in transferring an amino group from one amino acid to a keto acid (Zibaee et al., 2008). The AST and ALT serve as a strategic link between the carbohydrates and protein metabolism and are known to be changed during various physiological and pathological conditions (Etebari et al., 2005). Our results indicated a clear decrease in both enzymes and these were concurrence with Gaaboub et al. (2012), but Amirmohammadi et al. (2013) did not show differences in AST and ALT on treated insects. Selem and El-Sheikh (2015) showed significant decrease in ALT activity, but neemazal T/S only markedly decreased AST activity.

Lipid hydrolyzing enzymes showed converse trend as they were increased after treatment. Enhancement of midgut lipase activity might be the reason for a greater utilization of exogenous lipids and might result in the biomass production (Desai and Desai, 2000; Emam et al., 2009). Sujatha et al. (2010) mentioned that *Pedaliumm murex* L. extract increased lipase activity in *Spodoptera litura* (Fabricius). Yazdani et al. (2014) confirmed that lipase activity was not significantly changed. While remarkable decrease in lipase activity was stated by Zibaee et al. (2008); Zibaee and Bandani (2010); Mojarab-Mahboubkar et al. (2015).

Esterase (EST) is a vital detoxifying enzyme in vivo which hydrolyzes the esteric bond in synthetic chemicals. The response increases of EST enzymes to botanical extracts were significantly attributed to using different concentrations of extract and long exposure. The obtained herein results are harmony with what reported by Yazdani et al. (2014) who demonstrated the increased activity of general esterase in the *G. pylolais* larvae. Zibaee and Bandani (2010) mentioned the significant increased 24 h post-treatment of *Artemisia annua* extract in *Eurygaster integriceps* Puton hemolymph. On contrary, Mojarab-Mahboubkar et al. (2015); Abdel-Razi (2018) revealed a decreased amount of esterases.

Proteins are major biochemical components for the development of organisms, growing and performing their vital activities (Desoky et al., 2020; El-Saadony et al., 2021a; El-Saadony et al., 2021b; Saad et al., 2021c). The decline in protein content in the larvae was due to one or a combination of factors, like a reduction in proteins synthesis or an increase in the breakdown of proteins to detoxify the active principles present in the plant extracts or essential oils (Vijayaraghavan et al., 2010; El-Saadony et al., 2021c). Similar results were obtained by Schmidt et al. (1998) by using a methanolic extract of *Melia azedarach* L. on the hemolymph protein of *Spodoptera littoralis* (Boisduval) and *Agrotis ipsilon* (Hufnagel). Also, Mojarab-Mahboubkar et al. (2015); Abdel-Razi (2018) demonstrated the same result.

To meet the energy expenses under stress conditions, more sugars might be metabolized. This might be the reason for the carbohydrate level depletion in the treated larvae. This is agree with Khosravi et al. (2010) in *Glyphodes pyloalis* larvae treated with *Artemisia annua* extract (Yazdani et al., 2013) in *Glyphodes pyloalis* treated with essential oil of Summer Savory, *Satureja hortensis* L. (Family: Lamiaceae) and (Abdel-Razi, 2018).

Reduction of lipid levels in the larvae treated with plant essential oils, neemazal and pesticide may be due to their effect on the lipid metabolism, and due to the utilization of lipid reserves for energy production because of induced stress (Sancho et al., 1998; Sak et al., 2006). This result was in line with (Yazdani et al., 2013, 2014; Abdel-Razi, 2018).

Insect blood plasma is characterized by very high levels of free amino acids. It performs additional metabolic function in insects apart from protein synthesis. Marked changes occurred in larvae, when it was treated with different plant extracts can be referred to accelerated neuromuscular activity which resulted in greater needs for energy. So, high quantity of free amino acids entered into the Tricarboxylic acid cycle and oxidized. It results that the free amino acid of haemolymph is reduced substantially Gnanamani and Dhanasekaran (2014). Similar result was obtained by Gnanamani and Dhanasekaran (2014) when they studied five different plants extracts on free amino acids in the haemolymph of the last instar larvae of *Pericallia ricini*. Along with the depletion of amino acids, there was also a marked decrease in the protein content. It is evident that the plant extracts induced significant reduction in protein content and amino acids. This view is also go with that recorded by Pandey et al. (1986); Vijayaraghavan and Chitra (2002), but did not agree with Pathak and Tiwari (2017).

5. Conclusion

Our results clearly confirm that neemazal formulation and both essential oils were both toxic to *T. absoluta* 3rd instar larvae, and showed irreversible effects on key metabolic processes that impair the physiological fitness of the larva. Therefore, neemazal and tested oils may be considered a potent candidate in integrated pest management programs for controlling this pest.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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