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

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A B S T R A C T

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, LC25 and LC50 under constant laboratory conditions. Results showed that neemazal T/S is more toxic than detected oils compared with higher toxicity of coragen with LC50 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.

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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 larval 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 environmen-
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 available on the larvicidal effect of these oils on *Tuta absoluta*.

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 *Glyphpodes 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 (LC25 and LC50) 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 (3.9 cm in diameter and 6 cm in height) covered with muslin cloth for oviposition and fixed with a rubber band. Two droplets of 10% honey solution were added in the cup as food. The insects were kept for two days to copulate and lay eggs. Muslin 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 × 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. Muslin 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).

#### Table 1

| Common name                  | Scientific name                  | Family       | Used part (Formulation) | Source                  |
|-----------------------------|----------------------------------|--------------|-------------------------|-------------------------|
| Neemazal T/S formulation (1% azadirachtin) | *Azadirachta indica* (A. Juss.) | Meliaceae    | Seeds (Formulation)     | Trifolio-M GmbH-Germany Extracted |
| Ginger oil                   | *Zingiber officinale* *Roscoe*  | Zingiberaceae | Rhizomes                | Extracted               |
| Clove oil                    | *Syzygium aromaticum* *L.*       | Myrtaceae    | Flowers buds            |                         |
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 (Bio-diagnostic 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) using Diagnostic 29 Tahreer St., Dokki, Giza, Egypt) that was purchased from High Lab Company. 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 Statistik 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. absoluta 3rd instar homogenated larvae

The LC50 of both plant extracts and coragen on Tuta absoluta larvae were introduced in Table 2. The LC50 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 LC25 and LC50 of 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 LC25 and LC50 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. Carbohydrate hydrolyzing enzymes

Treatments with LC25 and LC50 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 LC25 and LC50 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 LC25, 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 LC50, 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 (LC25 and LC50) representing by 170.15 µg/mL and 132.28 µg/mL respectively. Ginger oil introduced the last category in reducing β-glucosidase (204.21 µg/mL) at LC50 concentration while clove oil recorded the lowest one (185.17 µg/mL) at LC50. 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).

| Treatment               | LC25 (95% CI) | LC50 (95% CI) | LC90 (95% CI) | Slope ± S.E. |
|-------------------------|--------------|--------------|--------------|-------------|
| Azadirachta indica      | 95.97 (76.68–110.09) | 159.94 (144.32–179.61) | 422.39 (327.20–668.49) | 3.0391 ± 0.46 |
| Zingiber officinale     | 241.58 (172.52–312.14) | 633.38 (510.87–781.43) | 3958 (2801–6426) | 1.61 ± 0.16 |
| Syzygium aromaticum     | 283.79 (190.73–380.40) | 930.71 (726.39–1217) | 8902 (5395–18989) | 1.31 ± 0.15 |
| Coragen                 | 27.91 (20.13–34.48) | 57.52 (49.08–66.70) | 227.54 (168.74–370.46) | 2.15 ± 0.28 |

LC25, LC50 and LC90 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.
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 all used substances at both doses (F = 6.41, df = 4, p = 0.0022). It is noticeable that activity of this enzyme markedly decreased by concentration increase with 14.72 µg/ml in control, followed by drastic decline at LC50 (F = 32.86, df = 1, p = 0.0000) except for ginger oil. Ginger oil caused no significant decrease in the activity of GOT in the treated larvae than control and the increase was 166.42 ± 3.91µg/ml in case of coragen (645.35 µg/ml) at LC50 and LC25.

### 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 all used substances at both doses (F = 6.41, df = 4, p = 0.0022). It is noticeable that activity of this enzyme markedly decreased by concentration increase with 14.72 µg/ml in control, followed by drastic decline at LC50 (F = 32.86, df = 1, p = 0.0000) except for ginger oil. Ginger oil caused no significant decrease in the activity of GOT in the treated larvae than control and the increase was 166.42 ± 3.91µg/ml in case of coragen (645.35 µg/ml) at LC50 and LC25.

### 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 LC25 compared to control, followed by drastic decline at LC50 (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 LC25, then followed by significant raise at LC50 (196.27 µg/ml). The difference between coragen, clove oil and neemazal treatments at LC50 was not significant.

### 3.1.3. Lipid hydrolyzing enzymes

As shown in Table 5, lipase and α-esterase enzyme level activities increased while, β-esterase revealed different attitudes in the larvae fed on the tested substances at both concentrations, LC25 and at LC50.

#### 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) and which have a 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 LC50 compared with 247.33 µg/ml of control. On the contrary, the least activity occurred in ginger oil at LC25 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 LC50.
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 h as compared with control. This organic component greatly influenced by increasing dose (F = 189.44, df = 4, 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 30.26 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 LC25 and LC50 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 LC25 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 LC50 concentration. This reduction at LC50 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 LC25 and LC50 of all tested substances compared to control (Table 7) (F = 33.86, df = 4, p = 0.0000). At LC25 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 LC50 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 5

| Enzymes          | Treatments   | Mean β –Esterase | Mean γ –Esterase | Mean Lipase |
|------------------|--------------|------------------|-----------------|-------------|
|                  | LC25         | LC50             | LC25            | LC50        |
| Control          | 6.29         | 10.37            | 9.27            | 7.67        |
| Neemazal T/S     | 9.10         | 12.70            | 11.40           | 10.80       |
| Ginger oil       | 5.60         | 9.10             | 8.20            | 7.40        |
| Clove oil        | 4.10         | 7.60             | 6.70            | 5.80        |
| Coragen 20% SC   | 6.56         | 10.07            | 9.37            | 8.67        |
| LSD ≤ 0.05 Treat.| 14.67        | 8.89             | 13.12           | 10.47       |
| Conc.            | 8.56         | 11.96            | 10.76           | 9.86        |
| Treat. × Conc.   | 5.87         | 9.17             | 8.37            | 7.57        |

### Table 6

| Enzymes          | Treatments   | Mean total proteins | Mean total carbohydrates | Mean total lipids |
|------------------|--------------|---------------------|-------------------------|-------------------|
|                  | LC25         | LC50                | LC25                    | LC50              |
| Control          | 33.45 ± 0.91a| 35.45 ± 0.91       | 33.85 ± 1.16            | 30.24 ± 1.24      |
| Neemazal T/S     | 25.51 ± 0.90d| 27.21 ± 0.90c      | 25.02 ± 1.13            | 22.87 ± 1.44      |
| Ginger oil       | 30.03 ± 1.25a| 31.70 ± 1.25        | 29.69 ± 1.35            | 27.73 ± 1.11c     |
| Clove oil        | 22.03 ± 1.31e| 24.07 ± 1.31        | 21.89 ± 1.35            | 20.24 ± 1.41a     |
| Coragen 20% SC   | 1.58         | 1.87               | 1.90                  | 1.90             |
| LSD ≤ 0.05 Treat.| 1.00         | 1.18               | 1.22                  | 1.22             |
| Conc.            | 2.24         | 2.64               | 2.72                  | 2.72             |
| Treat. × Conc.   | 5.87         | 9.17               | 8.37                  | 7.57             |
botanical compounds could be due to azadirachtin (a tetrarnotriterpenoid) as an active ingredient in neemazal. While volatiles, mostly monoterpines, 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.). Seleem and El-Sheikh (2015) represented normal trehalase activity in applying neemazal T/S, willow or chasteberry 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 shorted 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 synthesise 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. Seleem 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 Pedalium 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-Sadony et al., 2021a; El-Sadony 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-Sadony 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 (Boisduala) 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 pylolais larvae treated with Artemisia annua extract (Yazdani et al., 2013) in Glyphodes pylolais 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. This view is also to influence the work reported in this paper.

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 reduction in protein content and amino acids. This view is also to influence the work reported in this paper.

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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