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Biochemical influence of *Croton tiglium* oil and its nano-emulsion on larvae of the spiny bollworm *Earias insulana* (Lepidoptera: Noctuidae)

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ABSTRACT: ***This study aimed to evaluate effect of LC50 of Croton tiglium oil and its nano-emulsion on the biochemical response of the larvae of the spiny bollworm, Earias insulana. Treatments elicited decreasing in total carbohydrate, Glutamic pyruvic transaminase (GPT) and trehalase in the larvae of E. insulana as compared with control. The level of total lipid increased with nano-emulsion of C. tiglium oil and decreased with C. tiglium oil in the larvae of the spiny bollworm. Two treatments decreased levels of total protein in the larvae of the spiny bollworm. Tested two compounds elicited decreasing in Glutamic pyruvic transaminase (GPT) in the larvae of E. insulana as compared with control. Application of the two compounds reduced glutamic oxaloacetic transaminase (GOT) levels in the larvae of E. insulana. Croton tiglium oil decreased invertase enzyme levels, while its nano emulsion increased its levels in the larvae of E. insulana. Treatments caused inhibition in trehalase levels in the larvae of E. insulana as compared with control. C. tiglium oil increased levels of lipase enzyme, but nano-emulsion of C. tiglium oil caused inhibition of its level in the larvae of E. insulana. The treatments decreased levels of protease enzyme in the larvae of the spiny bollworm as compared with control. The two compounds under study increased acetyl cholinesterase activities in the larvae of the spiny bollworm as compared with control.***

Keywords: ***C. tiglium, Fixed oil, Nano-emulsion***

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I. INTRODUCTION

Cotton is one of the most significant commercial crops in Egypt and serves as a strategic crop by contributing to Gross domestic product (GDP) **(Ahmed and Delin, 2019).** The spiny bollworm, (SBW) *Earias insulana* (Lepidoptera: Noctuidae) is considered one of the most important pests in the world. It causes severe damage, resulting in a great loss in both quality and quantity of yield (**Abdel-Raheem *et al.*, 2023)**. Excessive use of synthetic chemicals has created harmful impacts on non-target organisms and environment. Plant-based insecticides have been evidenced as important alternatives to conventional synthetic insecticides **(Majeed *et al.*, 2021)**.

Some plant active compounds exhibit stomach and contact toxicity, whereas most of them have slow-acting actions such as larval growth inhibition and interruption of insect development **(Pavela, 2016; Ikbal and Pavela, 2019; Isman, 2020**). **Hu et al., (2010)** emphasized that *C. tiglium* L. seeds are rich in phorbol esters, crotonic acid and fatty acids in addition to active phytoconstituents that are responsible for severe purgative effect of the *C. tiglium* L. seeds extract. Due to the strong toxic effect

of the chemical synthetic products, components of natural essential oil are gaining frequent presence and increasing interest in the recent studies investigating their potential activity and functional utility **(Babahmad et al., 2018; Rakmai et al., 2018).** It was reported that linoleic acid, oleic acid and eicosenoic acid are the most abundant fatty acids exist in a methyl-esterified sample obtained by reflux method **(Mei et al., 2012).** *Croton tiglium* registered mortality with 57.39% to the 3rd instars larvae of *Spilarctia obliqua* Walk **(Srivastav & Chandel, 2021)**.

Since the oil is made into a nano-emulsion, the particle size is smaller and the surface area is larger, increasing the biological activity **(Massoud et al., 2018)**. Nano-emulsions are droplets that are uniform in size and extremelysmall, ranging from 20 to 200 nm **(Fernandes et al., 2014**). The amount required from nano-pesticides is very small for effective pest management and it can reduce the pesticide load on the environment **(Kumar et al., 2015). Marouf et al., (2021)** indicated that camphor nano-emulsion is more efficient than camphor essential oil against *S. Littoralis*. Also, castor oil nano-emulsion demonstrated the highest efficacy when compared to its bulk or standard emulsion, according to **(Abdel- Raheem, 2019)**. **Dinesh, et al. (2022)** recorded that the nano-matierals are effective and eco-friendly bio-insecticides for not only control bollworm and also other lepidopteran insects such as *Spodoptera litura* and *Plutella xylostella* at different crop ecosystem.

II. Materials and Methods

2.1. The spiny bollworm culture:

The laboratory strain of the spiny bollworm was taken from the Bollworm Research Department, Plant Protection Research Institute; Sharkia Branch, Agricultural Research Center (ARC). This strain has been reared for several generations away from any contamination with insecticides. Larvae of the spiny bollworm were reared on a modified artificial diet as described by **(Amer, 2015)** under laboratory conditions of 26 ± 1 ˚C and 65 ± 5 % R.H.

2.2. Tested materials

Croton tiglium oil is a fixed oil extracted from *Croton tiglium* seeds. Scientific name: is *Croton tiglium* plant. Common name: is purging croton plant. Nano-emulsion was also prepared.

2.2.1. Extraction technique of fixed oils.

Extraction of the *Croton tiglium* fixed oil was performed at room temperature using crushed seeds. Where, the dry powder seeds were steeped in petroleum ether (60/80). The petroleum-ether extracts were filtered over anhydrous sodium sulphate. A rotary evaporator apparatus was used to remove the solvent; oils were stored in dark brown bottles at 4C^{o} until use.

2.2.2 Preparation of Nano-emulsions

The oil-in-water nano-emulsions experiments were carried out in the laboratory of the National Research Center according to **EL-Medany, *et al*. (2022)**.

2.3 Biochemical effects of *C. tiglium* oil and its nano-emulsion in newly hatched larvae of *E. insulana*.

2.3.1 Preparation of samples for biochemical assay.

Following seven days of treatment with LC_{50}s (9.022 and 2.706%) of *C. tiglium* oil and its nano-emulsion, respectively and control, specimens of *E. insulana* larvae were obtained for biochemical analysis. For biochemical investigation, larvae were homogenized in a refrigerated glass Teflon tissue homogenizer (ST – 2 Mechanic-Preczyina, Poland). Supernatants were homogenized and then maintained at -20C^{o} in a deep freezer until they were needed for biochemical tests. UV/Visible double beam spectrophotometer (spectronic 1201, Milton Roy Co., USA) was utilized to evaluate the absorbance of pigments or metabolic components. The insects were prepared as described by **Amin (1998)**. This experiment was intended to elaborate variations in the activities of carbohydrate hydrolyzing enzymes, total protein, total carbohydrates, total lipids, trans-aminase enzymes (glutamic

oxaloacetic transaminase (GOT)& glutamic pyruvic transaminase (GPT)), proteolytic activity, Lipase activity and acetyl cholinesterase activities in the supernatant of the spiny bollworm homogenated larvae as affected by LC_{50}s as compared with untreated larvae.

2.3.2. Determination of total carbohydrates, total lipids and total protein content.

Using **Dubois et al. (1956)**'s phenol-sulphuric acid reaction, the total carbohydrates in the sample's acid extract were calculated. Total carbohydrates were extracted and prepared for assay according to **Crompton and Birt (1967).** Total lipids were estimated by the method of **Knight, et al. (1972)**.Total proteins were determined by the method of **Bradford (1976)**.

2.3.3. Determination of Transaminase enzymes.

Glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) were determined calorimetrically according to the method of **Reitman and Frankle (1957)**.

2.3.4. Carbohydrate digestive enzymes activity

Digestive enzymes were determined according to the modifications of **Amin (1998)** to the method described by **Ishaaya and Swirski (1976)** using trehalose, sucrose, and soluble starch as substrates for trehalase, invertase and α-amylase, respectively.

2.3.5. Determination of Lipase activity

Lipase activity was determined by a slight modification of the procedure of **Tahoun and Abdel-Ghaffar (1986)**.The method was based on the determination of the decrease in ester content of triolein as substrate.

2.3.6. Determination of protease activity

Protease activity was measured as described by **Tatchell et al. (1972)**, with some modifications, by measuring the increase in free amino acids split from substrate protein (albumin), for one hour incubation at 30 C^0. Amino acids were calorimetrically assayed by ninhydrin reagent according to the method described by **Lee and Takabashi (1966)**.

2.3.7. Acetyl cholinesterase activity

AchE (acetyl cholinesterase) activity was measured according to the method described by **Simpson et al. (1964)**, using acetylcholine bromide (AchBr) as substrate.

2.4. Statistical analysis

Data were analyzed using costat statistical software. One way analysis of variance (ANOVA) was used to test for significant differences between mean values. The values of proper "F" and LSD were counted as described by **Finney, (1971).**

III. Results

Newly hatched larvae were fed on diet treated with LC_{50} of 9.022 & 2.706 % of *C. tiglium* oil and its nano-emulsion, respectively, for one day, then the survived larvae from treatment and those from control were transferred singly into glass tubes containing untreated diet until reaching 7 days old under constant conditions (26 ± 1°C and 65±5 %R.H.)

3.1. Total carbohydrate:

High significant reduction ($p<0.0001$) in level of total carbohydrate in larvae of *E. insulana* was estimated as 13.16 ± 0.4977 and 18.30 ± 0.6506 mg/ g.b.wt by *C. tiglium* oil and its nanoemulsion compared with 32.71 ± 1.7578 mg/ g.b.wt in control experiment (Table 1).

3.2. Total lipid:

Data in Table (1) recorded disturbance in the activity of total lipid between elevation and reduction depending on treatment. The level of total lipid in larvae of *E. insulana* was significantly ($p = 0.0022$)

decreased by *C. tiglium* oil (1.25 ± 0.0288 mg/ g.b.wt) meanwhile, significant increase estimated by its nanoemulsion (2.46 ± 0. .2185 mg/ g.b.wt) in comparison to control 1.97 ± 0.0881 mg/ g.b.wt.

3.3. Total protein:

Data in Table (1) elicited high significant ($p < 0.0001$) decrease in level of total protein in larvae of *E. insulana* by *C. tiglium* oil (30.46 ± 1.0088 mg/ g.b.wt), nanoemulsion of *C. tiglium* oil (52.00 ± 0.5773 mg/ g.b.wt) compared to 65.04 ± 0.6064 mg/ g.b.wt in control.

Table (1): Effect of LC_{50} concentrations of *C. tiglium* oil and its nano emulsion on total carbohydrate, total lipid and total soluble protein in *E. insulana* larvae.

Treatments	Total carbohydrate (mg/ g.b.wt)	Total lipids (mg/ g.b.wt)	Total protein (mg/ g.b.wt)
***C. tiglium* oil**	13.16 ^{c} ± 0.4977	1.25 ^{c} ± 0.0288	30.46 ^{c} ± 1.0088
NE of *C. tiglium*	18.30 ^{b} ± 0.6506	2.46 ^{a} ± 0. 2185	52.00 ^{b} ± 0.5773
Control	32.71^{a} ± 1.7578	1.97 ^{b} ± 0.0881	65.04 ^{a} ± 0.6064
***P* values**	<0.0001***	0.0022 **	<0.0001 ***
$LSD_{0.05}$	3.8746	0.4744	2.6193

Each datum represents the mean and standard Error (SE) of four replicates. a, b and c: statistically significant with reference to control. Means pursued by the same superscript were not significantly different at level of $P \leq 0.05$ (SE, Standard error; NE, nano emulsion; *LSD*, the least significant difference ** significant, *** high Significant)

3.4. Transaminase activities

The changes in glutamic pyruvic transaminase (GPT) activity in the supernatant of the homogenated larvae of *E. insulana* illustrated in Table (2) revealed that *C. tiglium* oil and its nanoemulsion significantly (P=0.0002) decreased (GPT) within (3.16 ± 0.1763 and 2.90 ± 0.1527 U/ g.b.wt), respectively while control recorded 4.84 ± 0. 1527 U/ g.b.wt. Table (2) showed significant decrease (P<0.0001) at glutamic oxaloacetic transaminase (GOT) in all treatments compared with 6.64 ± 0.3179 U/ g.b.wt) control. *C. tiglium* oil and its nano emulsion recorded (1.69 ± 0.07234 and 6.11 ± 0.1481 U/ g.b.wt), respectively.

Table (2): Effect of LC_{50} concentrations of *C. tiglium* oil and its nano emulsion on transaminases enzymes activities of the *E. insulana* larvae

Treatments	Glutamic pyruvic transaminase(GPT) (U/ g.b.wt)	Glutamic oxaloacetic transaminase (GOT) (U/ g.b.wt)
***C. tiglium* oil**	3.16 ^{b} ± 0.1763	1.69 ^{b} ± 0.07234
NE of *C. tiglium* oil	2.90 ^{b} ± 0.1527	6.11 ^{a} ± 0.1481
Control	4.84 ^{a} ± 0.0881	6.64 ^{a} ± 0.3179
***P* values**	0.0002 ***	<0.0001 ***
$LSD_{0.05}$	0.4983	0.7155

Each datum represents the mean and standard Error (SE) of four replicates. a, b and c: statistically significant with reference to control. Means pursued by the same superscript were not significantly different at level of $P \leq 0.05$ (SE, Standard error; NE, nano emulsion; *LSD*, the least significant difference, *** high Significant)

3.5. Carbohydrate hydrolyzing enzymes

3.5.1. Trehalase

Data given in Table (3) showed the changes in trehalase activity in the supernatant of the homogenated larvae of *E. insulana*. The treatments recorded inhibitory action by significant (P<0.0001) decreasing in trehalase activity. *C. tiglium* oil and its nano emulsion gave (74.00 ± 3.1797 and 102.33 ± 2.8480 ug glucose/min/ g.b.wt), respectively while control recorded 269.34 ± 5.8118 (ug glucose/min/ g.b.wt).

Table (3): Effect of LC_{50} concentrations of *C. tiglium* oil and its nano emulsion on carbohydrate hydrolyzing enzymes in *E. insulana* larvae.

Treatments	Trehalase (ug glucose/min/ g.b.wt)	Invertase (ug glucose/min/ g.b.wt)
***C. tiglium* oil**	74.00 ^{c} ± 3.1797	297.33 ± 1.2018
NE of *C. tiglium*	102.33 ^{b} ± 2.8480	301.00 ± 2.0816
Control	269.34 ^{a} ± 5.8118	298.67 ± 4.0960
***P* values**	<0.0001 ***	0.6529 ^{ns}
$LSD_{0.05}$	14.6209	9.4885

Each datum represents the mean and standard Error (SE) of four replicates. a, b and c: statistically significant with reference to control. Means pursued by the same superscript were not significantly different at level of $P \leq 0.05$ (SE, Standard error; NE, nano emulsion; *LSD*, the least significant difference, *** high Significant ^{ns} non-significant)

3.5.2. Invertase

Data presented in Table (3) recorded different action in the activity of invertase after treatment with the two tested compounds. Non-significant increase in invertase activity recorded ($P \geq 0.05$) in case of nano emulsion of *C. tiglium* with (301.00 ± 2.0816 ug glucose/min/ g.b.wt), on the other hand *C. tiglium* oil provoked non-significant decrease in invertase activity with 297.33 ± 1.2018 ug glucose/min/ g.b.wt, compared to 298.67± 4.0960 ug glucose/min/ g.b.wt in control.

3.6. Lipase enzyme

All treatments showed significant (P<0.0001) decrease in lipase enzyme activity with 170.6 ± 2.905, 279.00 ± 0.577 (mU/ g.b.wt) for *C. tiglium* oil & its nanoemulsion, respectively compared with control 280.04 ±0.454 (mU/ g.b.wt) (table, 4).

Table (4): Effect of LC_{50} concentrations of *C. tiglium* oil and its nano emulsion on lipase, Protease and Acetyl cholinesterase enzymes in *E. insulana* larvae.

Treatments	Lipase (mU/ g.b.wt)	Proteases (Ug alanine/min/ g.b.wt)	Acetyl choline esterase (Ug AchBr/min/mg protein)
***C. tiglium* oil**	170.6^{b} ± 2.905	72.66 ^{c} ± 2.1858	115.00 ± 2.3094
NE of *C. tiglium*	279.00^{a} ± 0.577	90.33 ^{b} ± 2.6034	118.66 ± 5.2068
Control	280.04^{a} ± 0.454	128.34 ^{a} ± 6.0092	107.03 ± 3.605
***P* values**	<0.0001 ***	0.0002 ***	0.1764 ^{ns}
$LSD_{0.05}$	5.9883	13.7936	-

Each datum represents the mean and standard Error (SE) of four replicates. a, b and c: statistically significant with reference to control. Means pursued by the same superscript were not significantly different at level of P

≤0.05 (SE, Standard error; NE, nano emulsion; *LSD*, the least significant difference, *** high Significant ^{ns} non-significant)

3.7. Protease enzyme

The treatments showed high significant (*P*=0.0002) inhibitory action by decreasing in protease enzyme activity. *C. tiglium* oil recorded 72.66 ± 2.1858 ug alanine/min/g.b.wt. followed by its nanoemulsion which recorded 90.33 ± 2.6034 ug alanine/ min/ g.b.wt as compared with control (128.34 ± 6.0092 Ug alanine/min/g.b.wt.) (Table, 4).

3.8. Acetyl cholinesterase activities

The changes in Acetyl cholinesterase activities in the supernatant of the homogenate spiny bollworm larvae were manifested in Table (4). *C. tiglium* oil recorded 115.00 ± 2.3094 ug AchBr/min/mg protein while its nanoemulsion gave 118.66 ± 5.2068 ug AchBr/min/mg protein compared with 107.03 ± 3.6055 ug AchBr/min/mg protein in control.

IV. Discussion

In our study *Croton tiglium* and its nanoemulsion caused decreasing in total carbohydrate in the larvae of *E. insulana* as compared with control. The level of total lipid in larvae increased with nano-emulsion of *C. tiglium* and decreased with the other treatment as compared with control. The treatments decreased total protein in the larvae of *E. insulana* as compared with control. Our results are in harmony with **Elhadek, et al. (2015)** who reported that total protein content was reduced by *Nigella Sativa, Eucalyptus camaldulensis* and *Sesamum indica* but increased in case of using *Trigonella foenum* on *Spodoptera littoralis* 4th larval instar. **Ali, et al. (2017)** assessed that the total proteins and lipids contents were decreased in larvae of the *Spodoptera littorals* treated with garlic and lemon oils. Lipase and α-amylase were decreased in treated larvae. **Hegab, (2018)** showed increasing in total soluble protein content in the larvae of *E. insulana* treated with *C. limon* and *M. spicata*. **Said, et al. (2019**) assessed that LC_{50} of camphor, lavender, mint, rosemary and clove oils elevated the total protein content in the third instar larvae of wax moth. **El-Din, et al. (2020)** noticed that LC_{50} of jojoba oil and flaxseed oil decreased protein levels in pink bollworm. The total soluble lipids were reduced by Jojoba and elevated by flaxseed oil for pink bollworm, while they were decreased in flaxseed and elevated by jojoba oil for spiny bollworm. **El-Gendy& Sabry (2021)** The *Ruta angustifolia* and *Moringa oleifera* oils caused elevation in contents of total lipids and total carbohydrate of *Spodoptera littoralis*. **Aboulthana, et al. (2022)** found that *C. tiglium* ethanolic, aqueous and petroleum ether extracts provided with high contents of total protein, carbohydrate and lipid. All activities increased after incorporating ZnO-NPs. **El-Bassouiny et al. (2022)** reported that the use of tetramic acid against *Earias insulana* caused decrease in the activities total protein and lipids. **Fayez, et al. (2021)** indicated that Syzygium and Turpentine oils decreased the level of total protein and increased total lipid in pink bollworm. **Wahba, et al. (2022)** noticed that clove, eugenol oil and nanoemulsions of clove & eugenol oil decreased protein levels in larvae of *S. littorals*. **Asadi, (2023)** found that protein content activity in *Ephestia kuehniella* reduced by using sublethal concentration of *Salvia officinalis* essential oils. **Sabry, et al. (2023)** assessed that total soluble protein were increased as result of treating larvae of the spiny bollworm with *Acremonium* sp.

The two treatments decreased levels of glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT in the larvae of the spiny bollworm as compared with control. Similar results obtained from **Elhadek, et al. (2015)** who stated that *Sesamum indica* and *Eucalyptus camaldulensis* elevated GOT enzyme; in contrast, it decreased by using *Trigonella foenum graceum* and *Nigella Sativa* against 4th larval instar of *Spodoptera littoralis*. **Hegab, (2018)** showed increasing aspirate amino transferase enzymes and alanine amino transferase in the larvae of the spiny bollworm treated with *C. limon* and *M. spicata*. **El-Din, et al. (2020)** noticed general elevation in GOT transaminae activity was recorded in the pink bollworm and the spiny bollworm by using flaxseed and jojoba oil. **El-Bassouiny et al. (2022)** reported that the use of tetramic acid against *E. insulana* caused decrease in the activities of AST and ALT. **Wahba, et al. (2022)** noticed that AST activity increased

with clove oil and nanoemulsion clove oil but decreased activity with eugenol and nanoemulsion of eugenol. ALT activity decreased with clove, eugenol oil and nanoemulsions of clove oil and eugenol in larvae of *S. littorals*. **Kandil, et al. (2023)** emphasized that the activity of ALT and AST decreased on the spiny bollworm larvae treated with closer.

All treatments decreased levels of trehalase enzyme. *C. tiglium* oil decreased invertase enzyme levels, while the other treatment increased it in the larvae of the spiny bollworm as compared with control. **El-Din, et al. (2020)** noticed that the activity of carbohydrate hydrolyzing enzymes was elevated by both treatments on the spiny bollworm, while variations impacts on pink bollworm were recorded decreasing for jojoba oil and increasing for flaxseed oil. **Sabry, et al. (2023)** assessed that amylase and trehalase were reduced as result of treating larvae of the spiny bollworm with *Acremonium* sp.

C. tiglium oil increased levels of lipase enzyme, but its nano-emulsion caused inhibition of its level in the larvae of the spiny bollworm as compared with control. Treatments decreased levels of protease enzyme in the larvae of the spiny bollworm as compared with control. **Ali, et al. (2017)** assessed that lipase activity decreased in larvae of the *Spodoptera littorals* treated with garlic and lemon oils. **Dinesh, et al. (2022)** recorded decline in the digestive enzymes activities of *H. armigera, P. xylostella* and *S. litura* after treatment with encapsulated Chitosan-nanoparticles. **Asadi, (2023)** found that Proteolytic activity in *Ephestia kuehniella* reduced by elevated by *Salvia officinalis* oil and decreased by *Piper nigrum*, *Allium sativum* and *Rosmarinus officinalis* oils.

The treatments with *C. tiglium* and its nano emulsion increased acetyl cholinesterase activities in the larvae of *E. insulana* as compared with control. **Hegab, (2018)** showed increasing in acetyl cholinesterase activities in the larvae of the spiny bollworm treated with *C. limon* and *M. spicata*. **Said, et al. (2019**) assessed that acetylcholinesterase increased by camphor and mint in the third instar larvae of wax moth. **Fergani et al. (2020)** assessed that LC_{50} of crane's-bill, basil, dill, citronella, and clove oils increased the AChE activity in 3rd instar larvae of *S. littoralis*. **Aboulthana, et al. (2021)** found that acetyl-choline esterase, β-amyloid content and inflammatory markers increased by nano-extract of *C. tiglium*. **Khalil, et al. (2023)** found that using the LC_{50} of mustard, citronella, and sage oils caused elevation in the activities of acetylcholinesterase (AChE) against 4th instar larvae of *Galleria mellonella*. **Sabry, et al. (2023)** assessed that the activity of acetyl-choline esterase activity were increased as result of treating larvae of the spiny bollworm with *Acremonium* sp.

V. Conclusion

At the end of the present study, we concluded that all treatments affect the biochemical activities of the spiny bollworm larvae, which leads to disturbance in total carbohydrate, lipid & protein levels and some enzyme activities in each treatment. The findings of our study suggested that *C. tiglium* oil and its nanoemulsion can be used as an effective and safe alternative in the controlling of the spiny bollworm instead of traditional pesticides.

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