The potential of cinnamon, *Cinnamomum zeylanicum* **essential oil as a natural ovicide against cotton leafworm,** *Spodoptera littoralis*

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ABSTRACT

The present work deals with the extraction of volatile oil from dried bark of *Cinnamomum zeylanicum.* The volatile oil was extracted by the common hydro distillation method and determined by GC–MS. A total of 12 components were identified. The major component in the essential oil was the aromatic compound E, Z–cinnamaldehyde (31.91%), followed by the main groups, monoterpenoids or oxygenated monoterpenes (27.25%), sesquiterpene hydrocarbons (15.29%) and sesquiterpenoids (10.69%). The essential oil possessed ovicidal activity against three-day old eggs of *Spodoptera littoralis* (Boisduval) with LC₅₀ and LC₉₀ values of 1.64 and 7.42%, respectively. Dipping eggs in LC₅₀ values might be indicated the presence of abnormalities in the external morphology of eggshell, chorion surface and micropyle area as compared to untreated eggs using a scanning electron microscope. Additionally, the tested oil significantly reduced total soluble protein, total lipids and activities of both acid phosphatase and phenoloxidase enzymes as compared to control. Moreover, the oil affected some biological parameters such as incubation period of the eggs and subsequent offspring, i.e., larval mortality, larval and pupal duration and pupal weight compared to the control. This information might help in developing IPM strategies against this serious pest

Keywords: *Spodoptera littoralis*, *Cinnamomum zeylanicum,* ovicide, essential oil

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INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), also known as tobacco cutworm, African cotton leafworm or Egyptian cotton leafworm, is one of the most destructive agricultural pests in subtropical and tropical regions. This species is a prolific highly polyphagous organism, feeding on a multitude of host plants covering over 40 families containing at least 87 plant species of economic importance, that diminish the quality and quantity of the crop yields (EPPO, 1990). Given that the pest may spread even to the temperate zone due to international transport of vegetables and ornamental plants, European and Mediterranean Plant Protection Organization (EPPO) has listed *S. littoralis* as an A2 quarantine pest (OEPP/EPPO, 2015).

Despite conventional insecticides can be effective in protecting agricultural crops because they rapidly decimate dense this pest population, causing collateral effects. For example, pest resistance, toxic waste and the emergence of new pests. Most synthetic pesticides marketed have harmful effects on human health and the environment. Notably, these products may also destroy natural enemies allowing an exponential amplify of pest populations (Naqqash *et al*., 2016). Increased awareness of environmental pollution and the demand for safe food production have led to growing interests in the use of using natural products in plants' protection. In this context, investigating plant derivatives offer novel routes in the exploration of alternatives to conventional pesticides causing no damages to the environment and non–target organisms (Pavela, 2016; Benelli *et al*., 2017). Interestingly, the application of botanical pesticides for crop protection and insect pests control has been paramount. However, numerous researchers have experimented and developed plant-derived products as pesticide alternatives to be used against insects (Dwi Sutanto *et al*., 2017).

Essential oils are volatile secondary metabolites that plants construct for their own needs other than nutrition (e.g. attractant, otherwise protectant). In general, they are complex mixtures of organic compounds that give a characteristic odor and flavor to the plants. In the attempt to find new plant–borne compounds with efficacy against herbivore enemies affecting crops, the secondary metabolites produced from medical and aromatic species were considered. A limited number of plant families including (Lauraceae) capable to elaborate the chemical compounds that constitute essential oils. The main group of the volatile essential oils is composed of terpenes, terpenoids and the other aromatic and aliphatic compounds. Monoterpenes are the most representative one; they are ten carbon (C_{10}) and sesquiterpenes (C_{15}) . The aromatic compounds occur less frequently than the terpenes and derived from phenylpropane, aldehyde (cinnamaldehyde), alcohol (cinnamic alcohol) and methoxy derivatives (Tripathi *et al*., 2009)

There were many searches interested to study the ovicidal action of the essential oil. Khedr and El–Kawas (2013) studied the role of coriander, *Coriandrum sativum* L.essential oil as ovicidal against both *S. littoralis* and *Tetranychus urticae*. At the same connection, Moawad and Sadek (2018) confirmed that all the tested concentrations of moringa, *Moringa oleifera* L. and Bitter almond, *Prunus amygdalus* Va amara oils reduced the egg hatchability of *S. littoralis* at different levels. For the present study, cinnamon, *Cinnamomum zeylanicum* a species belonging to Lauraceae

family was selected. Its bark has a long history for being used as traditional medicine, cooking spices and perfumes (Al–Sahlany, 2016). The cinnamon essential oil has both antibacterial and antifungal compounds used to prevent food spoilage (Mahmoud, 2012). Thus, the main aim of this study was to utilize the essential oil of cinnamon, *C. zeylanicum* to control *S. littoralis* eggs. Therefore, the current investigation was designed to identify the different active chemical constituents present in *C. zeylanicum* dried inner bark. Furthermore, using the essential oil to explain its mode of action on *S. littoralis* eggs via ultrastructure, biochemical and biological responses.

MATERIALS AND METHODS

Culture of the Cotton Leafworm, *Spodoptera littoralis* **(Boisd.) (Rearing Technique)**

A laboratory (susceptible) strain of *S. littoralis* reared under no insecticidal contamination for over 30 generations, at the division of cotton leafworm, Branch of Plant Protection Research Institute at Zagazig, Sharqia Governorate, Egypt. Egg masses were reared on leaves of castor bean, *Ricinus communis*, L. according to El–Defrawi *et al*. (1964) under constant conditions of 26 ± 1°C and 70 \pm 5% relative humidity (RH).

Tested Plant

The dried bark of *C. zeylanicum* purchased from the local market of Sharqia Governorate, Egypt (30°34'00"N, 31°30'00"E). This dried bark grounded in a mechanical grinder to get uniform particle size distribution. Then, the grounded powder was sieved.

Hydro Distillation Method

This method most commonly used for the extraction of volatile compounds from the different matrix (Golmakani and Rezaei, 2008). The grounded powder (500 g) and two liters of distilled water were mixed in a round bottom glass flask. The essential oil was extracted using a Clevenger-type apparatus, according to the method of (Marcus and Lichtenstein, 1979), where the cinnamon bark powder was subjected to hydrodistillation for 24 h.

The cinnamon oil was separated, dried over anhydrous sodium sulfate to remove water after the extraction and kept in dark glass bottles at 4°C until used. The isolated oil (3 mL) is slightly reddish liquid with a distinguished odor and taste of cinnamon.

Characterization of *C. zeylanicum* **Oil Sample**

Gas chromatography–mass spectrum (GC/ MS) was used at the National Research Center, Giza, Egypt for identifying the chemical constituents of *C. zeylanicum* volatile oil according to the methods of Bernhard *et al*. (1983). These analyses were performed using GC/MS analysis technique on a series 115890. Hewlett Packard (Germany) gas chromatogram which has the following specifications: A Column HP-I (cross-linked methyl silicon), 12 m × 0.2 mm × 0.33 µm, high-speed capillary column programmed. Temperatures ranging from 30°C to 180°C at 50°C/min, carrier gas at a flow rate of 1 mL/min, and ionization voltage of 70 eV. Empirical identification of the essential oil components was conducted by comparison of their relative retention times (RT) and their relative retention index (RRI) to a series of n-alkanes. Mass spectrum matching was aided by commercial libraries which were NIST, Replib, wiley9 and mainlib.

Ovicidal Activity of Cinnamon Essential Oil

The cinnamon essential oil efficacy was determined by calculating the lethal concentrations that killed 50% (LC $_{50}$) and 90% of insects (LC $_{90}$), under laboratory conditions and conducted in triplicate. There were five concentrations of cinnamon oil beside the control (water) which were 0.5, 1.0, 2.0, 4.0 and 5.0% v/v. Three-days old egg masses were collected from a laboratory-reared population and dipped in each tested concentration as well as control for ten seconds. The tested egg-masses were left to dry in the air until full dryness, then transferred to Petri dishes (five egg-masses/dish). The inspection was performed daily until the eggs hatched. Mortality percentages of egg-masses were recorded using Abbott's formula (1925). Also, the incubation period was calculated in this trial.

Preparation of Egg Samples for Scanning Electron Microscopy

After 48 h of treatment, egg-masses of *S. littoralis* were prepared for scanning electron microscopy and biochemical assays by using LC_{50} of cinnamon as well as control. The external surface morphology of the *S. littoralis* egg masses was illustrated using scanning electron microscopy (JEOL–JSM–5500 LV) high vacuum mode. Specimens were fixed in 2.5% glutaraldehyde for 10 min and were dehydrated in a series of ascending concentrations of alcohol solutions (30, 50, 70 and 95%) for 10 min each and finally 100% for 5 min, three times using automatic tissue processor (Leica EM TP), then dried using $CO₂$ critical point drier (Tousimis Audosamdri–815). Next, the samples were coated with a gold coater (SPI–Module). Preparation and photographing were done at the Regional Center of Mycology and Biotechnology, Al-Azhar University.

Biochemical Assays

Five milligrams of egg-masses per each treatment were homogenized in distilled water using a Teflon homogenizer (ST–2 Mechanic–Preczyina, Poland) surrounded with a jacket of crushed ice for 3 min. The homogenates were centrifuged at 8,000 rpm for 15 min in a microcentrifuge (Hettich, Germany) to remove haemocytes. The supernatant was assayed to determine total soluble protein, acid phosphatase and phenoloxidase. While, the haemocytes were assayed to determine the total lipids.

Total Soluble Protein

Colorimetric determination of total soluble protein in the total homogenate of eggs was carried out as described by Bradford (1976). The principle of this method is based on that protein in the presence of an alkaline cupric sulphate, produces a violet purple color. The intensity of which is proportional to their concentration. The absorbance of the sample against a blank Biuret reagent was measured at a wavelength of 546 nm.

Acid Phosphatase

The activity of acid phosphatase was determined using the method of Powell and Smith (1954). In this method, the phenol released by enzymatic hydrolysis of disodium phenol phosphatase reacts with 4–aminoantipyrine, and by the addition of potassium ferricyanide, the characteristic brown color is produced.

Phenoloxidase

The activity of phenoloxidase was determined according to a modification of Ishaaya (1971). The reaction mixture consisted of 2.0 mL phosphate buffer, 0.2 dL, 1.0 mL $\rm H_2O$, 0.5 mL aqueous catechol solution and 0.5 mL enzyme solution in $\mathsf{H}_2\mathsf{O}.$ The pH, substrate and enzyme concentrations, and temperature of the reaction were at the optimum values found experimentally. The reaction mixture except for the enzyme was incubated for 5 min at the assay temperature and the enzyme solution was added to initiate the reaction. The absorbency at 410 nm was recorded for 2 min.

Total Lipids

The total lipids were estimated by the method of Knight *et al*. (1972), using kits from Diamond Diagnostics.The absorbance of the obtained pink color was measured at 525 nm.

Biological Aspects of Tested Oil on the Successive Stages of *S. littoralis* **Resulted from Treated Eggs**

This experiment was designed to evaluate the latent effect of LC_{50} of tested oil and the control against three-day old eggs. The treated and untreated eggs (control) were kept in Petri dishes until hatching. Fifty newly hatched larvae (10 larvae/replicate) for each tested groups (treated and untreated) were selected randomly and separately transferred into the glass rearing jars. The larvae were supplied daily with fresh castor bean leaves and checked daily. The rearing jars were kept under laboratory conditions as mentioned earlier. Larval mortality, larval duration, pupal duration and pupal weight represented the parameters of long-term bioactivity of each treatment.

Statistical Analysis

To estimate LC values of *C. zeylanicum* oil, the corrected mortality percentages which calculated using the computed percentage of mortalities versus corresponding concentrations were subjected to Probit analysis according to Finney (1971). The data of biochemical and biological parameters were statistically analyzed for significant differences between control and treated group using the Student's T–test. The P–values less than 0.05 were considered for significant differences (Snedecor and Cochran 1980). All values were expressed as means ± standard error.

RESULTS AND DISCUSSION

Chemical Characterization of *C. zeylanicum* **Essential Oil**

As shown in Table 1, GC–MS technique revealed that the presence of 12 compounds in the dried bark of cinnamon, *C. zeylanicum* essential oil. The major chemical constituent was E, Z– cinnamaldehyde (31.91%), and the major groups were monoterpenoids or oxygenated monoterpenes (27.25%), sesquiterpene hydrocarbons (contain only hydrogen and carbon; 15.29%) and sesquiterpenoids (10.69%). Data obtained from GC–MS analysis showed that, *C. zeylanicum* essential oil is rich in mono and sesquiterpene hydrocarbons and the aromatic compound (cinnamaldehyde) which comprises the major component in the essential oil. It has antioxidant and antibacterial functions (Aminzare *et al*., 2015; Al-Fekaiki *et al*., 2017). The composition of *C. zeylanicum* oil may vary depending on climatic and soil conditions, used parts, extraction method, orientation, seasonal variations and degree of maturity. Therefore, depending on its qualitative contents and major components, the activities of the essential oil will also vary.

Table 1 Common name and chemical structure of Cinnamon*, Cinnamomum zeylanicum* volatile oil isolated components using GC–MS

Note: RT = retention time. Monoterpenoid or oxygenated monoterpenes contain ten atoms of carbon (C10), hydrogen and oxygen. Sesquiterpene hydrocarbons contain (fifteen atoms of carbon (C15) and hydrogen only. Sesquiterpenoids contain C15, hydrogen and oxygen.

Different Impacts of Essential Oil on Eggs using SEM Ovicidal action

The essential oil of *C. zeylanicum* had ovicidal effects against three-day old eggs of *S.*

littoralis using dip technique. The LC₅₀ and LC₉₀ values were 1.64 and 7.42%, respectively (Table 2).

Table 2 Percentage ovicidal activity of *C. zeylanicum* essential oil against three-day old eggs of *S. littoralis*

Note: LCL = lower confidence limit, UCL = upper confidence limit, X² = chi square value, LC₅₀ = lethal concentration that kills 50% of insects, LC_{on} = lethal concentration that kills 90% of insects

Incubation period

The incubation period of three-day old eggs of *S. littoralis* treated by different tested concentrations of cinnamon oil was retarded from 2 days for control eggs to 5 days for the highest used concentration (4%) as shown in Figure 1.

Figure 1 Incubation period of three-day old eggs of *S. littoralis* using cinnamon oil

Ultrastructure Examination

A scanning electron microscope was used to investigate the effect of *C. zeylanicum* volatile oil on the external surface of *S. littoralis* eggs. The normal egg external surface (Figure 2A) illustrated with highly decorated exochorion, which covered with polygons made of the follicular cells imprints. There is a single micropylar opening in the anterior pole, while those of treated eggs with LC_{50} of tested oil showed many deformed features like losing the external polygons, shrinking of exochorion and wrapping of exochorion into many rolls (Figure 2B–D).

Figure 2 Stereo Electron Microscopy (SEM) morphological chorionic surface for the whole egg of *S. littoralis,* untreated normal egg seems to have a spherical shape (A), treated with LC₅₀ of *C. zylanicum* volatile oil (Deformed eggs) (B–D)

There is a single micropylar area in the anterior pole in normal egg characterized by the distal micropyle and the collar (Figure 3A). Applying the oil film on the outer surface may close the micropylar opening and aeropyles, preventing oxygen entrance and moisture (Figure 3B). The exochorion ornamentation of treated eggs differs considerably in the morphology of the chorionic cells, external chorionic reticulum, micropylar collar, and micropyle, respectively (Figure 3C). The endochorion and exochorion layers surrounding the embryo were damaged after breaking down of egg capsule. That is due to the inhibition of the eggshell with tested volatile oil causes flair in protecting the oocyte and the development of the embryo. Many embryos were noticed during scanning of the treated eggs to be deformed or even died (Figure 3D).

Figure 3 Stereo Electron Microscopy (SEM) of the top view of micropyle in detail, normal micropyle area and collar (white arrow) (A), treated micropyle area covered with a thin film of *C. zylanicum* volatile oil (B), folded micropyler area (C) and deformed larvae failure in emerging from the treated egg (D)

The external morphology or the surface chorion is covered with polygons in a network pattern (Figure 4A). Polygons are imprints of the follicular epithelial cells that secrete the chorion protein. Each polygon has aeropyle at each ridge. During the development of eggs from laying to hatch, the chorionic surface reveals a significant difference in surface architecture between treated and untreated eggs like irregularities and folding in their distributions (Figure 4B). The disappearance of cell imprints (Figure 4C), and complete separation between endo and exochorion while the latter was dried and convoluted forming wrapping around itself (Figure 4D). Another specialized form of deformity recorded in (Figure 4E–F) was the external formed deep fissure in between more crooked exochorion.

The ovicidal activities of *C. zeylanicum* essential oil present in this study against *S. littoralis* may be attributed to its unique mixture of several bunches of oxygenated monoterpenes (27.25%), sesquiterpenes either hydrocarbon or oxygenated (25.98%) as well as aromatic compound (E, Z– cinnamaldehyde; 31.91%). Most monoterpenes act on the nervous system causing symptoms suggested as a neurotoxic mode of action (Kostyukovsky *et al*., 2002). Additionally, cytotoxic to insect tissue, impairing respiration, reducing cell membrane permeability and acting as a chemical messenger for insects and other animals (Tripathi *et al*., 2009). Adding cinnamon oil to the pesticides (rotenone) improves its toxicity to *S. littoralis* and apparently affected midgut cell spacing and membrane permeability compared to rotenone alone (Li *et al*., 2017). The same trend recorded by Pavela (2010) who found that monoterpenoids (ã–terpinene, p–cymene and 1.8 cineole) had acute toxicity and mutual synergistic effect on *S. littoralis* larvae.

The embryos that develop inside the eggs need oxygen to survive and develop, which comes from the atmosphere through aeropyles. The ovicidal action of cinnamon oil which presented in this paper were photographed. Hence, its mode of action could be due to the impairment of egg respiration via 1) Formation of a thin layer of oil film covering the outer egg surface and sealing the aeropyles or 2) Formation of irregular dried and convoluted wrapping around itself, resulting gyrations on the external surface of the treated eggs (deformed eggs). This deformation can contribute to physiological and developmental deterrence inside eggs. Additionally, it can prevent the oxygen from reaching the internal embryos and affect the embryo's metabolism, eventually death. Plata–Rueda *et al*. (2018) found that the insecticidal action of cinnamon and clove essential oils against *Sitophilus granarius* maybe have been caused by the synergy of its constituents. Also, the ability to enter the insect or respiratory system often leads to low respiratory. Khedr (2016) recorded several ultrastructure deformations on the outer chorionic structure of *S. littoralis* eggs that treated with *Sesamum indicum* oil, where some eggs lost the decoration of polygonal cell imprints while others had multiple projections expressed at wall junctions. In addition, Mead *et al*. (2016) observed anomalies in the external morphology of eggshell imprints and aeropyles of *S. littoralis* eggs treated with mahlab fixed oil and KZ mineral oil as compared to control using a scanning electron microscope.

Figure 4 Stereo Electron Microscopy (SEM) comparison between normal chorionic egg surface (A) and those treated with LC₅₀ of *C. zylanicum* volatile oil (B–F), normal chorionic surface covered with polygons which has aeropyle (arrow) (A), arrows refer to several abnormal chorionic structures like: fissure (B), shrunken exochorion (C), raping of exochorion into many gyrations (D) and c rooked and fragmented chorionic surface (E–F)

Biochemical Aspects

As a general trend, the results obtained in Table 3 showed a remarkable highly significant decrease in the effect of both total soluble protein and total lipids, besides the activities of acid phosphatase and phenoloxidase enzymes on the treatment of three-day old eggs of *S. littoralis* with LC₅₀ of *C. zeylanicum* oil compared to untreated eggs. In this study, another potential ovicidal mechanism of the cinnamon volatile oil has been recorded. Compared to control, the tested oil significantly reduced the levels of total soluble protein, total lipids and activities of acid phosphatase and phenoloxidase enzymes. Insect eggs, like eggs of other animals, have to contain all substances which are necessary for the independent development of the embryo. Therefore, in addition to nucleic acids, mature eggs have to contain large amounts of proteins and lipids that can serve as building blocks and as a source of energy (Ziegler and Van Antwerpen 2006). The decrease in total soluble protein may reflect the decrease in various enzymes (El–Kordy *et al*., 1995). While the reason for the observed reduction in the effect of total lipids may be due to its conversion to proteins to replace the reduction in protein content or reduction of supplemented energy. Furthermore, Brattsten (1983) recorded that essential oils are lipophilic in nature and interfere with insect biochemical, physiological and behavioral functions.

Note: Each datum represents the mean of three replicates. Data expressed as mean ± standard error (SE). Means under each variety having different letters in the same row denote a significant different $(P < 0.05)$

Acid phosphatase enzyme associated with nutrition and egg maturation (Tsumuki and Kanehisa 1984). Detoxification enzyme, acid phosphatase (ACP) in insects is generally demonstrated as the enzymatic defense against foreign compounds and plays significant roles in maintaining their normal physiological functions (Li and Liu, 2007). In this context, Khedr (2016) recorded a reduction in the total soluble protein and an increase in the acid phosphatase enzyme after treating *S. littoralis* eggs with *Sesamum indicum* oil. Moreover, the phenoloxidase enzyme literally plays a key role in the production of eggs. While this role seems to be limited to the tanning of chorion, which is secreted just before the eggs are laid (Kim *et al*., 2005). The cinnamon essential oil exhibited a valuable tool with potential effects against *S. littoralis* eggs.

Biological Aspects

The latent effects of LC₅₀ of *C. zeylanicum* oil against three-day old eggs of *S. littoralis* were evaluated as larval duration, larval mortality, pupal duration and pupal weight. Data in Table 4 showed a highly significant elongation of the larval duration from 17 (control larvae) to 20 days (cinnamon essential oil). The cumulative mortality percentage up to the period of prepupal was 8% for cinnamon oil, whereas the control was 0% (Table 4). The pupal duration was 9.00 ± 0.45 days for pupae developed from untreated larvae and was 11.00 ± 0.54 days for pupae developed from treated one. This increase was statistically significant when compared to pupal control. For pupal weight, the cinnamon oil reduced significantly the average weight of the resulted pupae that developed from treated larvae $(0.3121 \pm 0.004 \text{ g})$ than pupal control $(0.3401 \pm 0.007$ g).

Table 4 Changes in some measured biological parameters using *C. zeylanicum* oil

Note: Each datum represents the mean of three replicates. Data expressed as mean ± standard error (SE). Means under each variety having different letters in the same row denote a significant difference $(P < 0.05)$

The mentioned biological aspects are possibly attributed to the presence of sesquiterpenes which are interfering with the metabolism of juvenile hormones and ecdysone that regulating growth and development of insects (Tsao and Coats, 1995). In this study, *C. zeylanicum* essential oil constituents cause acute ovicidal action against *S. littoralis* eggs. This effect may still present in the resulting subsequent stages by reducing the fitness of survival larvae and pupae via disruption larval development, reduction in pupal weight and failure in pupal eclosion. Jumbo *et al*. (2018) used the cinnamon essential oil to control *Callosobruchus maculates*. Bioassay results revealed that the essential oil exhibited insecticidal activity, decreased the growth rate, offspring emergence was almost abolished and significantly affect the oviposition of treated adult females.

CONCLUSIONS

ASST

The present results point to the possibility with respect to the essential oil of *C. zylanicum* bark as an alternative approach of ovicidal insecticide against *S. littoralis* eggs. Such pests known as one of the most destructive pests on so many crops and plants, to enhance the role of naturally origin compounds in plant protection and pest control. Additional studies including the feasibility and economic sides under field conditions are needed. Eventually, it is in developing countries that are wealthy in endemic plant biodiversity that these pesticides may ultimately have their greatest influence in future integrated pest management (IPM) programmes due to their safety to non–target organisms and the ecosystem.

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