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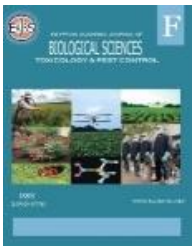

Some Toxicological and Physiological Aspects Induced by Camphor oil, *Cinnamomum Camphora* on the Cotton Leafworm, *Spodoptera littoralis* (Boisduval). (Lepidoptera: Noctuidae).

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ABSTRACT

The cotton leafworm, (*Spodoptera littoralis*) is considered as one of the most serious polyphagous pests not only in Egypt but also all over the world, infesting a wide host range. So, it was a vital demand to abolish this destructive pest. the harmful and traditional insecticides are frequently used so it was necessary to search for alternative eco-friendly Bio-pesticides. Natural products, to a large extent, are considered safer and have fewer side-effects. In the present study, the essential camphor oil (*cinnamomum camphora*) was investigated against 4th instar larvae of *S. littoralis* showing considerable toxicity with accumulative larval mortality with LC_{50} 20000 ppm. Moreover, when its biochemical effects were studied on *S. littoralis*, camphor oil not only caused a significant decrease in the main components: total proteins, total carbohydrates, and total lipids but also caused a significant reduction in the activities of invertase, trehalase, chitinase, and transaminases enzymes. However, camphor oil had no significant effect on amylase activity. Also, GC (gas chromatography) analysis was done for camphor oil. From this study, it was concluded that camphor oil is one of the promising tools for pest management as a lot of its partners from essential oils.

INTRODUCTION

The cotton leafworm, *S. littoralis* is a major limiting factor affecting vegetable crop production causing considerable damage to these hosts in Egypt, the Mediterranean, and Middle East countries (Rizk *et al.*, 2010). Currently, synthetic chemicals application has led to unintended hazards such as ozone depletion, pest resistance, environmental pollution, and toxicity on non-target organisms (Jembere *et al.*, 1995 and Okonkwo *et al.*,1996). Besides, increasing costs of application of chemical insecticides have directed the need for effective biodegradable pesticides (Abd El-Aziz and Ezz El-Din, 2007). Plant extracts, particularly the essential oils, have been considered as doable control agents against different insect pests for their selectivity, bio-degradability, non-toxic

products with a slighter effect on non-target organisms and safer for the environment (Isman, 2000). Essential oils are secondary metabolites, volatile, with a strong odor, and are formed of a mixture of several up to dozens of mono, di, sesqui-terpenes (Pavela, 2005). Recently, many studies have cleared that essential oils (EOs) derived from plants have proven to have toxicity for different pests (` Kumar *et al.*, 2012). Furthermore, several essential oils from different plants expressed high fumigant activity against *S. littoralis* (Dhen, 2014 and Pavela, 2005). Moreover, Fergani *et al*., (2020) indicated that the toxic effect of some essential oils proved its possible implementation as a bio-control method in IPM programs to control *S. littoralis*. Hence essential oils were a potent control method when integrated into control programs (Yazdani *et al.*, 2013). Also, Lingathurai *et al.,* (2011) found that many essential oils have strong larval toxicity, developmental retard, repellency, and feeding disorders to insects. Insects did not develop resistance to essential oils as general (Liao, *et al* 2017). As well as the essential oils were appraised intensively against stored product pests, plant chewing and sucking insect pests, health pests, and honey bee parasitic mites (Regnault-Roger, *et al* 2012). Natural pesticides, especially the ones derived from plants, are promising elements for pest control (Malarvannans *et al.,* 2008). Essential oils are very important botanicals that can act as fumigants, insecticides, repellents, and antifeedants (Wang *et al.*, 2014).

The camphor (*C. camphora*) is a white, crystalline substance with a strong odor and pungent taste, derived from the wood of camphor laurel (*C. camphora*) and other related trees of the laurel family. The leaf of *C. camphora* contains camphor as the main component along with cineol, linalool, eugenol, limonene, safrole, α¬pinene, β- pinene, B-myrecene, α- humulene, p-cymene, nerolidol, borneol, camphene, and some other components (Ho *et al.,* 2009). Analysis for camphor oil using GC- Mass which was done by Ali and Ibrahim (2018) showed that camphor oil comprises many insecticidal and antifeedant compounds. Also, Fu *et al.* (2015) investigated the insecticidal activity of camphor oil against red imported fire ant (RIFA) *Solenopsis invicta* and the latter showed fumigant toxicity and repellence against RIFA. The current study revealed that the toxic effect of camphor oil could be involved in any IMP programs as a biocontrol agent to *S. littoralis.*

Therefore, the present study aims to evaluate the efficiency of camphor oil on *S. littoralis* larvae with special reference to its toxicity and its effect upon main components as well as the transaminases and digestive enzymes.

MATERIALS AND METHODS

Tested Insect Species:

A sensitive laboratory strain of the cotton leafworm, *S. littoralis* was obtained from Syngenta company Qaha, Qalyubia Governorate, and reared in the physiology research Department, Plant Protection Research Institute, Dokki, Giza for several generations without any insecticidal and /or microbial pressure under constant laboratory conditions at 25±2°C and 60±5% R.H as described by El-Defrawi *et al.* (1964). Fourth and late sixth instar larvae were used in the present investigation.

Essential Oil Used:

Commercial camphor oil was purchased from El-Captain Co., Al-Obor city, Cairo, Egypt, and approved for human use from the Egyptian Ministry of Health.

Toxicity Assay:

To assess the activity of the camphor oil under investigation, a series of six acetone concentrations were prepared as 5886.7, 19957.1, 67658, 203028.1, 391874.1, and 1345217.6 ppm. Treatment was conducted by the dipping technique according to

Abo-El-Ghar *et al.* (1994) where Castor leaves were immersed in the prepared concentrations of camphor oil for the 30s. The leaves then left to dry at room temperature before being offered to 4th instars *S. littoralis* larvae. Larvae were fed on treated leaves for 48h. The treatment comprised 25 larvae and was replicated three times. A similar number of larvae were considered as a control in which larvae were offered castor leaves dipped in acetone. Accumulative Larval mortality was recorded after 48 h. till the end of the larval stage. The data were subjected to probit analysis ("LdPLine®" software (http://www. ehabsoft.com/ ldp line) to obtain LC_{50} and slope of camphor oil according to a method adopted by Finney (1971).

Effect of Essential Oil on Biochemical Parameters:

1. Preparation of Homogenate Samples of *S. littoralis* for Biochemical Analysis:

After feeding on leaves treated with LC_{50} of camphor oil for 48 h., larvae were collected and had been allowed to feed on untreated leaves. At the end of the larval stage, the larvae of the late sixth instar were homogenized and centrifuged using Beckman J2-Mc at 8000 rpm for 15 minutes at -2°C. After centrifugation, the supernatant fluid was divided into small aliquots (1 ml) and stored at -20 °C until the analysis of enzyme activities and determination of the main component. Three replicates were carried out for each biochemical determination.

2. Determination of main components

2.1. Total Protein Assessment:

Total protein content was estimated according to Bradford (1976) using bovine serum albumin as a standard.

2.2. Total Carbohydrates Assessment:

Total carbohydrates were determined by the method described by Singh and Sinha (1977) using anthron reagent.

2.3. Total lipids assessment:

Total lipid content in *S. littoralis* larval homogenate was estimated according to Knight *et al*. (1972) using phosphovanillin reagent.

3. Determination of Enzyme Activities:

3.1. Chitinase:

Chitinase was assayed using 3, 5-dinitrosalicylic acid reagent to determine the free aldehydic groups of hexosamine liberated on chitin digestion according to the method described by Ishaaya and Casida (1974).

3.2. Carbohydrates Hydrolyzing Enzymes Activities (Digestive enzymes):

The determinations of invertase, amylase, and trehalase activities were based on the digestion of sucrose, starch, and trehalose, respectively by spectrophotometric methods (Ishaaya *et al*. 1971 and Ishaaya and Swirski 1976).

3.3 Transaminases Enzymes:

Alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) activities were assayed according to the method of Reitman and Frankel (1957).

3.4 Analysis (GC) of Essential Oil:

GC analysis was performed by using a Perkin Elmer auto system XL equipped with a flame ionization detector (FID). A fused silica capillary column ZB5 (60m x0.32mm i.d.) was used the oven temperature was maintained initially at 50^0c at a rate of 3^0c /min. Helium was used as the carrier gas, at a flow rate of 1.1ml/min., the injector and detector temperatures were 220 and 250^0 C, respectively.

Data Analysis:

The obtained data were subjected to analysis of variance using proc. ANOVA in SAS, (SAS Institute, 1998). Mean separation was conducted using LSD in the same program at a significant level ($P < 0.05$).

RESULTS AND DISCUSSION

Toxicity of the Essential Oil:

The essential oil of *C. camphora* was a concentration dependant against 4th instar larvae of *S. littoralis* with different concentrations 5886.7, 19957.1, 67658, 203028.1, 391874.1, and 1345217.6 ppm, this was in agreement with El-Kholy *et al.* (2014) who revealed that the camphor oil has insecticidal activity against *S. littoralis* larvae. LC_{50} value of camphor oil (20000 ppm) was determined by plotting larval mortality versus concentrations, Figure (1). Moreover, Fergani *et al*. (2020) recorded that *C. camphora* expressed noticeable toxicity against the 5th instar larvae of *S. littoralis* after 48 hours. Also, Shalaby *et al.* (2020) stated the toxic effect of some essential oils on 2nd instar larvae of *S. littoralis*. In addition, Camphor oil showed high insecticidal activity and antifeedant index against *S.littoralis.* Furthermore, camphor affected on some physiological aspects on the larvae of *Spodoptera littoralis* (Ali and Ibrahim, 2018).

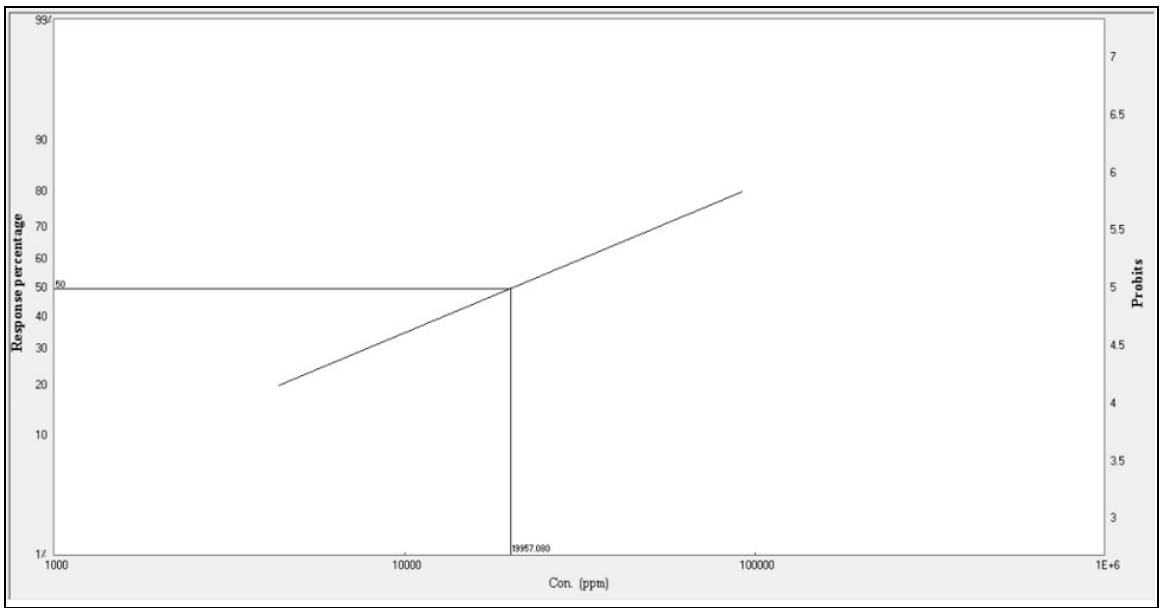


Fig. 1: Regression line of accumulative toxicity, (LC_{50}) of camphor oil (*C.camphora*) against 4th larval instar of the sensitive strain of *S.littoralis*.

Biochemical Changes Induced by Camphor Oil.

The latent effect of camphor oil (LC_{50}) on some biochemical parameters in the late 6th instar larvae of S. *littoralis* treated as 4th instar larvae including lipids, carbohydrates, proteins, and changes in some enzymatic activities were studied as follow:

1 Main Components:

Data in Table (1) indicated that camphor oil at LC_{50} significantly decreased (P<0.05) total lipids, carbohydrates, and proteins content in late 6th instar larvae of *S. littoralis* (401.98± 4.46, 252.45 ± 7.34 and 419.59 ± 8.91, respectively) compared with control (299.32± 7.9, 182.67 ± 2.76 and 250.67±13.89, respectively).

Table 1: Effect of *C. camphora* oil on the total contents of lipids, proteins, and carbohydrates of late 6th instar larvae of *S. littoralis* treated as 4th instar larvae with LC_{50}

Treatment	T. lipids (mg/gm) Mean ± SE	Carbohydrates (mg/gm) Mean ± SE	T. proteins (mg/gm) Mean ± SE
Control	401.98 ± 4.46 a	252.45 ± 7.34 a	419.59 ± 8.91 a
C. camphora	299.32 ± 7.9 b	182.67 ± 2.76 b	250.67 ±13.89 b

Values within a column followed by the same letter do not differ significantly (Duncan's test: $P< 0.05$). LSD= Least significant difference.

In the present study, total proteins *in S. littoralis* larvae were significantly decreased after treatment with camphor oil and this was in agreement with Fetoh *et al.* (2013). Moreover, Mahmoud and Ibrahim (2018) indicated that camphor oil decreased total proteins and carbohydrates in the larvae of *S. littoralis*. They also found that camphor oil did not affect the number of total lipids in *S. littoralis* larvae in contrast with the present study that showed a significant reduction in the number of total lipids. In the present study, the camphor oil caused a significant reduction in the total protein content; this was in agreement with Osman *et al*, (2012) who found that total protein content decreased in the 4th larval instar of *S.littoralis* when treated with camphor oil. The same result was obtained by Fetoh *et al*., (2013) who investigated total protein *in S. littoralis* larvae which was significantly decreased after treatment with camphor extract. Moreover, Shahriari *et al.* (2017) found that the amounts of protein, glycogen, and triglyceride in *E. kuehniella* larvae were treated by α-pinene (one of the principal constituents of camphor oil) were significantly decreased versus control.

2 Enzymatic Changes:

2.1. Digestive Enzyme:

Results deal with changes in the activity of the digestive enzyme presented in Table (2). Statistically, LC_{50} camphor oil didn't affect ($P>0.05$) the activity of amylase enzyme in the late 6th instar larvae (90.58± 6.1) compared with control larvae (88.06± 6.7). On the other hand, there was a significant decrease ($P<0.05$) in the activities of invertase, trehalase, and the Chitinase enzymes in the late 6th instar larvae (23.45±2.1, 18.33±0.89 and 0.640±0.190, respectively), as compared to control larvae (32.03± 2.5, 26.64 ±1.7 and 1.366±0.547, respectively).

Table 2: Effect of *C. camphora* extracted oil on the activities of amylase, invertase, trehalase, and chitinase of late 6th instar larvae of *S. littoralis* treated as 4th instar larvae with LC_{50} levels

Treatment	Amylase (µg glucose/ min/ml) Mean ± SE	Invertase (µg glucose/ min/ml) Mean ± SE	Trehalase (µg glucose/min/ml) Mean ± SE	Chitinase (µg glucose/min/ml) Mean ± SE
Control	88.06 ± 6.7 ab	32.03 ± 2.5ab	26.64 ±1.7 ab	1.366 ±0.547
C. camphora	90.58 ± 6.1 bd	23.45 ±2.1 d	18.33 ±0.89 d	0.640 ±0.190

Values within a column followed by the same letter do not differ significantly (Duncan's test: $P< 0.05$). LSD= Least significant difference.

In the present study, a significant decrease in invertase, trehalase, and chitinase activities were recorded with the latent effect of camphor oil on 6th larval instar. So, the present investigation that adopts the hypothesis reduction effect on the digestive enzyme herein except amylase could be attributed to the direct toxic effect of camphor oil and irregularity of enzymatic activity. Camphor oil decreased the amount and activity of chitinase (Fetoh and Asiry, 2013). Abdel-Aziz *et al*. (2013) indicated that there was highly significant stimulation in chitinase activity in 2nd and 4th *S. littoralis* instar larvae with thyme and bitter essential oils. Mahmoud and Ibrahim (2018) agreed with our study that there is no effect on the amylase enzyme was done by camphor oil on the *S.littoralis* larvae.

2.2. Transaminases:

The obtained results in Table (3) revealed that there was a significant decrease in AST(Aspartate amino Transferase) and ALT (Alanine aminotransferase) enzymes activities(29.76 ± 2.1 and 9.62 ± 0.53, respectively) of late 6th instar larvae upon exposure to the camphor oil compared to control larvae (48.23 ± 1.7 and 36.82 ± 2.98, respectively).

Table 3: Effect of *C. camphora* oil (at LC_{50}) on the activities of AST (Aspartate amino Transferase) and ALT (Alanine aminotransferase) of *S. littoralis* late 6th instar larvae (mean values ± SE)

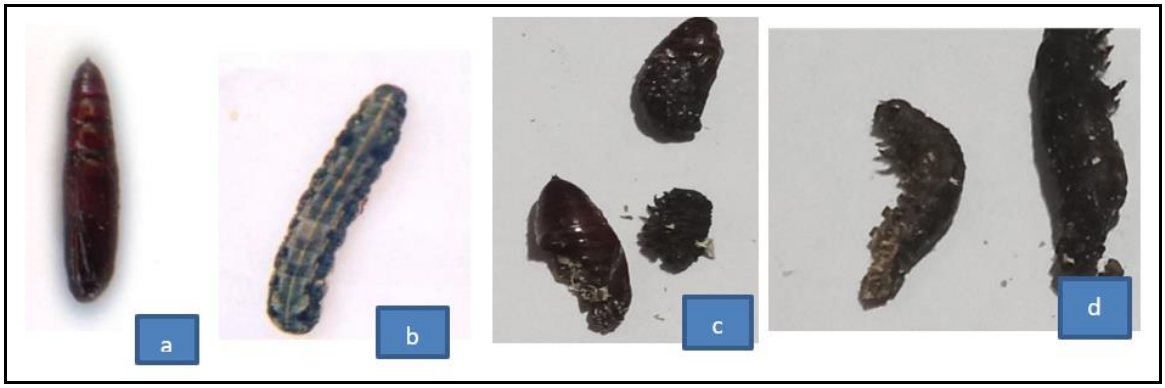
Treatment	AST (µg oxaloacetate/min/ml)	ALT (µg pyruvate/min/ml)
C. camphora	29.76 ± 2.1 c	9.62 ± 0.53cd
Control	48.23 ± 1.7a	36.82 ± 2.98a

Means within a row followed by the same letter do not differ significantly (Duncan's test: P< 0.05). LSD= Least significant difference.

3. Forms of malformations induced by camphor oil treatment

Figure (2) indicated the potentiality of camphor oil treatment to induce a shrank or incomplete pupal stage, where, pupae could not complete or develop a normal pupal cuticle.

Camphor oil-induced stages between the pupae and adults including the head capsule and thorax of abnormal emerged adult and remnant of the pupa, also the appendages were severely shrunk and reduced.



a) Normal pupa (c) normal pre- pupa (b) malformation in pupae (d) malformation in pre-pupa

Fig.1: different malformations in pre-pupae and pupae, of *S. littoralis* which were treated with camphor oil (*C.camphora*) compared with control.

The significant decrease in the total soluble protein may be due to binding with foreign compounds, as the plant extract treatment. This was supposed by Ahmed *el al*. (1985) when investigated dieldrin against *Periplaneta americana* (L.), Or maybe due to the inhibition of synthesis of DNA and RNA, as proposed by Mitlin *et al*. (1977) for boll weevils treated with chitin synthesis inhibitors and by Qadri and Narsaih (1978) for last nymphal instar of *P. americana* injected with the plant extract, azadirachtin . In addition, the decrease in total soluble protein might be due to the breakdown of protein into amino acids, so with the entrance of these amino acids to TCA cycle as keto acids, they will lead to supply energy for the insect. So, protein depletion in tissues may cause physiological mechanisms and might play a role in compensatory mechanisms under insecticidal stress, to provide intermediates to the Krebs cycle, by keeping free amino acids content in haemolymph (Nath *et al*., 1997 and Kamel *et al*., 2010).

Glutamate oxaloacetate transaminase (GOT) and glutamine pyruvate transaminase (GPT) are also known as aspartic transferase (AST) and alanine transaminase (ALT) respectively. The transaminases are key enzymes in the formation of non-essential amino acids, gluconeogenesis, metabolism of the nitrogen compound, and associated with protein metabolism (Mordue & Goldworthy, 1973). Hence, the reduction in total protein content may be related to the reduction in the activities of both AST and ALT which were resulted in the present study, as these enzymes responsible for the transfer of amino groups from kettoacids resulting in the formation of amino acids which in turn considered the structural unit of protein. And vice versa, the reduction in those enzymes might be due to the decrease in total protein as the enzymes are protein in nature (Mitlin *et al*., 1977).

Lipids are essential structural components of the cell membrane and cuticle (Rafea, 2014). In the present study, the total lipids content was significantly decreased due to the treatment of camphor oil and this can be attributed to the conversion of total lipid contents to protein to produce supplementary energy (Abuldahab *et al*., 2011).

Carbohydrates are of vital importance as they are utilized by the insect body for the production of energy or conversion to lipids or proteins. Metabolism of carbohydrates is controlled mainly by trehalase, amylase, and invertase enzymes that play a principal role in the digestion and utilization of carbohydrates by insects (Wigglesworth, 1972). Amylase and invertase which have been reported to occur in the digestive tract of several insects are important for digestion and utilization of starch and sucrose, respectively. It is known that in insects, trehalase degrades trehalose to glucose for internal energy supply and generation of glucose needed for chitin build-up (during moulting). Chitinase enzyme has been demonstrated in the moulting fluid which appears in the space between the old and the new cuticles during ecdysis and secreted by the hypodermis (Kimura, 1976), and in the integument (Koga *et al*., 1989) it has a role in the degradation of chitin via chitobiase to N-acetylglucosamine. Therefore, the reduction in chitinase activity obtained in the present study might be related to the reduction in total carbohydrate content in our study. Furthermore, reduction in chitinase activity may cause the malformation found in the pre-pupae and pupae as a result of the treatment of *S.littoralis* with camphor oil. Trehalase has the important function for liberating glucose for energy and is activated during moulting to generate glucose for chitin build-up (Meisner *et al.,* 1978), so the inhibition of trehalase activity observed in the present work might affect chitin build-up, and therefore, may reflect the abnormalities which were resulted in the present study.

From the mentioned above, it could be concluded that the significant reduction in invertase and trehalase activities obtained in the present study may be related to the significant reduction in total carbohydrate. On the other hand, the reduction in chitinase

may result in the malformation found in the larval developmental stages for *S.littorais* against *C.camphora* oil. This malformation presented in our study might be due to the reduction of lipid content, carbohydrate, total protein, and chitinase enzyme, the latter responsible for the degradation of the old cuticle to develop a new one for the developmental stages of the pest which were Obtained in the present study.

Chemical Constituents of Camphor Essential Oil:

Data in Table (4) summarize the GC analysis of *C. camphora.* The essential oil mainly composed of 12 major components which were identified and also quantified (liquid and solid monoterpenes). The main components of *C. camphora* essential oil were *D*-camphor (39.10%), α-Terpineol (24.75%), 1,8-cineole (10.48%), and Terpin-4-ol (6.46%). The toxic effect of camphor against *S. littoralis* larvae could be attributed to such compounds. These results may coincide with other reports. For example, fumigant toxicity of linalool against *Tribolium castaneum* (mortality 70 % after 7 days), *R. dominica* (highly effective and produced 100% mortality), and *Sitophilus oryzae* (Rozman *et al*. ,2008). The contact toxicity tests indicated that *(*+)-camphor showed feeble activity against *T. castaneum* and *Sitophilus oryzae*. So the toxic effects of the extracted oil of C. *camphora* might be attributed to *D*-camphor and other components Abdelgaleil *et al.* (2009). In other studies, (+)-camphor and 1,8-cineole had potent fumigant toxicity against *S. littoralis*. Moreover, (-)-limonene and (+)-camphor could be used as alternative control agents for *T. pisana*. In addition, 1-8-cineole could be useful as fumigants for control *T. pisana* and *S. littoralis (*Abdelgaleil, 2010). Linalool toxic effect was estimated by Davoudi *et al*. (2011) against the two most common stored-product insects such as *Callosobruchus maculatus* and *Rhyzopertha dominica*. Camphor is also found to have powerful contact toxicity and fumigant toxicity against *Liposcelis bostrychophil*a (Liu *et al*., 2012). Furthermore, the *D*-camphor was also effective as a fumigant against *Sitophilus zeamais* (Chu *et al.*, 2013). Chen *et al*. (2014) found that the camphor and linalool compounds showed potent fumigant toxicity against *Lasioderma serricorne* adults with LC_{50} values. Moreover, *D*-camphor has insecticidal activity. In total, 435 binary combinations were tested only 6 substances were identified as being able to create a synergic effect with more than 20 substances: γ-terpinene, limonene, *p*-cymene, *trans*-anethole, borneol, and camphor (Pavela, 2014).

In conclusion, camphor oil can be successfully used as larvicid against *S. littoralis* (Boisduval). So, the development of new pesticides from this essential oil could be possible. Their safety on the non-target organisms and their stability should be studied in further investigation.

Table 4: Chemical composition of the essential oil of *C. camphora* by GC

Compound	RT	Relative concentration%
α-pinene	17.011	5.39
Camphene	17.734	3.08
β-pinne	19.021	3.32
ρ-Cymene	21.148	1.43
Limonene	21.395	3.59
1,8-Cineol	21.570	10.48
Terpinolene	24.287	0.68
Linalool	25.093	1.34
Camphor	27.224	39.10
Terpin-4-ol	28.348	6.46
α-Terpineol	29.514	24.75
β-Citronellol	30.463	0.38

REFERENCES

Abd El-Aziz, S. E. And Ezz El-Din, A. A. (2007). Insecticidal activity of some wild plant extracts against cotton leafworm, Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae). *Pakistan Journal of Biological. Sciences*; 10(13): 2192-2197.

Abo El-Ghar, G.E.S.; M.S. Khalil and T.M. Eid (1994). Effect of plant extracts on development and fecundity of *Agrotis ipsilon* (Lepidoptera: Noctuidae). *Bulltein of the Entomological Society of Egypt, Econ. Ser.*, 21: 171-190.

Abuldahab, F.F.; N.Y. Abozinadah and N.S. Al- Haiqi (2011). Impact of Bacillus thuringiensis β– exotoxin to some biochemical aspects of Musca domestica (Diptera: Muscidae). *Journal of Bacteriolgy Research*, 3(6): 92-100.

Ahmed, M.S.; F.A. Ali and AR. Shakoori (1985). Effect of dieldrin on the whole-body protein content of Periplaneta americana. *Pakistan Journal of Zoology,* 17 (1): 105-109.

Bradford, M.M. (1976). A rapid and sensitive method for the quantization of Microgram quantities of protein utilizing the principle of protein- dye binding Analatical. *Biochemistry*, 72; 248-254.

Dhen N, Majdoub O, Souguir S, Tayeb W, Laarif A,Chaieb I (2014). Chemical composition and fumigant toxicity of Artemisia absinthium essential oil against Rhyzopertha dominica and Spodoptera littoralis. *Tunisian Journal of Plant Protection.* 9:57-65.

El-Defrawi, M.E.; A. Toppozada; N. Mansour and M. Zeid (1964). Toxicological studies on Egyptian cotton leafworm, Prodenia litura (F.), 1. Susceptibility of different larval instar to insecticides. *Journal of Economic Entomology,* 57 (4): 591-593.

El-Kholy, R.M.A., M.M.M. El-Bamby, M.F. El-Tawil and W.L. Abouamer (2014) . Effect of Three Plant Extracts on Some Biological Aspects of Cotton Leafworm, Spodoptera littoralis (Boisd.). *Middle East Journal of Applied Sciences*, 4(2): 243-251.

Fergani, Y. A.; Elbanna, H. M. and Hamama, H. M. (2020). Genotoxicity of some plant essential oils in cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae): the potential role of detoxification enzymes. *Egyptian Journal of Zoology*, 73: 53-66

Fetoh, B.E.S.A. and K.A. Asiry (2013). Biochemical effects of chlorpyrifos organophosphorous insecticide, camphor plant oil and their mixture on Spodoptera littoralis (Boisd.). *Archive Phytopathology Plant Protection*, 46: 1848-1856.

Finney DJ. Probit analysis. Cambridge University Press.1971, 333.

Ishaaya, I. and E. Swirski (1976). Trehalase, invertase and amylase activities in the black scale, Saissetia *oleae* and their relation to host adaptability. *Journal of Insect Physiology*, 22: 1025-1029.

Ishaaya, I. and J.E. Casida (1974). Dietary TH 6040 alters composition and enzyme activity of housefly larval cuticle. *Pesticide Biochemistry and Physiology*, 4: 484-490.

Ishaaya, I.; I. Moore and D. Joseph (1971). Protease and amylase activity in larvae on the Egyption cotton worm, *Spodoptera littoralis*. *Journal of Insect Physiology*, 17: 45-953.

Isman, M. B. (2000). Plant essential oils for pest and disease management. *Crop Protection*, 19(8-10): 603-608.

Jembere, B; Obeng-Ofori, D.; Hassanal. and Nyamasyo, G. N. N. (1995). Products derived from the leaves of *Ocimum kilim* and scharicum (Labiatae) a spost-harvest grain protectant against the infestation of three major stored product insect pests.*Bulletin of Entomological Research*, 85: 361–367

Kamel, S.A.; F.A. Abd-El Aziz and M.N. El-Barky (2010). Biochemical effects of three commercial formulations of Bacillus thuringiensis (Agerrin, Dipel 2X and Dipel DF) on Spodoptera littoralis larvae . *Egyptian Academic Journal of Biological*

*Sciences, (A.Entomology)*Vol.3 (1): 21-29.

Knight, J. A.; Anderson S. And Rawle J. M. (1972): Chemical basis of the sulfophospho-vanillin reaction for estimating total serum lipids. *Clinical Chemistry*, 18: 199-202.

Kumar, P., S. Mishra, A. Malik, and S. Satya. (2012). Compositional analysis and insecticidal activity of Eucalyptus globulus (family: Myrtaceae) essential oil against housefly (Musca domestica). *Acta Tropica*, 122: 212–218.

Liao, M.; Xiao, J.-J.; Zhou, L.-J. *et al*. (2017). Chemical composition, insecticidal and bio-chemical effects of *Melaleuca alternifolia* essential oil on the *Helicoverpa armigera*. *Journal of Applied Entomology*, 141(9): 721-728.

Lingathurai, S.; Ezhil Vendan, S.; Gabriel Paulraj, M. *et al*. (2011). Antifeedant and larvicidal activities of *Acalypha fruticosa* Forssk. (Euphorbiaceae) against *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) larvae. *Journal of King Saud University (Science)*, 23: 11-16.

M Shahriari, N Sahebzadeh, A Zibaee (2017) . Effect of Teucrium polium (Lamiaceae) essential oil on digestive enzyme activities and energy reserves of Ephestia kuehniella (Lepidoptera: Pyralidae). *Information Systems Journal*, 14: 182-189.

Meisner, J.; I. Ishaaya; K.R.S. Asher and M. Zur (1978). Gossypol inhibits protease and amylase activity of Spodoptera littoralis larvae. *Annals of the entomological Society of America.*, 71: 5-8.

Mitlin, N.; G. Wiygul and J.W. Haynes (1977). Inhibition of DNA synthesis in boll weevil (Anthonomus grandis Boheman) sterilized by Dimilin. *Pesticide Biochemistry and Physiology*, 7: 559-563.

Nath, B.S.; A. Suresh; M.B.R. Varma and P. Kumar (1997). Changes in protein metabolism in haemolymph and fat body of the silkworm, Bombyx mori L., in response to organophosphorus insecticides toxicity. *Ecotoxicology and Environmental Safety*, 36: 169–173.

Okonkwo, E.U. and Okoye, W. I. (1996). "The efficacy of four seed powders and the essential oils as protectants of cowpea and maize grains against infestation by *Callosobruchus maculatus* (Fabricus) (Coleoptera: Bruchidae) and *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) in Nigeria,". *International Journalof Pest Management*,.42(3):143–146

Osman, H.H.; Fetoh,B.A.; Mohammed, A.M.(2012). The potency of Chloropyrifos and Camphor extract on *Spodoptera littoralis* (BOISD.). *Egyptian Academic Journal of Biological Sciences, (A.Entomology)*Vol.5(2) : 131-139.

Qadri, S. S. H. and Narsaih, S. (1978). Effect of azadirachtin on the moulting process of last instar nymph of Periplaneta americana (L.). *Indian Journal of Exprimental Biology*, 16: 1141-1143.

R. Pavela,(2014) . Acute, synergistic and antagonistic effects of some aromatic compounds on the Spodoptera littoralis Boisd. (Lep.,Noctuidae) larvae. *Industrial Crops and Products*, vol. 60, pp.247–258.

Rafea, H.S A. (2014): Combined Effects of Two Biocides and an Insect Growth Regulator on the Biological and Biochemical Aspects of Cotton Leafworm Spodoptera Littoralis (BOISD.).

Regnault-Roger, C.; Vincent. C. and Arnason, J. T. (2012). Essential oils in insect control: low-risk products in a high stakes world. *Annual Review of Entomology*, 57: 405-424.

Reitman, S. And Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, 28(1): 56-63.

S. A. M. Abdelgaleil, M. I. E.Mohamed, M. E. I. Badawy, and S.A. A. El-Arami, (2009)

.Fumigant and contact toxicities of monoterpenes to Sitophilus oryzae (L.) and Tribolium castaneum (Herbst) and their inhibitory effects on acetylcholinesterase activity, *Journal of Chemical Ecology*, vol. 35, no. 5, pp. 518–525.

Samir A. M. Abdelgaleil, (2010). Molluscicidal and insecticidal potential of monoterpenes on the white garden snail, Theba pisana (Muller) and the cotton leafworm, Spodoptera littoralis (Boisduval). *Applied Entomology and Zoology*, 45 (3): 425–433

SAS, 1996. Statistical analysis system. SAS user`s guide: statistic SAS institute Inc. Editors, cary, Nc.

Shalaby, M.M.; El-Sherif, A.G.; Borham, S.G. and El-Bialy, F. M. (2020). Toxicity of Some Essential Plant Oils Against Cotton Leaf Worm, *Spodoptera littoralis* (BOISD.). *Egyptian Academic Journal of Biological Sciences*, (F.Toxicology and Pest control) Vol. 12(1): 1-7.

Singh, N. B. And Sinha, R. N. (1977). Carbohydrates, lipids and protein in the developmental stages of Sitophillus oryzea and Sitophillus grannarius. *Annual Entomology, Society America.* 107-111.

Wang X, Li Q, Shen L, Yang J, Cheng H, Jiang S et al .(2014). Fumigant, contact, and repellent activities of essential oils against the darkling beetle, Alphitobius diaperinus. *Journal of Insect Science*, 14:1-11.

Wigglesworth, V.B. (1972): The Principles of Insect Physiology. The Analysis of Time Series: An Introduction with R (Chapman & Hall/CRC Texts in Statistical Science)

Yazdani, E.; Sendi, J. J. and Aliakbar, A. (2013). Chemical composition, toxicity and physio-logical effects of essential oil of *Rosemarinus officinalison* lesser mulberry pyralid, *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae). *Journal of Crop Protection*, 2(4): 461-476