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CHEMICAL COMPOSITION AND BIOASSAY OF ESSENTIAL OILS FROM TWO *EUCALYPTUS* SPECIES AGAINST THE COTTON LEAF WORM AND THE PINK CORN BORER LARVAE

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ABSTRACT

The insecticidal, biological, latent and antifeedent activities of the volatile oils (V.O) obtained from the hydrodistillation of the aerial parts of *Eucalyptus camaldulensis* and *Eucalyptus citriodora* was evaluated against the two tested insects, the cotton leaf worm, *Spodoptera littoralis* and pink corn borer *Sesamia cretica.* Our study indicated that the volatile oil of *E. citriodora* possessed more potent effect than the former. This fact supported our phytochemical analysis performed in this study using gas-chromatography-mass spectrometry (GC/MS) technique–coupled by a Triple – Axis Detector- which indicated that *E. citriodora* oil was more abound in compounds known to have a high insecticidal activity such as Spathulenol, α and β Pinene, Limonene, 1, 8-cineole and others. In addition, a number of compounds having a more potent biological activities was detected only in *E. citriodora* oil such as: Geranial (3.74%), α-pinene (3.41%), Citronellal (1.60%), β-selinene 2.70%, Piperitone (2.22%), Verbenone (1,86%), γ-eudesmol (1.38%), σ-cadinenene (1.06%) which may contribute to its significantly higher insecticidal activity observed in this study.

Key words: *Eucalyptus camaldulensis*; *Eucalyptus citriodora*; Geranial; Piperitone; eudesmol; Verbenone .

INTRODUCTION

Insect pests considered important factor of loss production (20-30%) in agriculture and horticulture, but in some cases they provoke a total loss. In addition, many populations of already > 550 species have developed resistance against most current insecticide groups, implying a high demand for novel insecticide targets. So, many scientists in industry and academia are currently trying to obtain useful compound from plants as new, natural insecticides. Plants constitute a rich source of bioactive compounds such as phenolics, terpenoids, coumarins and alkaloids (Harborne, 1993; and Ahn *et al.,* 1998). Since these compounds which are often active against a limited number of species including specific target insects, are biodegradable to non toxic products and potentially suitable for use in integrated pest management programs, they could lead to the development of new classes of safer insect control agents (Park *et al.,* 2002; and Mansour *et al.,* 2004).

Several workers reported that many plants are considered to contain materials efficient for pests control. Such agents may be used as toxicants, repellents, synergists, growth regulators or antifeedants for many insect pests (Kubo and Clocke, 1982).

Essential oils may have attractive or repellent effects and in some cases they showed insecticidal action against certain insect pests. Oils isolated from plants which consist of cyclic and monocyclic mono-terpens proved effective repellents against insects (Rodriguez and Levin, 1975). It was found that these bioactive compounds are potentially toxic to insects and mites but relatively safe to human and wildlife. Recently, there is a great need to find alternative pesticides instead the traditional chemical insecticides which proved to have toxic effect on human, animal and on the whole environment.

The present work deals with the identification and quantification of compounds responsible for latent and antifeedant activities of the volatile oils of both *E. camaldulensis* Dehn and *E. citriodora* Hook (Family: Myrtaceae) and to evaluate their efficacy against the tested insects, *Spodoptera littoralis* Boisd. and *Sesamia cretica.* Led (Lepidoptera: Noctuidae) larvae.

MATERIALS AND METHODS

The aerial parts of the two plants, *Eucalyptus camaldulensis* and *Eucalyptus citriodora,* were collected at full flowering stage in Autumn from the Montaza Botanical garden, Alexandria, Egypt and identified by Dr. Hosny Abdel-Azem Abou-Gazia, Professor of Timber trees and wood Technology, Faculty of Agriculture, Alexandria University. A voucher specimen was deposited in the Applied Entomology Department, Faculty of Agriculture Alexandria University.

Adult emergency percentages

All treatments caused highly significant differences in adult emergency percentages. For *E. citridora* volatile oil the values obtained were 21.7 ± 0.2 and 17.0 ± 0.0 % (V.O). While values obtained from the volatile oil of *E. camaldylensis* were 23.3±0.4 and 16.7±0.4 % (V.O) as compared to 89.7 ± 1.2 % of control one.

Adult longevity

All treatments caused significant reduction in pre-, ovi-, and post-ovipostion periods (day). The shortest results obtained from *E. citridora* V.O using 10µg/larvae, while *E. camaldylensis* volatile oil doses of 10µg/larvae of V.O was less affected by all treatment. The observed reduction in adult emergence was in conformity with the findings of Shonoda *et al* . ,(2000) and Farrag, (2000) after treatment of *S. littoralis* larvae with different extracts. The results showed that a significant reduction of imaginal longevity and was more pronounced for adults emerged from 4th larval instar treated with V.O *E. citriodora* more than that of *E. camaldulensis*.

Chemosterilant effect

Results in Tables (8 and 9) cleared a significant difference between adults insects obtained from 4th larval instar treated with V.O and control in the number of deposited eggs, (expressed as female fecundity) and also in percentage of egg hatchability since control females deposited an average of 1783.33±12.3 egg/female with hatchability of 94.21±0.25%. Reduction in female fecundity reached 329.33±15.5 and 255.67±24.9 egg/female in *E. citriodora* V.O. with doses of 5 and 10µg/L. In *E. camaldulensis* V.O. the reduction reached 286.67±27.7 and 146.0±17.9 egg/female with the same doses. The reduction reached 16.1±0.19 and 10.6±3.04% hatchability of eggs in *E.citriodora* V.O with doses 5 and 10 µg/L. In *E. camaldulensis* the reduction reached 20.0±8.33 and 14.85±0.59 hatchability of eggs when V.O was used at the same doses comparing with 97±26±0.7% for control one.

It is obvious that all treatments of V.O of *E. citridora* and *E. camaldulesis* not only affect adult longevity but also exhibited a chemosterilant effect by reducing female fecundity and egg hatchability which were more pronounced in *E. citriodora* treatment. The previous effect may be explained according to the explanation of Smagghe and Deyheele (1994) and Smaggho *et al.,* (1996) who illustrated an explanation about the chemosterilizing effect of V.O obtained from the two plant species on female of Lepidoptera by interfering with ovulation and oviposition. Their studies indicated that the reduction in egg laying is a result of inhibition of new-oocyte formation and induction of oocyte resorption.

The suppression of fecundity which associated with the treatment of V.O can be explained by the inhibition of Oogensis and ovarian development. This effect is due to the presence of sesquiterpenoids which was identified in Tables (1 and 2) and was structurally related juvenile hormone (JH). So, it plays an important role as insect growth inhibition and interferes with the developmental processes such as metamorphosis and reproduction (Pascual and Robledo, 1998 and Bede *et al.,* 1999). Similar results were obtained by Pathak and Krishna (1993) who found that treatment of *Earias vittello* with vapour oils of *Eucalyptus sp*. larvae reduced number of eggs (inhibited fecundity) and egg hatchability of treated adults. Egg hatchability was also significantly reduced when the parents was derived from 4th larval instar treated whereas some egg batches from the treated pairs did not hatch at all.

The reductions of fecundity and egg hatchability of the parents (adults) derived from treated 4th larval instar compared with control which caused sterility may indicate that both sexes are responsible for the chemosterilant effect. Accordingly, treatment with V.O of *E. citridora* and *E.camaldulensis* appeared in this final evaluation more efficient in reducing the reproductive potential of *S. littoralis*.

Malformations occurred

Data obtained in Tables (10 and 11) and illustrated in Figures (3 and 4) showed that the treatment of fourth larval instars of both *S. littoralis* and *S. cretica* with both V.O from both plants *E.citridora* and *E. camaldulensis* induced significant pupal and adult malformations than control in all tested doses ranged from 5µg - 80µg/L. The average mean of the pupal malformation rates in the case of *E. citridora* V.O for *S. littoralis* was 47.8%, while in *S. cretica* it was 61.5 %. While the average mean of the pupal malformation rates in the case of *E.camaldulensis* V.O for *S. littoralis* was 46.8%, while in *S. cretica* it was 52.8 %. The average mean of adult malformation rates in *E. citridora* V.O for *S. littoralis* was 52.8% and in *S. cretica* was 55.8 %, while in *E. camaldulensis* V.O it was 55.0 % for *S. littoralis* and 51.6 % for *S. cretica*. Statistical analysis using the calculated L.S.D. at 0.01 emphasized that there no significant differences observed between the effects of doses or between V.O of the two plants on both insects.

Pupal abnormalities observed as a result of larval treatment with V.O were deformed pupal with larval cuticle platches, larval pupal intermediate with larval head capsule and thoracic legs, reduced pupal size with body shrinkage and inability to shed larval cuticle during metamorphosis, pupae often had difficulties in shedding the pupal cuticles sheaths of cuticle sometimes involved the head, mouth parts or legs, the old cuticle shrinked and remained attached to posterior region of the abdomen. The malformation in adults include moths that could not free their bodies from pupal skin, abnormal adults with deformed mouth parts, moths with pupal abdomen, moths developed normally but with malformed crumpled wings (Figs. 3 and 4). Which failed to undergo complete metamorphosis. So, *E. citridora* and *E. camaldulensis* extracts play an important role as insect growth inhibitor. This finding was in agreement with Raghuraman *et al.,* (2007) who demonstrated that *E. citridora* extract against *Helicoverpa armigera*, *Earias vittela* and *Pectinophora gossypiella* caused reducing the incidence in shed reproductives and growth inhibitor. Therefore, the authors suggest that the biopesticide formulations based on *E. citridora* could be recommended as a compound of sustainable management in cotton crop.

Fig. (3): Adult: *Spodoptera littoralis.* Malformed adults with abnormal body, shorten wings, crumpled wings

Fig. (4): *Spodoptera littoralis.* A-E: types of abnormal and malformed pupae failed to cast the old cuticle. F: Gaint pupae

Table (10): Malformation due to the treatment of *Spodoptera littoralis* 4th larval instar with different doses of volatile oils extract (V.O)

Dose of volatile oil µg/larvae	Malformed (V.O)			
	Pupal malformation %		Adult malformation %	
	Mean±SE/ replicat	Total	Mean±SE/ replicate	Total
1-*Eucalyptus citriodora*				
5µg/L	13.7±2.0	41.2	6.7±3.3	20.0
10µg/L	13.7±2.0	41.2	13.3±3.3	40.0
20µg/L	11.1±2.8	33.3	12.5±0.0	37.5
40µg/L	16.7±0.0	50.0	11.1±11.1	33.3
80µg/L	22.2±11.1	66.7	33.3±33.4	100.0
mean	15.9±4.0	47.8	17.6±12.0	52.8
2-*Eucalyptus camaldulensis*				
5µg/L	11.9±2.4	35.7	7.4±3.7	22.2
10µg/L	18.5±1.9	55.6	8.3±4.2	25.0
20µg/L	13.3±3.3	40.0	9.5±4.8	28.6
40µg/L	8.3±4.2	25.0	22.2±5.6	66.7
80µg/L	22.2±11.1	66.7	33.3±33.4	100.0
mean	15.6±5.1	46.8	18.4±12.0	55.0

Table (11): Malformation due to the treatment of *Sesamia cretica* 4th larval instar with different doses of volatile oils extract (V.O)

Dose of volatile oil µg/larvae	Malformed (V.O)			
	Pupal malformation %		Adult malformation %	
	Mean±SE/ replicat	Total	Mean±SE/ replicate	Total
1-*Eucalyptus citriodora*				
5µg/L	19.3±1.8	57.9	16.7±4.2	50.1
10µg/L	17.9±2.6	53.7	16.7±0.0	50.1
20µg/L	19.4±2.8	58.2	13.3±6.7	40.0
40µg/L	22.2±6.4	66.7	11.1±11.1	33.3
80µg/L	22.2±11.1	66.7	33.3±33.4	100.0
mean	20.5±5.7	61.5	18.6±12.8	55.8
2-*Eucalyptus camaldulensis*				
5µg/L	15.0±0.0	45.0	15.4±2.8	46.2
10µg/L	16.7±0.0	50.1	22.2±0.0	66.6
20µg/L	19.4±2.8	58.3	13.3±6.7	40.0
40µg/L	20.8±4.2	62.4	11.1±11.1	33.3
80µg/L	13.3±6.6	40.0	22.2±11.1	66.6
mean	17.6±3.4	52.8	17.2±7.2	51.6

Antifeeding effect

Larvae fed on castor bean leaf discs (7 cm in diameter) either untreated or treated with V.O of each plant on both surfaces of the discs four concentrations of 80, 40, 20 and 10 µg/cm^2 was determined. Differences in larval weights after 24 hours were recorded and the starvation percent was calculated. Data in Tables (12 and 13) show that as the concentration of the V.O increase the percent starvation increase, starting from 14.8 % for *E. citridora* and 12.1 % for *E. camaldulensis* using 10 µg/cm^2. The starvation increased to 39.8 % for *E. citridora* and 23.3 % for *E. camaldulensis* on doubling the concentration to 20 µg/cm^2. The highest tested concentration of 80 µg/cm^2 resulted in 70.4 % for *E. citridora* and 62.9 % for *E. camaldulensis*. Volatile oil of *E. citridora* had the highest activity on larval weight and percentage of starvation.

Concerning the treatment of 4th larval instar of *S. littoralis* with the volatile oil of *E. citridora* and *E. camaldulensis* (Tables 12 and 13), it is obvious that the effect may be due to a combination of antifeedant action and post digestive toxicity. So, larval mortality constituted the major portion of mortality attained. So, the deleterious effects of the volatile oils were due to the toxicity as well as the antifeedant activities and possessed high insecticidal activities in varying degrees. Also, *E. citridora* and *E. camaldulensis* showed satisfactory activity either with feeding or topical application treatment. It could be concluded that the volatile oil of *E. citridora* and *E. camaldulensis* have the potential to be used as natural pest control agent.

Table (12): Effect of *Eucalyptus citrodora* volatile oil (V.O.) on larval weight and percentage of starvation of the 4th larval instar of *Spodoptera littoralis*.

Concentration of volatile oil µg/cm^2	Zero time average weight gm/larva (A)	After 24 hrs average weight gm/larva (B)	Difference (B-A) gm/larva	Starvation %
80	0.186	0.197	0.011	70.4
40	0.189	0.208	0.019	63.0
20	0.182	0.226	0.044	39.8
10	0.179	0.250	0.071	14.8
Starved (s)	0.182	0.161	-0.021	-
Control (C)	0.186	0.273	0.087	-

Table (13): Effect of *Eucalyptus camaldulensis* volatile oil (V.O) on larval weight and percentage of starvation of the 4th larval instar of *Spodoptera littoralis*.

Concentration of volatile oil µg/cm^2	Zero time average weight gm/larva (A)	After 24 hrs average weight gm/larva (B)	Difference (B-A) gm/larva	Starvation %
80	0.176	0.200	0.024	62.9
40	0.184	0.224	0.040	49.1
20	0.194	0.264	0.070	23.3
10	0.179	0.262	0.083	12.1
Starved (s)	0.188	0.169	-0.019	-
Control (C)	0.190	0.267	0.097	-

Hence, toxicological, phytochemical and biochemical further studies indicated that treated larvae were normal but failed to eat (antifeedant effect) which ultimately resulted in their death. Therefore, the first noticeable effect of the tested extracts was their interference with the ability of larvae to eat. The lack of feeding may result in the reduction of chitin biosynthesis, thereby weaking the mouth parts or in complete clearance of the foregut and midgut at the moult (Abdel-Moneam *et al.*, 1980). Also, Mordue *et al.*, (1986) indicated that treatment with azadirachtin showed insect regulatory effects of prolonged instars and moult aberration which suggest an interference with the insect's endocrine system. This plant extract have oily properties, so the defatted non polar extract was more potent. Finally, we can assumed that these plant extracts were toxic as stomach poisons and/or contact action against 4th larval instar of *S. littoralis*.

Synergistic phenomena of essential oils:

Plants produce a high diversity of secondary metabolites a prominent of protecting plants against predators or repellence to herbivores. Some metabolites are also involved in defense mechanisms against abiotic stress (e.g. UV-B-exposure) and are important in the interaction of plants with other organisms (e.g., attraction of pollinators). There are three major groups of secondary metabolites, including terpenes, phenylpropenoids and N- and S-containing compounds. These main groups include terpenes, terpenoids, aromatic and aliphatic constituents, all characterized by low molecular weights.

Essential oils, volatile oils, or aromatic plant essences, are volatile and fragrant substances with an oily consistency typically produced by all plant organs and have been long recognized for their insecticidal activity (Bassole

and Juliani, 2012). The interaction between essential oils compounds can produce four possible types of effects; indifferent, additive antagonistic, or synergistic effects. (Pei *et al.,* 2009 and Mulyaningsih *et al.,* 2010). Zore *et al.,* (2011) proposed two hypotheses for mechanism of action to explain synergistic effects of essential oils (could increase the permeability of the cytoplasmic membrane, and probably enable insecticidal compounds to be more easily transported into the cell and also could increase the number, size or duration of existence of the pores created by the binding to proteins in the cell membrane, so that a synergistic effect is achieved indicating a possible toxicity of combined essential oils or components.

Mechanism of action of the *Eucalyptus* extracts:

Terpens and monoterpenoids including thymol, eugenol, and citronellal combinations have been patented for pesticidal activity against cockroaches and the green peach aphid. Similarly, citronellal, citronellol or a mixture of these have been reported as pest treatment composition against the human louse (Ping, 2007). Eucalyptus, limonen, Carvones have been well documented as fumigants (Tripathi *et al.,* 2009). α-pinene, eugenol, limonene, terpinolene, citronellol, citronellal, camphor and thymol are repellents against mosquitoes; the sesquiterpenes, β-caryophyllene is repellent against *A. aegyptiphytol*, aphytol, a linear diterpene alcohol is repellent against *Anopheles gambiae* and phenylethyl alcohol. β-citronelol, cinnamyl alcohol, geraniol and α-pinene, terpenen 4-ol., are repellents against the tick, *Ixodes ricinus,* in some metabolites that have the hydroxyl group linked to a tertiary carbon (Linalool, α-terpineol and limonene), the repellant activity is suppressed against *A. gambiae* (Nerio *et al.,* 2010 and Merio *et al.,* 2010).

Volatile terpenoids (terpenen-4-ol) can act as attractants. In some cases, the same terpenoid can repel certain undesirable insects while attracting more beneficial insects, for example, geraniol will repel flies but attract honey bees (Dubey *et al.,* 2011). Essential oils of lemon *Eucalyptus citriodora* (3,8-p- methone-diol, citronellal, 2-phenethyl propionate para-menthane 3,8 diol = PMD) are effective against mosquitoes (Fradin and Day, 2002). Also, PMD provides very high protection from abroad range of insect vectors and due to its proven safety to human health (EPA, 2011) it is clinical efficacy to prevent malaria.

The essential oils camphor, cineole, geraniol and piperidine possess repellent properties towards cockroaches (Liu *et al.,* 2011). Also, limonene, linalool, β-Ocimene, carvone act as repellents and can protect the crops nearby (Isman, 2010). The author illustrated that females of Caribbean fruit flies, *Anastrepha suspense*, lay their eggs readily in ripe grape fruit but do not oviposit in immature grape fruit because of the presence of linalool, which is toxic to the eggs and larvae of insects, limonene is toxic and highly attractive, β- Ocimene is repellent, Carvone (monoterpene) is a non-toxic botanical insecticides used under the trade name TALENT. It enhances the shelf life of stored fruits and vegetables and inhibits microbial deterioration without altering the test and odor of the fruit after treatment.

CONCLUSION

The results obtained from our current study indicated that the insecticidal, biological, latent and antifeedent activities of the volatile oil (V.O) obtained from the hydrodistillation of the aerial parts of *Eucalyptus citriodora* possessed more potent effect as insecticidal, biological, latent and antifeedant activities than the volatile oil obtained from the hydrodistillation of the aerial parts of *Eucalyptus camaldulensis.* This fact supported our phytochemical analysis performed in this study using gas-chromatography-mass spectrometry (GC/MS) technique–coupled by a Triple Axis Detector which indicates that *Eucalyptus citriodora* oil was more richer in compounds known to have a high insecticidal activity than that present in the volatile oil of *E. camaldulensis* such as: Spathulenol, α and β Pinene, Limonene, 1, 8-cineole and others. To the best of our knowledge, our study was the first that reported the presence of a number of compounds having a more potent biological activities in *Eucalyptus citriodora* oil for the first time such as: Geranial (3.74%), α-pinene (3.41%), Citronellal (1.60%), β-selinene 2.70%, Piperitone (2.22%), Verbenone (1,86%), , γ-eudesmol (1.38%), σ-cadinenene (1.06%) (Table 3). These two reasons (different in composition qualitatively and quantitavely) may contribute to its significantly higher activity more than that of *E. camaldulensis.*

This study provides a reference for developing *E. citridora* and *E. camaldulensis* V.O as a new type of promising insecticidal activity according to its toxic, latent and antifeedant activities.

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Fig. (3): Adult: *Spodoptera littoralis.* Malformed adults with abnormal body, shorten wings, crumpled wings

Fig. (4): *Spodoptera littoralis.* A-E: types of abnormal and malformed pupae failed to cast the old cuticle. F: Gaint pupae

Fig. (3): Adult: *Spodoptera littoralis.* Malformed adults with abnormal body, shorten wings, crumpled wings

Fig. (4): *Spodoptera littoralis.* A-E: types of abnormal and malformed pupae failed to cast the old cuticle. F: Gaint pupae

Freshly collected plants were hydrodisitilled for recovery of its essential oil by steam distillation in a Clevenger-type apparatus, according to the literature. The essential oils obtained were dried over a layer of anhydrous sodium sulphate and submitted to chemical and biological analysis.

The identification of volatile constituents was conducted by gas-chromatography-mass spectrometry (GC/MS) technique–GC 7890A apparatus with Mass spectrometry the characteristic fragment ions reported in the NIST 98 and WILEY 138 databases. Relative amounts of individual components are based on peak areas obtained. MS 5975C VL MSD with Triple – Axis Detector-Automatic sampler–7683B Series injector and split/splitless injection system. The GC was coupled with MS capillary column (INNOWAX, 60 m, 0.25 mm I.D., 0.25 mm Film thickness). The temperature program was as follows: Temperature of injector 250°C, pressure 146.99 kpa, Temperature of MS detector 280°C, Temperature of oven 40°C for 4 min, then gradient 4°C/min to 260°C, 4 min hold time, 63 min was the final time, transfer line temperature was 200°C. Helium was used as carrier gas at kpa pressure with flow 1.3689 ml/min, linear velocity 30 cm/S. the mass spectrometer had a vacuum compensation ON, Solvent delay time 4 min to avoid the solvent peak, split ratio 1:10 and electronic pressure control on. Scan time was 29-650 m/z. Ionization energy was set at 70 eV. The essential oils and crude extracts from the two plants were tested against newly moulting 4th larval instar of both insects (0-24 hours after ecdysis) for toxic, biological activity, latent and antifeedant effects. For toxicity tests, a series of dilutions were prepared from the tested essential oils in water by using Tween 20 as emulsifier at different doses 80, 40, 20, 10 and 5 µg/larvae. The extractions were applied topically by the aid of accupipet (2 micro liter per larvae). For control group, topically applied of Tween 20 soluted in water was at the same volume 2µl/larvae. Experiments were carried out using three replicates for each dose each of which contained 10 larvae. Larval mortality in each jar was counted 72 hour after initial exposure and the accumulative percent mortality was also calculated till pupal formation. Mortality percentages were corrected by Abbott formula (Abbott 1925). Equations used for estimating different bio-metric records of *S. littoralis*:

According to El-Dewy (2006)

$$\text{1- Pupation \%} = \frac{\text{Total number of pupae}}{\text{Total number of treated larvae}} \times 100$$

$$\text{2- Pupal malformation \%} = \frac{\text{Total number of abnormal pupae}}{\text{Number of (normal + abnormal) pupae}} = 100$$

$$\text{3- Abnormalmoths emergence\%} = \frac{\text{Number of abnormal moths}}{\text{Total number emergent moths}} = 100$$

The probit analysis (Finney, 1971) was employed in analyzing the dose-mortality response to estimate LD_{50} values using SAS program (SAS Institute, 1997).

Latent effects:

Observation on the treated and control larvae were recorded. The different biological aspects such as larval and pupal duration, pupation and adults emergence percentages, pupal weight, pre, ovi, and post-oviposition period, number of eggs/female, and hatchability percentage were determined at the LD50 values of the tested compounds. Also, the observed malformations for each tested dose were recorded and photographed. The obtained data of the biology were statistically calculated through excel for windows computer program to determine the F-value, and LSD (least significant difference) at 0.01 probability level.

Laboratory feeding tests on *S. littoralis*:

The effect of different concentrations of each of the two extracts on feeding activity of fourth larval instar of *S. littoralis* were carried out. Volatile oils and methanolic extracts diluted in Tween 20 and water were applied by a pipette to both surfaces of castor bean leave discs. The diameter of each disc was 7 cm and then placed in convenient glass jars. Control discs were treated only with Tween 20 and water. *S. littoralis* larvae were left without feeding for 24 hours, and then their weights were recorded. Three replicates for each concentration (80, 40, 20, 10 µg/cm^2) and 10 larvae for each replicate were used. Ten larvae were exposed to each disk and left under 25±2°C and 65±5% RH for 24 hours. The larvae were then reweighed after 24 hours of feeding with the treated disk. Percentage of starvation in the tested larvae is calculated according to the following equation of Moustafa (1969) and Abdel-Mageed *et al*., (1975):

$$\text{\% starvation} = \frac{C - E}{C - S} \times 100$$

Where

E: mean weight gain of treated larvae at each tested concentration within 24 hours

C: mean weight gain of control larvae within 24 hours

S: mean weight gain of starved control larvae within 24 hours

RESULTS AND DISCUSSION

Pesticides based on plant essential oils have recently entered the marketplace, plant-based insecticides are not only toxic to pest but also deter and/or repel pests which may contribute to their overall efficacy against pests that cause great economic loss at the pre- as well postharvest stage of the crop production and reduce transmission of disease to plants and animals including humans (Khater 2012). Also, he illustrated that essential oils (Botanical insecticides are desirable alternative to synthetic chemical insecticides for controlling pests. They are best suited for uses in organic food protection in industrialized countries but can play a mush greater role in developing countries as a new class of ecofriendly products for controlling pests .

Chemical composition of the essential oils derived from the tested plants

In the present study a GC/MS analysis method was developed for accurate identification and quantification of the highest possible number of compounds in the essential oils obtained by hydrodistillation from the two selected plants. This study also evaluates their latent and antifeedent activities against the tested insects, *S. littoralis* and *S. cretica*.

Identification of the components was confirmed by GC-MS with the aid of the mass chromatograms of the characteristic fragment ions reported in the NIST 98 and WILEY 138 databases. Relative amounts of individual components are based on peak areas obtained.

Results showing the percentages of the highest possible number of identified components are summarized and listed in Table (1) and Fig. (1) for *Eucalyptus camaldulensis* and Table (2) and Fig. (2), *Eucalyptus citriodora*, respectively. Also, Table (3) illustrated the main differences in composition of the essential oils obtained from the two plants under study.

Table (1): Identification and quantification of chemical composition of the essential oil from *Eucalyptus Camaldulensis* Dehnh. (Egypt, Alexandria).

P. n	R.t (min.)	Name of compound	M.wt	Relative amount (%)
1	6.302	α-Pinene (2,6,6-trimethyl-Bicyclo [3.1.1]hept-2-ene).	136	2.35
2	8.328	2-.β-Pinene (6,6-dimethyl-2-methylene-Bicyclo [3.1.1]heptane).	136	9.48
3	11.313	Limonene (1-methyl-4-(1-methylethenyl)-Cyclohexene).	136	0.82
4	11.682	Eucalyptol; 1,8-Cineole (2-Oxabicyclo[2.2.2] Octane, 1,3,3-trimethyl).	154	2.80
5	13.722	ρ-Cymene (1-methyl-2-(1-methylethyl)-Benzene).	134	20.54
6	23.443	α- Phellamdrene (α-2methyl-5-(1-methyl)-1,3 cyclohexadiene	136	0.66
7	23.58	Sabinene (4-methylene-1- (1-methylethyl)-Bicyclo(3.1.0) hexane).	136	0.68
8	23.933	α- Thujone (15,4R,5R)-4-methyl-1-propan-2-ylbicycle (3.1.0) hexan-3-1).	152	0.27
9	24.662	4-Terpineol; Terpinene-4-ol (1-Terpinen-4-ol; 4-Carvomenthenol; 4-methyl-1- (1-methylethyl)-3-Cyclohexen-1-ol).	154	4.29
10	25.264	Myrtenal (6,6-dimethyl-Bicyclo[3.1.1]hept-2-ene-2-carboxaldehyde).	150	1.11
11	25.51	1-Terpineol; Terpenene-1-ol (2-(4-methyl-1-cyclohex-3-enyl) propan-2-o1).	154	0.42
12	26.044	Trans-Pinocarveol (6,6-dimethyl-2-methylene-Bicyclo[3.1.1]heptan-3-ol).	152	1.82
13	26.838	Crypton (4-(1-methylethyl)-2-Cyclohexen-1-one).	138	10.30
14	29.714	Hydroxy-2-methyl-3-phenyl-Propanal.	164	2.53
15	29.86	Linalool (3,7-dimethylocta-1,6 –dien-3-o1).	170	2.70
16	31.562	4-trimethyl-Benzeneethanol; Benzeneethanol, α,α-dimethyl	150	0.55
17	33.013	Cis –P-menth-2-en-1-o1 (Y-terpinene, α-terpineol, nerylacelate α-copaene).	138	0.48
18	34.546	Caryophyllene oxide (4,12,12-trimethyl-9-methylene-5-oxatricyclo[8.2.2.0.0]4,6)]dodecane).	220	12.27
19	35.354	Phellandral (4-propan-2-Y/cyclohexane-1-carbaldehyde).	152	0.35
20	35.7	Thymol (5 methyl-2-(1-methylethyl)-phenol).	150	0.20
21	35.9	Aromadendrene (1,1,7-trimethyl-4-methylidene-octahydro 1-aH-cyclopropa[e] azulene	204	0.92
22	37.668	Cuminol; p-Cymen-7-ol; Cumic alcohol; Cumyl alcohol (4-(1-methylethyl)-Benzenemethanol).	150	1.20
23	38.051	Spathulenol (1H-cycloprop [e] azulen-7-o1).	220	19.25
24	39.352	Nerolidol (3,7,11-trimethyl-1,6,10-dodecartrien-3-o1).	222	0.33
25	39.886	Ledol (1,4,7,7-tetramethyl-decahydro-1H-cyclopropol [e] azulen-4-o1 ozulen-4-o1	222	0.40
26	40.132	Carvacrol (2-methyl-5-(1-methylethyl)-Phenol).	152	0.62
27	40.393	m-Cumenol (3-(1-methylethyl)-Phenol).	150	0.31
28	41.816	β-Bisabolene (1-methyl-2-(4-methyl-3-cyclohexan-1-y).	204	0.89
29	42.13	α- Campholen aldehyde (2,2,3-trimethyl-3-cyclopentar acetaldehyde).	152	0.33
30	42.638	3- methyl-2(2-penthenyl) cyclopentanone.	156	0.37
31	42.965	4-(1-methyl ethyliden) cyclohexanone.	98	0.14
32	43.514	P-Ment-1-en-8-ol (2-(4-methylcyclohex 3-en-1-y1) propan-2-o1).	152	0.62
				100.00

P.n.: Peak number R.t: Retention time M. Wt: Molecular weight

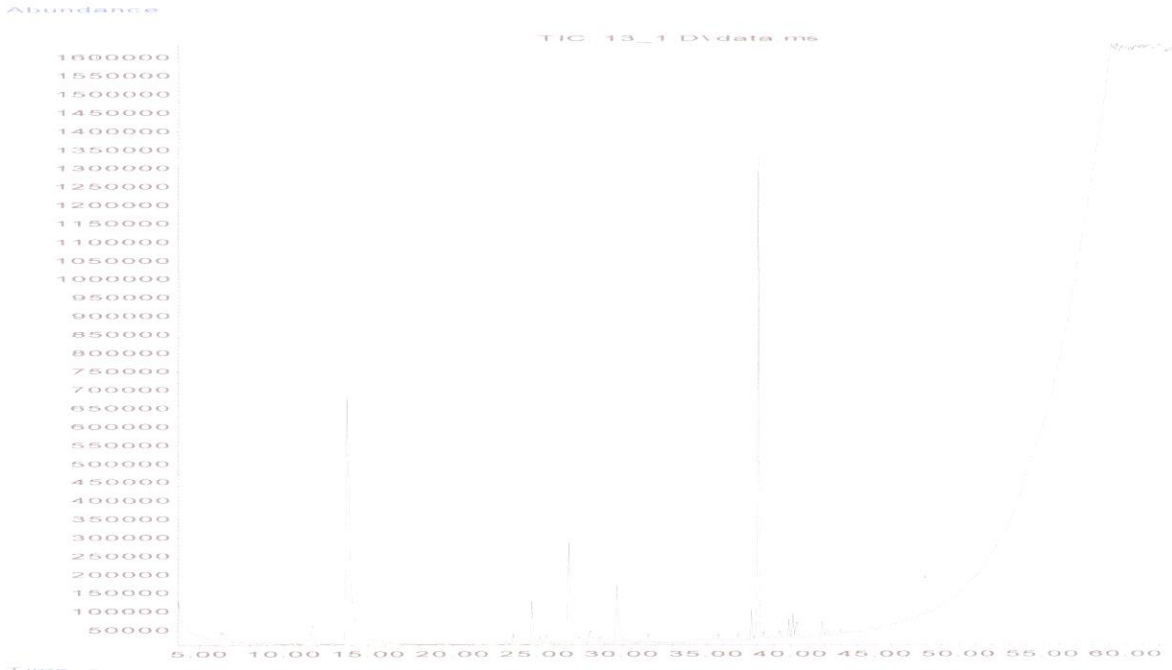

Fig. (1): Gas chromatogram of the essential oil of Eucalyptus Camaldulensis Dehnh.

Chemical composition of essential oils derived from:

Eucalyptus camaldulensis

In the present work, the chemical composition of the essential oil was investigated using GC/MS techniques. The relative amount percentages of the identified components are listed in Table (1). This study allowed the identification of thirty two compounds in the volatile oil of the aforementioned plant accounting for 100% of the oil.

The main constituents were found to ρ.cymene (20.54%), Spathulenol (19.25%), caryophyllene oxide (12.27%), crypton (10.30%), β-pinene (9.48%), 4-terpineol (4.2%), 1,8-cineole (2.80%), linalool (2.70%), 2-methyl-3-phenyl propanal (2.53), α-pinene (2.35%), Trans-pinocarveol (1.82%), cuminolin (1.20%), Myrtenal (1.11%), other components were present in amount less than 1.00% such as aromadentrene, bisabolene, limonene, sabinene, α-phellandrene, carvacrol, P-Ment-l-en-8-o1, 4-trimethyl-benzenemethanol, Cis-p-menth-2-en-1-o1, l-terpineol, ledol, 3-methyl-2 (2-penthenyl) cyclopentanone, phellan-dral, nerolidol, α-campholen aldehyde, m-cumenol, α-thyjone and thymol.

The composition of the essential oil from *E. camaldylensis* reported in this study was of great diversity in the oil composition, the results were quite different from the previous reports. Our results reported the presence of aromadentrene, limonene, ledol, phellamdral, nerolidol and thujone in the oil obtained from *E. camaldylensis*, while other several authors did not reported the presence of any of these compounds in the oil from *E. camaldylensis* obtained from different origins (Duke, 2004; Brooker and Kleining, 2006; Siramon and Ohtani, 2007; and Basak and Candom, 2010).

These differences might have been due to geographical origin, tissue explored, harvest time and local, climatic, genetic and seasonal factors as well as storage duration of medicinal herbs. The principal component of the oil was cymene, what also reported as the major constituents and dominated of the *E. camaldylensis* essential oil (Brooker and Kleining, 2006; Akin *et al.*,2010; Basak and Candom, 2010; and Grbovic *et al.*, 2010). Also, spathulenol, caryophyllene oxide, crypton, pinene, 4-terpineol, 1,8-cineole, linalool, and pinocarveol were found to be the most abundant components as reported by (Akin *et al.*, 2010, and Ben Marzoug *et al.*, 2011).

Grbovic *et al.*, (2010) showed that the main compounds presence in *E. camaldylensis* essential oil are 1,8-cineole, spathulenol, P-cymene and crypton. which are similar to those obtained by Pappas and Sheppard -Hanger, (2000), and Tsiri *et al.*, (2003).

Our results certainly suggest that the main characteristic of this chemotype of *E. camaldylensis* is the presence of a high amount of aromatic monoterpene hydrocarbon cymene, followed by monoterpenes: cryptone, β-pinene, 4-terpineol (terpinene-4-o1) and sesquiterpene, spathulenol. According to the high cymene, cryptone and 4-terpineol ratio, a great similarity was found with (Pappas and Sheppard -Hanger 2000, and Siramon and Ohtani 2007). In addition, our study was the only study that recorded the presence of aromadentrene, limonene, ledol, phellamdral, nerolidol and thujone compounds for the first time in the oil obtained from *E. camaldylensis*.

Eucalyptus citriodora

In this respect, the chemical composition of the essential oil was investigated using GC/MS techniques. The relative amount percentages of the identified components are listed in Table (2). This study allowed the identification of thirty four compounds in the volatile oil of a formentioned plant accounting for 100% of the oil.

Table (2): Identification and quantification of chemical composition of the essential oils from *Eucalyptus citriodora* Hook. (Egypt, Alexandria).

P.n	R.t (min.)	Name of compound	M.wt	Relative amount (%)
1	6,288	α-Pinene (2,6,6-trimethyl-Bicyclo [3.1.1]hept-2-ene).	136	3.41
2	8,314	2-.β.-Pinene; Pseudopinen; Pseudopinene (6,6-dimethyl-2-methylene-Bicyclo [3.1.1]heptane).	136	13.62
3	11,313	Limonene; 1, 8-p-menthadien (1-methyl-4-(1-methylethenyl)-Cyclohexene).	136	3.53
4	11,628	2-Thujone (4-methyl-1-(1-methylethyl)-Bicyclo[3.1.0]hex-2-ene).	152	2.59
5	13, 736	O-Cymene; o-Cymol (1-methyl-2-(1-methylethyl)-Benzene).	134	11.78
6	23,443	Geranial; Citral; lemonal (3,7 dimethyl-2,6-pctadienal).	154	3.74
7	23,799	β - Phellandrene (α-2-methyl-5-(1-methyl)-1,3 cyclohexadiene).	136	0.31
8	24,087	β- Myrcene (7-methyl-3- methylene-1,6-octadiene).	136	0.23
9	24,662	4-Terpineol; Terpinene-4-ol; 1-Terpinen-4-ol; 4-Carvomenthenol (4-methyl-1- (1-methylethyl)-3-Cyclohexen-1-ol).	154	1.44
10	25,154	Aromadendrene (1,1,7-trimethyl-4-methylendedecahydro-1H-Cycloprop[e]azulene).	204	1.22
11	25.25	Myrtenal (6,6-dimethyl-Bicyclo[3.1.1]hept-2-ene-2-carboxaldehyde).	150	1.35
12	26.044	Trans-Pinocarveol (6,6-dimethyl-2-methylene-Bicyclo[3.1.1]heptan-3-ol).	152	1.25
13	26.838	Crypton; Cryptone (4-(1-methylethyl)-2-Cyclohexen-1-one).	138	3.43
14	27.455	Citronellal (6-octanl, 3,7-dimethyl).	154	1.60
15	27.687	Bicyclogermacrene (3,7,11,11-tetramethyl-Bicyclo[8.1.0]undeca-2,6-diene).	204	0.89
16	28.125	n- Eicosan-3,12-diol	282	1.00
17	28.7	Viridiflorene (decahydro-1, 1,4,7-tetramethyl).	222	0.17
18	29.7	2-Methyl-3-phenylpropanal.	148	0.32
19	29.85	Verbenone ([3.1.1] hept-3-en-2-one,4,6,6-trimethyl-(IR))	150	1.86
20	31.548	Calamenene (15,4R-1,6-dimethyl-4-propan-2-y1-1,2,3,4 tetrahydronaphthalene).	202	0.13
21	34.546	Eucalyptol; 1,8- cineole (2-oxabicyclo[2.2.2] octane 1,3,3-trimethyl).	154	2.08
22	35.341	Piperitone (6R-3-methyl-6-propan-2-ylcyclonex-2-en-1-one).	152	2.22
23	35.710	Iso-Bornyl acetate [1R,45,6R]-1,7,7-trimethyl-6-bicyclo[2.2.1] heptanyl	196	0.58
24	36.901	σ- Cadinene (Naphalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethy).	204	1.06
25	27.668	Cuminol; p-Cymen-7-ol; Cumic alcohol; Cumyl alcohol (4-(1-methylethyl)-Benzenemethanol).	150	0.85
26	38.038	Spathulenol (1,1,7-Trimethyl-4-methylenedecahydro-1 H-cyclopropa[e]azulen-7-ol).	220	28.91
27	38.394	α-Terpineolene (1-methyl-4-propan-2-ylidene cyclohexanene).	136	1.81
28	38.914	Trans-2-Caren-4-o1(4,7,7-trimethylbicyclo [4.1.0] hept-4-en-3-o1).	152	0.27
29	39.34	P- Cymen-8-o1.	150	0.78
30	39.872	α-Farnesol (1,3,6,10-Dodecatetraene; 3,7,11-trimethyl).	204	0.89
31	40.119	Carvacrol; Antioxine; Isothymol; Karvakrol; p-Cymen-2-ol (2-methyl-5-(1-methylethyl)-Phenol).	152	0.96
32	40.310	Thujopsene (cyclopropa[d] naphthalene).	204	1.64
33	41.858	γ-Eudesmol (2-Naphthalene methanol).	222	1.38
34	42.104	β-Selinene (Naphthalene, decahydro-4a-methyl-1-methylene-7- (1-methyl-ethenyl).	204	2.70
				100.00

P.n.: Peak number Rt: Retention time M. Wt: Molecular weight

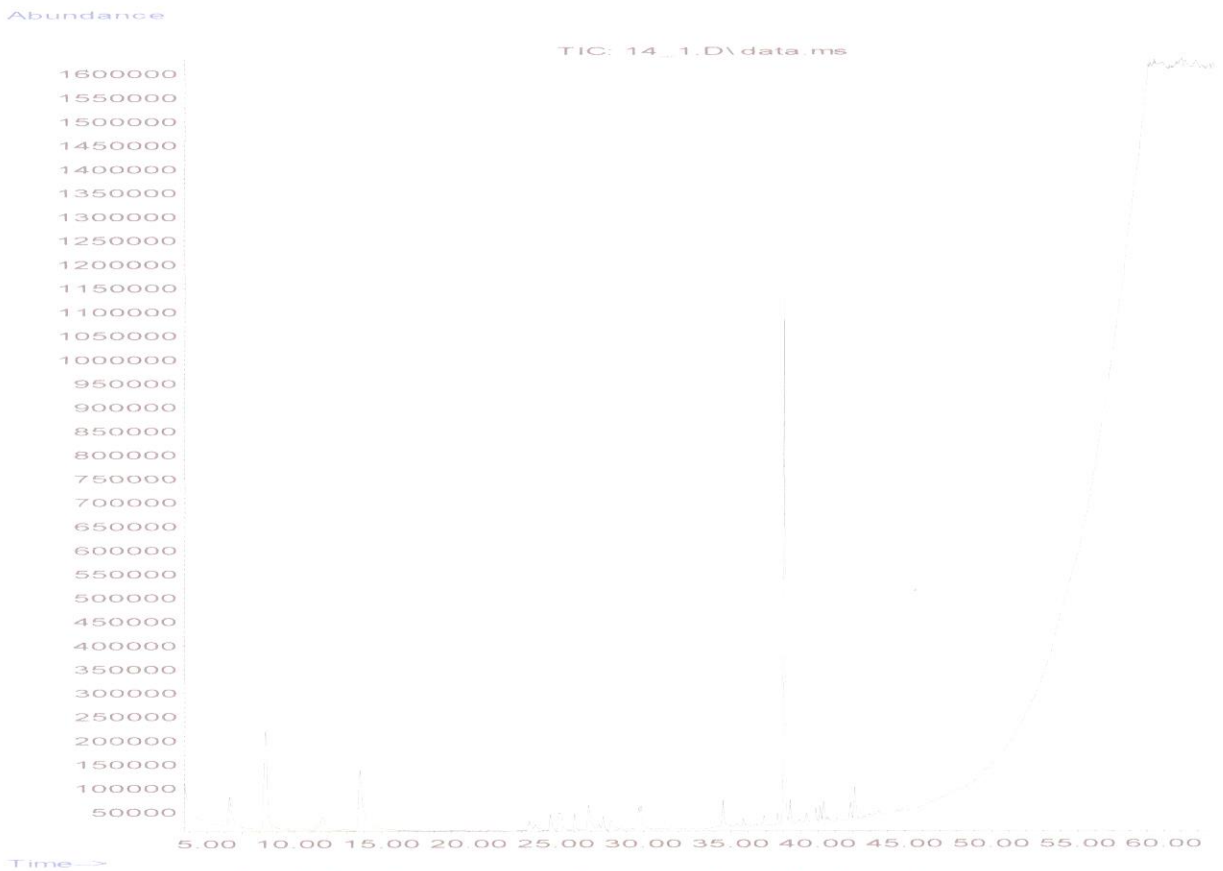

Fig. (2): Gas chromatogram of the essential oil of *Eucalyptus citriodora*

The major constituents of *E. Citriodora* volatile oil identified in this study were: spathulenol (28.91%), 2-β-pinene (13.62%), O-cymene (11.78%), Geranial (3.74%), Limonene (3.53%), Crypton (3.43%), α-pinene (3.41%), β-selinene (2.70%), 2-Thujone (2.59%), Piperitone (2.22%), 1,8-cineole (2.08%), Verbenone (1,86%), α-terpineolene (1.81%), Thujopsene (1.64%), Citronellal (1.60%), 4-terpineol (1.44%), γ-eudesmol (1.38%), Myrtenal (1.35%), Trans-pinocarveol (1.25%), aromadendrene (1.22%), σ-cadinenene (1.06%), and n-eicosan-3, 12-diol (1.00%), as well as compounds were found as a minor components (less than 1.00%) carvacrol (0.96%), bicyclogermacrene (0.89%), α-farnesol (0.89%), Cuminol (ρ-cymen-7-o1) (0.85%), P-cymen-8-o1 (0.78%), Iso bronyl acetate (0.58%), 2-methyl-3-phenylpropanal (0.32%), β-phellandrene (0.31%), Trans-2-caren-4-o1 (0.27%), β-Myrcene (0.23%), viridiflorene (0.18%), and Calamenene (0.13%).

It was found that our results contradicted with some of the previous reports of Akin *et al.,* (2007), Gilles *et al.,* (2010), Abhilasha and Mohammed (2011), and Abd El-Mageed *et al.,* (2011) whose reported 3-hexen-1-o1 (crypton) as the major components of volatile oil in *E.citriodora.* While in our study spathulenol (28.91%) was reported as the main active ingredient in this oil, while crypton in our study was found to represent only a minor component with a concentration of only 3.43%.

In addition, our oney was the only study that reported the presence of citronellal (52.88%) along with geraniol, citronellol, cetronellyl acetate and isopulegol, thujone, thujopsene, geranial and piperitone compounds for the first time in the oil obtained from *E.citriodora* (Table.2). This detected compounds in our study was not detected by other authors (Batish *et al.,* 2006; Ouedraogo *et al.,* 2009; Akin *et al.,* 2007, Gilles *et al.,* 2010; Abhilasha and Mohammed 2011; and Abd El-Mageed *et al.,* 2011).

The presence of appreciable percentage of 1, 8-cineole (eucalyptol) (2.08%), β-phellandrene (0.31%), cymene (0.85%) in our study was in agreement with Akolade *et al.,* (2012), who also reported the presence of these compounds that are known to be responsible for the high cytotoxic effect of the *Eucalyptus* oil investigated in this study. This suggests that it may be suitable for use as repellant or antifeedant in insecticidal formulation. In other report, Maciel *et al.,* (2009) found that the major constituents in *E. citriodora* oil were Z-citral, α-citral, citronellal, and 1,8-cineole. The *Eucalyptus* essential oils constitute alternative natural products for the control of *Lutzomyia longipalpis* (their insecticidal effects on egg, larva and adult phases were assessed).

Table (3): Main difference in composition of the essential oils from *Eucalyptus camaldulensis and Eucalyptus citriodora* which may be contributed to the difference in their activity

Eucalyptus camaldulensis		*E. citridora*	
Name of compound	**Relative amount (%)**	**Name of compound**	**Relative amount (%)**
Sabinene	0.68	Geranial	3.74
2-Methyl-3-phenylpropanal	2.53	β- Myrcene	0.31
Linalool	2.70	Citronellal	0.32
4-trimethyl-Benzeneethanol	0.55	Bicyclogermacrene; 3,7,11,11-tetramethyl-Bicyclo(8.1.0) undeca-2, 6-diene	0.89
Cis –P-menth-2-en-1-o1	0.48	n-Eicosan-3, 12-diol	1.00
Caryophyllene oxide; 4,12,12-trimethyl-9-methylene-5-oxatricyclo[8.2.2.0.0]4,6)]dodecane	12.27	2-Methyl-3-phenylpropanal	1.60
Phellandral	0.35	Verbenone	1.86
Thymol	0.20	Calamenene	0.13
Nerolidol	0.33	Piperitone	2.22
Ledol	0.40	Borny/ acetate	0.57
m-Cumenol; 3-(1-methylethyl)-Phenol	0.31	σ- Cadinene	1.06
Bisabolene	0.89	Trans-2-Caren-4-ol	0.27
α- Campholen aldehyde	0.33	P-Cymen-8-ol	0.78
3- methyl-2(2-penthenyl) cyclopentanone	0.37	β-Farnesol	0.89
4-(1-methyl ethyliden) cyclohexanone	0.13	Isospathulenol	1.64
P-Ment-1-en-8-ol	0.62	γ-Eudesmol	1.38
		β-Selinene	2.70

As shown in Table (3). There were main difference in composition of the essential oils from *Eucalyptus camaldulensis and Eucalyptus citriodora* which may be contributed to the difference in their activity. These results were in agreement with many researchers as Manzoor *et al.*, (2012) who tested laboratory bioassays on essential oil from *Eucalyptus citriodora* for its toxicity, repellency and fumigant activity against three economically important termite species. Eucalyptus oil proved to be toxic and repellent within 24 hours of exposure. So, present study investigated the potential of *E. citridora* oil from commonly used plants as an environmentally safe measure to control termites (have excellent termiticidal properties). Also, Adesina and Josephine (2012) using steam distillation method, reported that the volatile oil obtained from *Eucalyptus citriodora* possess high activities against a wide rang of insect species. Also, Shoraby *et al.*, 2012 tested the toxic effect of natural essential oils of *Eucalyptus globulus* on 1st nymphal instar of the *Heteracris littoralis*. The normal development of the grasshopper was exhibited. Results cleared that there was statistically variable numbers of increased the nymphal periods, life cycle, adult longevity and life span comparing with the control test. High reduction in the deposited eggs and egg fertility and marked malformation were observed. Histological changes on the alimentary canal and fat bodies of the remaining nymphs after treatment were detected. The results suggest that the natural plant essential oils of *Eucalyptus* may be used in IPM control program against *Heteracris littoralis*.

Toxicity assays

The insecticidal activity of different doses of volatile oils (V.O.) of *Eucalyptus citridora* and *E. camaldulensis* against the fourth larval instar of *Spodoptera littoralis* and *Sesamia critica* are shown in Tables (4, 5, 6 and 7).

It is well known that volatile oils (V.O.) need a longer period of observation after treatment to notice its full action (have delayed effect). So, it is important of considering the toxicity limits of mortality after 72 hours and cumulative inhibition till pupal formation. Also, the obtained results from the previous tables revealed that the toxic and growth inhibitory effect of V.O. for *E. citridora* and *E. camaldulensis* were very apparent, the mortality obtained during larval stage was less pronounced than the other till pupal formation, which generally occur during the moult following the first exposure to the tested volatile oils (V.O.) and extended at the following moult. This mortality in larval stages after topical application increased with increasing the doses.

The mortality in larval stage was clearly due to the moult disturbing effect of these extract, so the affected larvae failed to free themselves from the old skin and ultimately died. This result was in agreement with Bede *et. al.*, 1999 who reported that sesquiterpenoids are structurally related to the insect Juvenile hormones (JH) that regulate developmental processes such as metamorphosis and reproduction. This could explain the growth inhibition effect of the volatile oils (V.O.) observed in this study. These

compounds could interfere with the insect physiological functions (Rosenthal and Berenbaum, 1991) or it could act as antagonist by inhibition of JH biosynthesis (Bowers *et al.*, 1976).

The obtained results showed that the essential oil of *E. citridora* was more effective than that of *E. camaldulensis*. On both insects, there was a significant difference ($P< 0.01$) between mortalities of all doses and the control of V.O. of the two plants. The control treatment had no effect on the fourth-instars larvae of both insects.

Regarding, these results it appears that treatment 4th instar larvae of *S. littoralis* with volatile oils (V.O.) possessed high accumulativetality in pupal and adult stages which may be reflect its juvenilizing effects. This means that volatile oils (V.O.) need a longer period of time to express its full action as I nsect growth regulator. The larvicidal bioassay results as LD_{50} values calculated after 72 hours of exposure, indicated that *E. citridora* volatile oil exhibited highest activity against *S. littoralis* and *S. cretica* with LD_{50} values of 15.6 and 20.6 µg/L, respectively. *E. camaldulensis* volatile oil treatments had intermediate effect with LD_{50} values of 17.2 and 22.0 µg/L for the two tested insects, respectively (Table 4 and 5).

The results of toxicity calculated after cumulative inhibition till pupal formation shows that the two tested essential oils were very toxic to larvae of both insects *S. littoralis* and *S. critica*. LD_{50} volatile oils of *E. citridora* were 9.7 and 11.2 µg/L, while in case of *E. camaldulensis* LD_{50} values were 10.0 and 12.9 µg/L (Tables 6 and 7). These results agree with Stampini (2009) who studied the larvicidal activity of *E. globulus* against *Plodia interpunctella* larvae (Tunc *et al.*, 2000; and Singh *et al.*, 2006).

Latent effect:

We evaluated the effect of these volatile oils on 4th instar larvae to ascribe its effect on larval, pupal and adult stages. Results shown in (Tables 8 and 9) demonstrated that the treatment of the fourth larvae instar of *Spodoptera littoralis* with two doses; 5 and 10µg/Larvae of volatile oils (V.O) of *E. citriodora* and *E. camaldulensis* plants exhibited significant differences ($P< 0.01$).

Larval duration:

All treatments caused significant decrease in the larval duration with averages of 8.67±0.33 and 7.33±0.33 days for 5 and 10µg of V.O of *E. citriodora* and 9.67±0.33 and 8.33±0.87 days for V.O of *E. camaldulensis* as compared to 11.0±0.0 day of control (Tables 8 and 9).

Table (4): The toxic activity of the two tested volatile oils applied topicaly on 4th larval instar of *Spodoptera littoralis* after 72 hours.

Plant extract	LD50 µg/L	Confidence limits		Regression equation is Y=	Slope±SE	Probability	X^2
		Lower	Upper				
Volatile oils							
Eucalyptus citridora	15.6	13.2	18.6	-1.9+1.6X	1.6±2.4	0.36	5.4
Eucalyptus camaldulensis	17.2	14.5	20.5	-1.9+1.6X	1.6±2.3	0.32	5.9

Table (5): The toxic activity of the two tested volatile oils applied topicaly on 4th larval instar of *Sesamia cretica* after 72 hours.

Plant extract	LD50 µg/L	Confidence limits		Regression equation is Y=	Slope±SE	Probability	X^2
		Lower	Upper				
Volatile oils							
Eucalyptus citridora	20.6	16.9	24.9	-1.8+1.4X	1.4±2.1	0.53	2.9
Eucalyptus camaldulensis	22.00	17.9	26.9	-1.8+1.3X	1.3±2.1	0.59	2.4

Table (6): The toxic activity of the two volatile oils applied topicaly on 4th instar larval of *spodoptera littoralis* after cumulative inhibition till pupal formation.

Plant extract	LD50 µg/L	Confidence limits		Regression equation is Y=	Slope±SE	Probability	X^2
		Lower	Upper				
Volatile oils							
Eucalyptus citridora	9.7	7.5	12.5	-1.2+1.3X	1.3±2.2	0.36	6.6
Eucalyptus camaldulensis	10.0	7.9	12.7	-1.3+1.3X	1.3±2.2	0.50	3.3

Table (7): The toxic activity of the two tested volatile oils applied topicaly on 4th larval instar of *Sesamia cretica* after cumulative inhibition till pupal formation.

Plant extract	LD50 µg/L	Confidence limits		Regression equation is Y=	Slope±SE	Probability	X^2
		Lower	Upper				
Volatile oils							
Eucalyptus citridora	11.2	8.6	14.6	-1.2+1.1X	1.1±2.1	0.83	1.2
Eucalyptus camaldulensis	12.9	10.2	16.4	-1.4+1.2	1.2±2.1	0.81	1.2

Pupal duration:

All treatments significantly increased the pupal duration: 9.0±0.58 and 11.0±0.58 days (V.O.) for *E.citridora* as well as 8.33±0.33 and 9.0±0.00 days (V.O.) for *E. camaldulensis*, as compared to 8.0±0.0 day of control (Tables 8 and 9)

Pupation percentages:

Data in the same tables indicated that the larval treatment of 4th larval instar of *S. littoralis* showed that the previous treatments significant ($P<0.01$) highly reduced the pupation percentages in *E. citridora*, as they were 24.3 ± 0.0 and 21.7 ±0.2 % (V.O.). Other values recorded for *E. camaldulensis* were 30.3 ± 0.3 and 26.7 ± 0.4 % (V.O.) as compared to 90.0 ± 0.0 % of control one.

Pupal weight:

All treatments significantly shortened the pupal weight and recorded 0.190±0.01 and 0.111±0.00 g (V.O.), for *E. citriodora* as well as 0.219±0.0 and 0.144±0.01 g (V.O.), for *E. camaldulensis* as compared to 0.315±0.0, of control one. The volatile oils obtained from *E. citridora* and *E. camaldulensis* were evaluated for their effect on reproductive capability of *S. littoralis*, which previously treated in 4th larval instar with different doses (Tables 8 and 9). It is obvious that treatment with these volatile oils resulted in a decrease in the percentage pupation which was more pronounced in V.O of *E. citridora* than that of *E. camaldulensis*.

These findings are in agreement with those obtained by Shaurub *et al.*, (1998) and Shonoda *et al.*, (2000) who reported a significant effect of Volatile oil of *E. citridora* on the present pupation of *S. littoralis*.

The results also indicated that all treatments succeeded to suppress the pupal weight compared to control. This effect may be explained at least in part to the reduced physiological age of the larvae brought about by reduced food intake and weight gain. Breller and Schmidt (1995) observed the reduction in pupal weight of *Spodoptera frugiperda* and *S. littoralis* after treatment of Neem extracts.

Table (8): Averages (±S.E.) of some biological aspect of *Spodoptera littoralis* 4th larval instar treated with the volatile oil of *Eucalyptus citriodora*.

Developmental stages / Doses	Volatile oil		Control
	5µg/larvae	10µg/L	Mean±SE
Larval duration (day)	8.67±0.33 [b]	7.33±0.33 [a]	11.0±0.0
Pupal duration (day)	9.0±0.58 [ab]	11.0±0.58 [ab]	8.0±0.0
Pupation %	24.3±0.0 [a]	21.7±0.2 [a]	90±0.0
Pupal weight (gram)	0.190±0.01 [ab]	0.111±0.00 [a]	0.315±0.0
Adult emergency %	21.7±0.2 [a]	17.0±0.2 [a]	89.7±1.2
Pre-oviposition period (day)	1.67±0.33 [b]	1.0±0.58 [b]	2.0±0.0
Oviposition period (day)	1.00±1.16 [a]	1.0±0.0 [a]	3.0±1.16
Post-oviposition period (day)	1.0±0.0 [a]	0.67±0.33 [a]	2.67±0.88
Female fecundity egg/female	329.33±15.5 [a]	255.67± 24.9 [a]	1783.33±12.3
Mean no. of hatched egg/female	53.0±2.9 [a]	27±3.7 [a]	1680.33±48.5
Hatchability of eggs %	16.1±0.19 [a]	10.6±3.04 [a]	94.21±0.25

Table (9): Averages (±S.E.) of some biological aspect of *Spodoptera littoralis* 4th larval instar treated with the volatile oil of *Eucalyptus camalduleasis*.

Developmental stages / Doses	Volatile oil		Control
	5µg/larvae	10µg/L	Mean±SE
Larval duration (day)	9.67±0.33 [b]	8.33±0.67 [a]	11.0±0.0
Pupal duration (day)	8.33±0.33 [ab]	8.0±0.00	8.0±0.0
Pupation %	30.3±0.3 [a]	26.7±0.4 [a]	90±0.0
Pupal weight (gram)	0.219±0.0 [b]	0.144±0.01 [a]	0.315±0.0
Adult emergency %	23.3±0.4 [a]	16.7±0.4 [a]	89.7±1.2
Pre-oviposition period (day)	2.0±0.0 [ab]	0.67±0.67 [a]	2.0±0.0
Oviposition period (day)	1.0±0.58 [a]	0.0±0.58 [a]	3.0±1.16
Post-oviposition period (day)	1.33±0.67 [b]	0.00±0.00 [a]	2.67±0.88
Female fecundity egg/female	286.67±27.7 [a]	146.0±17.9 [a]	1783.33±12.3
Mean no. of hatched egg/female	57.33±6.5 [a]	36±0.00 [a]	1680.33±48.5
Hatchability of eggs %	20.0±8.23	14.63±0.0	94.21±0.25