Biological activities of natural terpenes on Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.)

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CONTENTS

SUMMARY

The increasing serious problems of resistance and residue to synthetic pesticides and contamination of the biosphere associated with large-scale use of broad spectrum synthetic pesticides have led to the need for effective biodegradable pesticides with greater selectivity. This awareness has created a worldwide interest in the development of alternative strategies, including the discovery of newer insecticides. However, newer insecticides will have to meet entirely different standards. They must be pest specific, nonphytotoxic, nontoxic to mammals, ecofriendly, less prone to pesticide resistance, relatively less expensive, and locally available. This has led to re-examination of the century-old practices of protecting crops using plant-derivatives, which have been known to resist insect attack. Plant derived materials are more readily biodegradable, less likely to contaminate the environment and may be less toxic to mammals. Therefore, today, researchers are seeking new classes of naturally occurring insecticides that might be compatible with newer pest control approaches. In the present study, the insecticidal activity seven monoterpenes, namely (-)-carvone, 1,8-cineole, cuminaldehyde, (-)-citronellal, *p*-cymene, α-pinene, and α-terpinene. Two phenylpropenes *trans*-cinnamaldehyde and eugenol, and two sesquiterpenes farnesol and nerolidol were evaluated against the second and fourth instar larvae of *Spodoptera littoralis*. The effects of these compounds on feeding, growth and development of insect were also examined. In addition, the inhibitory effects of tested compounds on four key enzymes, acetyl cholinesterase (AChE), adenosine triphosphatases (ATPases), total proteases and α-amylase isolated from *S. littoralis* larvae were studied to understand the possible modes of action of the tested compounds.

1. Fumigant toxicity of tested monoterpenes, phenylpropenes and sesquitepenes

The results of fumigant toxicity experiments on the second instar larvae of *S. littoralis* showed that, 1,8-cineole, *P*-cymene and α-terpinene were the most potent compounds. The LC_{50} values of these compounds were 2.32, 7.35 and 9.71 µl/L, respectively. Similarly, two monoterpenes, (−)-carvone and α-pinene, displayed pronounced toxicity, while compounds, *trans*-cinnamaldehyde, (-)-citronellal, cuminaldehyde, eugenol, farnesol and nerolidol showed weak or no toxicity where their LC_{50} values were higher than 100 µl/L. When examined against the fourth instar larvae of *S. littoralis*, 1,8- cineole and α-pinene also revealed the highest toxicity. The LC_{50} values of 1,8-cineol and α-pinene were 3.13 and 14.66 µl/L, respectively. Moreover, (−)-carvone, *p*-cymene, and α-terpinene had relatively high toxic effect against this instar. In general, monoterpenes were more toxic than phenylpropenes and sesquiterpenes against the 2^{nd} and 4^{th} instar larvae of *S. littoralis*. Also, the 2^{nd} larval instar was more susceptible than 4^{th} larval instar.

2. Contact toxicity of tested monoterpenes, phenylpropenes and sesquitepenes

The monoterpenes, phenylpropenes and sesquiterpenes showed different levels of toxicity when tested on the 4^{th} instar larvae of *S. littoralis* by using topical application assay. The results indicated that, (−)-carvone, cuminaldehyde, eugenol, *p*-cymene and nerolidol had the highest insecticidal activity against the larvae. Their

LD_{50} values were 0.15, 0.27, 0.32, 0.36 and 0.41 mg/larva, respectively. In addition, the other tested compounds displayed moderate insecticidal activity as their LD_{50} values ranged between 0.58 and 0.95 mg/larva, while *trans*-cinnamaldehyde was the least effective compound with LD_{50} value higher than 1.0 mg/larva.

3. Residual toxicity of monoterpenes, phenylpropenes and sesquitepenes

The toxicity of tested monoterpenes, phenylpropenes and sesquiterpenes were evaluated at concentrations (1000, 2000, and 4000 mg/L) on the 2nd instar larvae of *S. littoralis* by using residual film method. The results were recorded as mortality percentages after 2, 3, 4 and 5 days of treatment. With few exceptions, the toxicity of tested compounds improved with increasing the time of exposure and concentration. After 2 days, α-terpinene revealed the highest mortality percentage at concentrations of 1000 and 2000 mg/L, while *p*-cymene displayed the highest mortality percentage at concentration of 4000 mg/L. After 3 days of treatment, (-)-citronellal revealed the highest insecticidal activity at concentrations 1000 and 2000 mg/L, while *p*-cymene displayed the most potent toxic effect at concentration 4000 mg/L. (-)-Citronellal, *trans*-cinnamaldehyde and *p*-cymene caused the highest mortality at the three tested concentrations after 4 and 5 days of treatment.

The results of the insecticidal activity of monoterpenes, phenylpropenes and sesquiterpenes on the 4th instar larvae of *S. littolaris* indicated that, (−)-carvone caused the highest mortality at 1000 mg/L and α-terpinene had the highest insecticidal activity at 2000 and 4000 mg/L after 2, 3, 4 and 5 days of treatment.

4. Growth inhibitory effect of monoterpenes, phenylpropenes and sesquitepenes

The tested monoterpenes, phenylpropenes and sesquiterpenes showed remarkable growth inhibition of *S. littoralis* 2nd instar larvae after 5 days of feeding in leaves treated with three tested concentrations (1000, 2000 and 4000 mg/L). The tested compounds exhibited growth inhibition ≥ 80%. 1,8-Cineole showed the highest larval growth inhibition at the three tested concentrations with 92.6, 93.6 and 96.9 % growth inhibition. In contrary, *trans*-cinnamaldehyde, α-pinene and nerolidol revealed the lowest inhibitory effect at concentrations of 1000, 2000 and 4000 mg/L, respectively. When examined on *S. littoralis* fourth instar larvae, all of the tested compounds strongly inhibited the larval growth after 5 days of treatment. α-Pinene (growth inhibition = 93.2%) was the most potent growth inhibitor at concentrations of 1000 and 2000 mg/L, while cuminaldehyde was the least potent growth inhibitor. At 4000 mg/L, 1,8-cineole (growth inhibition = 95.2%) caused the greatest reduction of growth inhibition. Similarly, the other tested compounds exhibited potent growth inhibition at this concentration.

5. Antifeedant activity of monoterpenes, phenylpropenes and sesquitepenes

The tested compounds showed a significant deterrence of food consumption by *S. littoralis* larvae fed on the semi-artificial diet treated with 500, 1000 and 2000 mg/Kg. The results showed that, the antifeeant activity of tested compounds improved significantly with increasing the treatment time. *trans*-Cinnamaldehyde revealed the strongest antifeedant activity at the three tested concentrations after 3 days of treatment with antifeeant indices of 33.3, 44.4 and 44.4 % at 500, 1000 and 2000, respectively. After 6 days, α-terpinene, *trans*-cinnamaldehyde, eugenol, and (−)-

carvone caused the highest feed deterrence at 500 mg/Kg. In addition, 1,8-cineole revealed the highest antifeedant activity at 1000 and 2000 mg/Kg. Furthermore, *trans*-cinnamaldehyde caused the highest antifeedant activity at 500 and 2000 mg/Kg, while citronellal was the most potent at 1000 mg/Kg after 9 days. The tested monoterpenes, phenylpropenes and sesquiterpenes were more potent antifeedant than pyriproxifen.

6. Growth inhibitory effect of monoterpenes, phenylpropenes and sesquitepenes

The tested compounds showed promising growth inhibition of the 2nd instar larvae of *S. littoralis* at 500, 1000 and 2000 mg/Kg after for 3, 6 and 9 days. After 3 days, the larval growth inhibition ranged between 45.4 and 100% at 500 mg/Kg. Cuminaldehyde and 1,8-cineole revealed the strongest larval growth inhibition at 500 mg/Kg. These two compounds caused higher growth inhibition than pyriproxyfen at this concentration. 1,8-Cineole, eugenol and cuminaldehyde were the most potent growth inhibitors at 1000 mg/Kg, while 1,8-cineole and cuminaldehyde had the highest growth inhibition at 2000 mg/Kg. 1,8-Cineole revealed the highest growth inhibition at the three tested concentrations after 6 days. Similarly, 1,8-cineole was the most potent growth inhibitor among the compounds at the three tested concentrations after 9 days of treatment.

7. Insecticidal activity of monoterpenes, phenylpropenes and sesquitepenes

The tested monoterpenes, phenylpropenes and sesquiterpenes showed various levels of toxicity on the 2nd instar larvae of *S. littoralis* after feeding on treated semi-artificial diet for 3, 6 and 9 days. It was obvious that, the mortality percentages improved by increasing the concentrations and exposure time. The results showed that, cuminaldehyde, 1,8-cineole and (−)-carvone were the most potent compounds at concentrations of 500,1000 and 2000 mg/Kg, respectively, after the three times of exposure These three compounds were more toxic than pyriproxifen.

8. Effect of monoterpenes, phenylpropenes and sesquitepenes on biological parameters

The effects tested compounds on life stages of *S. littoralis*, such as larval and pupal duration, pupation and adult emergence percentages, oviposition and egg hatchability have been studied. The tested compounds slightly increased larval duration at all concentrations except *trans*-cinnamaldehyde which increased larval duration to be 20.4 days compared with 13.5 days in control. Moreover, the tested compounds exhibit relatively moderate increasing in pupal duration at all concentrations compared with control. Pyriproxifen prolonged pupal duration more than the tested compounds. In addition, the tested compounds decreased the body weights of pupae females. *trans*-Cinnamaldehyde displayed highest reduction on the weight at concentration of 500 mg/Kg. Farnesol caused the greatest reduction of pupae female weights at concentrations of 1000 and 2000 mg/Kg. Similarly, monoterpenes, phenylpropenes and sesquiterpenes caused reduction in pupae male weights at the tested concentrations compared with control. Farnesol and 1,8-cineole were the most effective compounds in weight reduction at 500 mg/Kg. Cuminaldehyde, nerolidol and (-)-citronellal produced the height weight reduction at 1000 mg/Kg, while *trans*-cinnamaldehyde, farnesol and α-terpinene displayed the highest reduction in pupae male weights at 2000 mg/Kg.

On the other hand, the tested compounds caused a significant reduction of pupation percentages compared with control. *trans*-Cinnamaldehyde had the highest reduction effect on pupation at 500 mg/Kg. Cuminaldehyde and 1,8-cineole were the most effective compounds in reduction of pupation at 1000 mg/Kg. 1,8-Cineole, (-)-citronellal, *cuminaldehyde* and eugenol had the highest reduction in pupation at 2000 mg/Kg. In addition, the tested compounds caused significant reduction of adult emergence. 1,8-Cineole displayed the strongest reduction on adult emergence at the three tested concentrations. Similarly, the tested compounds produced significant reduction in number of eggs laid by female. *trans*-Cinnamaldehyde showed the highest reduction effect at 500 mg/Kg, while (-)-citronellal was the most effective compound in oviposition reduction at 1000 and 2000 mg/Kg. Finally, the tested compounds significantly reduced egg hatching at the three concentrations, particularly at the higher concentrations of 1000 and 2000 mg/Kg**.** 1,8-Cineole caused the highest reduction in egg hatching at 1000 mg/Kg, while trans-cinnamaldehyde caused the highest reduction in egg hatching at 500 and 2000 mg/Kg.

9. Inhibitory effect of monoterpenes and sesquitepenes on acetyl cholinesterase

The results of *in vitro* inhibitory effect of (−)-carvone, cuminaldehyde and nerolidol on acetyl cholinesterase (AChE) of *S. littoralis* larvae confirmed that the tested compounds exhibited remarkable inhibitory effect on the enzyme activity. Cuminaldehyde (IC_{50} = 1.04 mM) was the most effective inhibitor of AChE activity, followed by nerolidol (IC_{50} = 6.28 mM) and (−)-carvone (IC_{50} = 6.34 mM).

10. Inhibitory effect of monoterpenes and sesquitepenes on adenosine triphosphatases

(−)-Carvone, cuminaldehyde and nerolidol gave a remarkable inhibitory effect on adenosine triphosphatases (ATPases) isolated form *S. littoralis* larvae. Nerolidol (IC_{50} = of 0.02 mM) caused the highest inhibitory effect, followed by cuminaldehyde (IC_{50} = 0.05 mM) and (−)-carvone (IC_{50} = 0.22 mM).

11. Inhibitory effect of phenylpropenes and sesquitepenes on α-amylase

The phenylpropenes (*trans*-cinnamaldehyde and eugenol) and a sesquiterpene (farnesol) had potent inhibitory effect on the activity of α-amylase isolated from *S. littoralis* larvae. *trans*-Cinnamaldehyde showed higher inhibitory activity than farnesol and eugenol.

12. Inhibitory effect of phenylpropenes and sesquitepenes on total proteases

The results of *in vitro* inhibition of *trans*-cinnamaldehyde, eugenol and farnesol on total proteases isolated from *S. littoralis* larvae indicated that, these compounds had inhibitory effect on the enzyme activity. Eugenol (IC_{50} = 0.24 mM) caused the greatest inhibition of the total proteases activity, followed by *trans*-cinnamaldehyde (IC_{50} = 1.12 mM) and farnesol (IC_{50} = 2.33 mM).