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Anticholinesterase Potential of Monoterpenoids on the Whitefly *Bemisia tabaci* and Their Kinetic Studies

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ABSTRACT

B-biotype *Bemisia tabaci* is a severe insect pest worldwide in many ornamental, agricultural, and horticultural crops. Control of this insect is obstructed by resistance to many AcetylCholinEsterase (AChE)-inhibiting insecticides, such as organophosphates and carbamates. In the present work, we evaluated the acetylcholinesterase inhibitory activity of six monoterpenoids namely α-pinene, terpineol, linalool, ß-myrcene, nerol and geraniol *in vitro* and *in vivo*. Inhibition of AChE of *B. tabaci* was measured by colorimetric method. The results showed that all of the monoterpenoids produced AChE inhibitory activity, with IC_{50} values ranging from 0.96 to 26.85 mM. Alpha-pinene showed the most potent inhibitory activity (IC_{50}= 0.96 mM). Kinetic analysis showed reversible non-competitive type inhibition, revealing that these components might bind both the enzyme alone and the enzyme-substrate. Results demonstrate the AChE inhibitory activity as mode of action of these monoterpenoids at relatively high concentrations. Thus, this could be useful for investigation of new ecofriendly natural insecticidal compounds.

Keywords: Acetylcholinesterase, *Bemisia tabaci*, , IPM, Monoterpenoids, Non-competitive inhibition.

INTRODUCTION

The whitefly *Bemisia tabaci* Genn. (Hemiptera: Aleyrodidae), is one of the most destructive insect pests of agriculture and horticulture in Tunisia and worldwide (Chermiti *et al*., 1997; Oliveira *et al*., 2001). *B. tabaci* has an extensive host-plant range, transmits several plant viruses and is a highly invasive species (Gonzalez-Zamora and Moreno, 2011; Parrella *et al*., 2012). Chemical control has been widely used for the management of whiteflies (Palumbo *et al*., 2001); however, negative impacts resulted such as extermination of natural enemies and rapid development of resistance by insects (Wilson *et al*., 2007, Roditakis *et al*., 2009). This resistance to organophosphorus and carbamate insecticides in *B. tabaci* is due to insensitivity of the target enzyme AcetylCholinEsterase (AChE) (Byrne and Devonshire, 1997).

On the basis of these problems, there is an urgent demand to reduce the use of the conventional pesticides and develop alternatives with fewer harmful effects on the environment and lower toxicity to non-target organisms.

Among natural products used for pest control, one of the most successful botanical

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pesticide groups are monoterpenoids (Benner, 1993), which are mostly found in plant essential oils (Isman, 2000). These compounds were identified to be good insecticides, acaricides, and insect repellents (Paluch *et al*., 2009; Isman et *al*., 2001). Although, in a recent research, monoterpenoids have been widely investigated owing their capacity to inhibit acetylcholinesterase, the key enzyme in the breakdown of acetylcholine, and they are considered as a promising strategy for the treatment of neurological disorders such as Alzheimer's Disease (AD) (Orhan *et al*., 2004). For example, many studies concerning the AChE inhibitory activity of commercial essential oils performed by Dohi *et al*. (2009) demonstrated for the first time that eugenol from *Ocimum sanctum* L. essential oils was a potent AChE inhibitor. Amongst various components, α-pinene, linalool and terpineol frequently found in mint and lavender oils, ß-myrcene that often occurs in bay, cannabis and thyme essential oils, nerol originally isolated from neroli oils and geraniol which is the primary part of palmarosa and citronella oils were recommended by several researches as alternatives to chemical insecticides for controlling a wild range of pests (Park *et al*., 2005; Alzogaray *et al*., 2013; Gallardo *et al*., 2015).

In the present work, we evaluated *in vitro* and *in vivo* the mode of action of six monoterpenoids (α-pinene, terpineol, linalool, ß-myrcene, nerol and geraniol) on insect acetylcholinesterase activity from the whitefly *Bemisia tabaci*.

MATERIALS AND METHODS

Chemicals

The six monoterpenoids (α-pinene, terpineol, linalool, ß-myrcene, nerol and geraniol) were purchased from Acros Organics BVBA/SPRL. Acetylthiocholine iodide and the Ellman's reagent 5,5′-DiThio-bis(2-NitroBenzoic) acid (DTNB) were purchased from Aldrich Co. (UK).

Insect

Unsexed *B. tabaci* adults were collected from pepper culture in experimental greenhouse in Chott-Mariem region (Sousse, Tunisia), which were identified as the B biotype (Saleh *et al*., 2012). The whitefly population was reared on tomato plants (*Solanum lycopersicum* L.) placed in a rearing room. The rearing conditions were: 26±2°C, 60±5% RH, and photoperiod of L: D 16 h: 8 h.

Assay of AChE Activity

In Vitro Inhibition of AChE

One and half grams of whole insects were homogenized in 7ml of phosphate buffer (0.1M, pH 7). The homogenate was centrifuged at 4°C for 90 minutes and the supernatant containing AChE was filtered through glass wool. The AChE extracted was pre-incubated with monoterpenoids (1–100 µM) at 37°C for 30 minutes and the inhibition of AChE was determined.

In Vivo Inhibition of AChE

Bemisia adults were exposed to 1, 10, 50 and 100 mM concentrations of monoterpenoids. Respective batches of solvent treated controls were also employed. After 45 minutes exposure insects were removed. Whole insects were homogenized and the AChE was extracted as described above for enzyme assay.

Inhibition of AcetylCholinEsterase (AChE) was assessed by the colorimetric method of Ellman *et al*. (1961). The effects of increasing concentrations of monoterpenoids (1m, 10, 50 and 100 mM) on AChE were tested and typical concentrations of substrates were used (1, 2, 5 and 10 mM). Thus AChE (0.1 mL) was mixed with substrate (ATChI) (0.2 mL), DTNB (0.2 mL) and phosphate buffer (2.4 mL). To this mixture, monoterpene test solutions (1 mL) dissolved in absolute ethanol were added. Control treatments were prepared by the addition of absolute ethanol

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(1 mL) in place of a monoterpene. Tests and control assays (without monoterpenoids) were corrected by blanks for non-enzymatic hydrolysis. Each assay was triplicated. Level of AChE activity was estimated by PharmaSpec uv-1700 Shimadzu Spectrophotometer set at 412 nm and measured at 25°C.

Data Analysis

Enzyme kinetic constants: Michaelis Menten constants (Km) and maximum Velocity (Vmax) were determined by Lineweaver Burk plots. Data are presented as means. They were analyzed through one-way analysis of variance using Statistical Package for Social Sciences (version 20.0; SPSS, Chicago, III). Probit analysis (Finney, 1971) was used to estimate IC_{50} values.

RESULTS AND DISCUSSION

Inhibition of AChE by Monoterpenoids

The AChE inhibitory effects of different concentrations 1, 10, 50 and 100 mM, of six monoterpenoids (α-pinene, linalool, ß-myrcene, terpineol, nerol, geraniol) were assessed using typical concentrations of substrates (1, 2, 5 and 10 mM).

As shown in Table 1, all the monoterpenoids were potent inhibitors of AChE. The inhibitory potential of this enzyme decreased in the following order: Alpha-pinene (Ki= 2.22 mM)> linalool (Ki= 2 mM)> ß-myrcene (Ki= 1.75 mM)> terpineol (Ki= 1.26 mM)> geraniol (Ki= 1.17 mM)> nerol (Ki= 1.02 mM).

Table 1. Acetylcholinesterase inhibitory effects of six monoterpenoids Inhibition constant (Ki).

Monoterpenoids	Ki (mM)
α-pinene	2.22
Linalool	2
ß-myrcene	1.75
Terpineol	1.26
Geraniol	1.17
Nerol	1.02

These results supported the hypothesis that insect AChE is a potential target for some monoterpenoids (Mukherjee *et al*., 2007). The capacity to inhibit AChE can be explained by monoterpenoids chemical structure. Although many authors showed that a bicyclic monoterpene hydrocarbon containing an allylic methyl group was a strong inhibitor of AChE activity, and they also reported the importance of the position of the double bond on the activity (Orhan *et al*., 2008).

Previously, a similar attempt has been made by López and Pascual-Villalobos (2010) to find another AChE inhibitor of plant origin. They investigated 8 monoterpenoids [(−)-linalool, camphor, ɣ-terpinene, geraniol, *S*-(+)-carvone, *E*-anethole, fenchone and estragole] and found many fold variations in AChE inhibitory activity; fenchone, S-carvone and linalool produced the highest inhibition. Moreover, Chaubey (2011) reported that essential oil components like cuminaldehyde, limonene, α-pinene and α-phellandrene of *Cuminum cyminum* and *Piper nigrum* might be responsible for AChE inhibitory activities of the rice weevil *Sitophilus oryzae*.

Our data show that among the pure compounds tested, *in vitro* and *in vivo* tests α-pinene showed the best acetylcholinesterase inhibitory activity. In Figure 1 the progress of *in vitro* inhibition is illustrated by different concentrations of α-pinene (1 mM= 57.07% inhibition at 15 minutes, 10 mM= 78.53% at 15 minutes, 50 mM= 83.92% at 15 minutes and 100 mM= 95.45% at 15 minutes) and using different concentrations of substrate ATCh (1, 2, 5 and 10 mM). These findings were in agreement with Kim *et al*. (2013) who found that α-pinene showed the highest AChE inhibition rate of *S. oryzae* (97.36%), followed by β-pinene (54.96%) and limonene (51.23%) at a concentration of 1 mg mL^{-1}. Besides, Miyazawa and Yamafuji

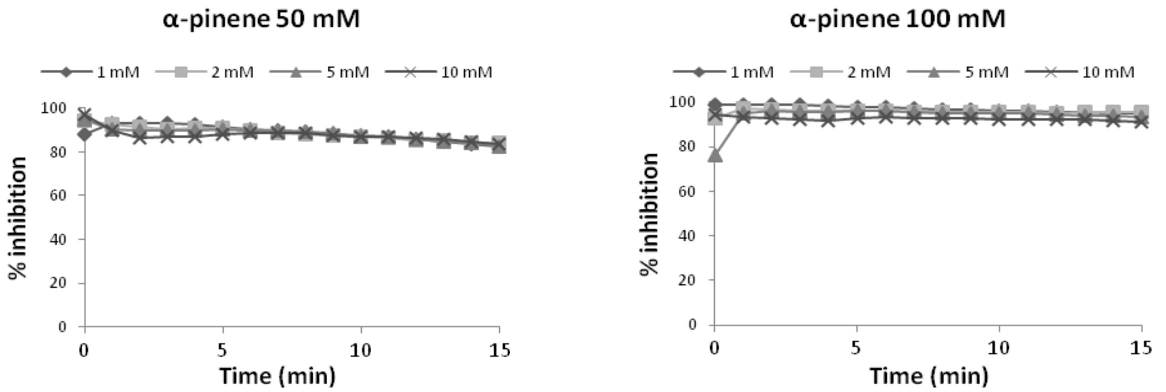

Figure 1. Progress of *in vitro* inhibition of AChE by α-pinene (1, 10, 50 and 100 mM) using substrate ATCh (1, 2, 5 and 10 mM).

(2005) worked on the anti-AChE activity of bicyclic monoterpenoids commonly encountered in *Melaleuca alternifolia* essential oils. Results pointed out that α-pinene was a potent inhibitor of AChE with 57.1 µg mL^{-1}, which was in accordance with our data.

On the basis of this study, we estimated that the inhibition produced by all the monoterpenoids tested was dose dependent. The percent of *in vitro* inhibition of AChE activity at the highest concentration tested (100 mM), was around 90% (between 87.53 and 97.08%) (Figure 2). Lower concentration (1 mM) of monoterpenoids was also found to be effective in AChE inhibition (between 70.77 and 84.73%) (Figure 3). The *in vivo* enzyme inhibition of AChE activity following the six monoterpenoids was dose dependent (Figure 4). α-pinene was the most effective inhibitor of *B. tabaci* AChE activity (32.73, 63.13, 80.93 and 90.54% at 1, 10, 50 and 100 mM respectively), followed by linalool (28.93, 49.7, 76.68 and 84.16% at 1, 10, 50 and 100 mM respectively). In contrast, nerol was the least effective inhibitor of *B. tabaci* AChE at the 4 concentrations (14.39, 51.39, 63.7 and 70.68% at 1, 10, 50 and 100 mM respectively).

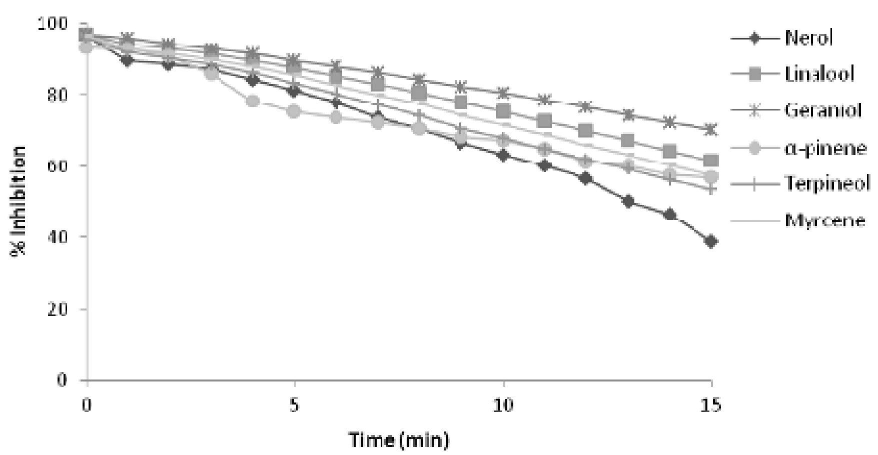

Figure 2. Progress of *in vitro* inhibition of AChE by six monoterpenoids (1 mM) using substrate ATCh (1 mM).

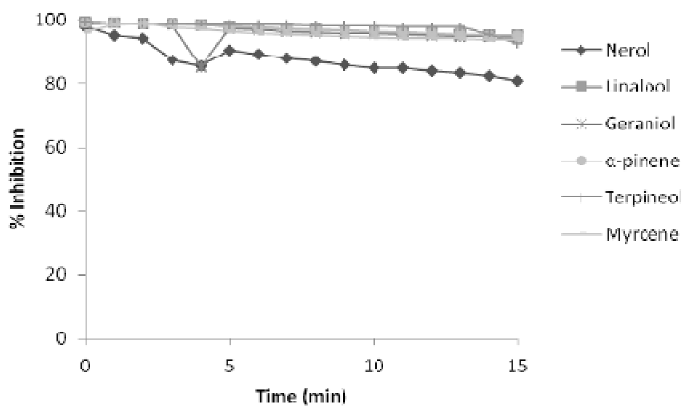

Figure 3. Progress of *in vitro* inhibition of AChE by six monoterpenoids (100 mM) using substrate ATCh (1 mM).

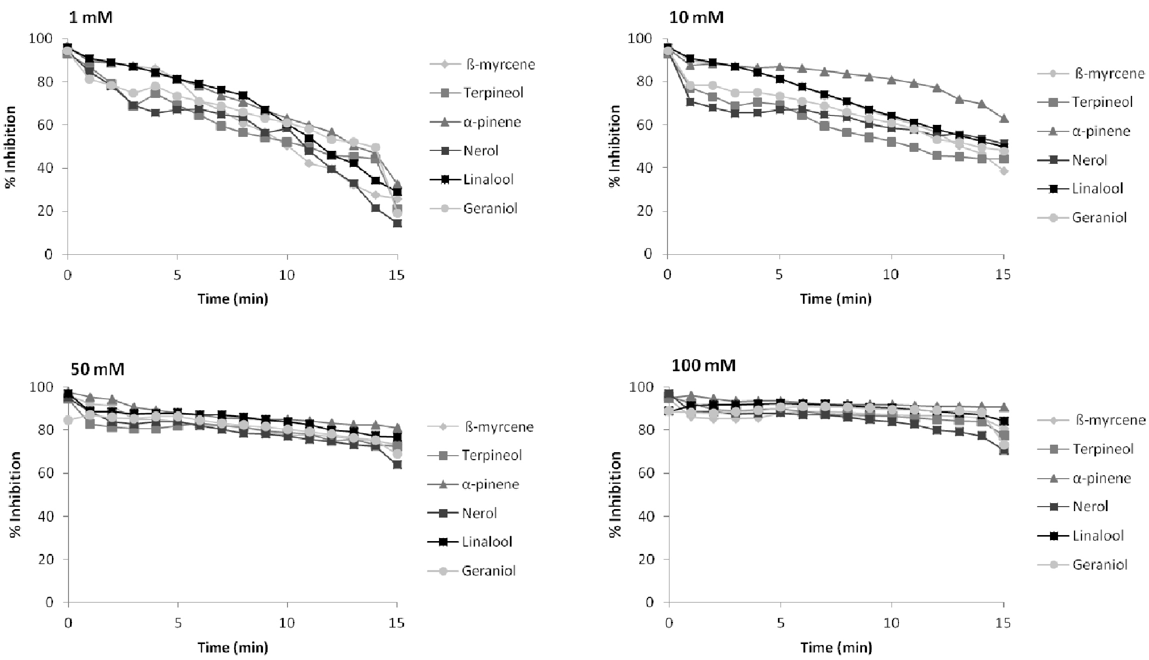

Figure 4. *In vivo* inhibition of *B. tabaci* adult acetylcholinesterase by six monoterpenoids.

Table 2. IC_{50} values (mM) obtained for the six monoterpenoids.

Monoterpenoids	*IC50*	*χ2*
α-Pinene	0.96	9.50
Linalool	8.11	19.63
ß-Myrcene	10.47	17.24
Terpineol	10.96	9.30
Geraniol	12.40	13.62
Nerol	26.85	11.87

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The IC_{50} values obtained from the AChE inhibition assay for the monoterpenoids are shown in Table 2. The strongest inhibition was displayed by α-pinene, followed by linalool and ß-myrcene with IC_{50} values ranging respectively between 0.96 and 26.85 mM. Thus, IC_{50} values confirm that these monoterpenoids have proved to be a potential as inhibitor of AChE at relatively high concentrations. These results indicate that the monoterpenoids tested are much weaker inhibitors (IC50 values of 1-26 mM) than the alkaloids physostigmine and galanthamine (IC50 values of 0.02–0.09 μM) (Jukic *et al*., 2007, Bhadra *et al*., 2011). In this respect, previous studies have shown that α-pinene is a potent AChE inhibitor with IC_{50} values ranging from 0.086 to 0.090 mg mL^{-1} (Miyazawa and Yamafuji, 2006) and (Savelev *et al*., 2003). Moreover, Kim *et al*. (2013) indicated that α-pinene exhibited strong AChE inhibition activity with IC_{50} value of 0.019 mg mL^{-1}.

Kinetic Analysis of the Acetylcholinesterase Activity

All the monoterpenoids investigated in this work were shown to behave as non-competitive inhibitors of AChE. The Lineweaver-Burk plots are shown in Figure 5. The inhibition revealed that the inhibitor decreased the activity of the enzyme and bound equally to the enzyme, whether or not it was already bound to the substrate and lead to acetylcholine accumulation, hyperstimulation of nicotinic and muscarinic receptors, and disrupted neurotransmission (Dvir *et al*., 2010). These findings indicated that these six monoterpenoids are all positive modulators of the insect AChE activity, and they could cause inhibitory effects on the insect nervous system. Previous studies have shown that fenchone, ɣ-terpinene, geraniol and linalool showed a reversible competitive inhibition of AChE activity of three stored-product insect pests, *S. oryzae*, *Rhyzopertha dominica* and *Cryptolestes pusillus*. Although, *S*-(+)-carvone, estragole and camphor produced a mixed inhibition for this enzyme (López and Pascual-Villalobos, 2010). Moreover, Perry *et al*. (2002) indicated that *in vitro* inhibition of AChE by *Salvia lavandulifolia* essential oil and its major monoterpenes, α-pinene, 1,8-cineole, and camphor were found to be the competitive reversible inhibitors of AChE, and it was suggested that the inhibitory activity of this essential oil was primarily due to its main terpenoids, which showed a major synergistic effect. Besides, linalool and citral were revealed to be the reversible competitive inhibitors of AChE (Ryan and Bryan, 1988). Recently, López and Pascual-Villalobos (2015a) pointed up that the *S. oryzae* susceptible and tolerant strains showed a competitive inhibition of AChE for linalool and estragole. Whereas, *C. pusillus* susceptible and tolerant populations presented a competitive inhibition for linalool and a non-competitive inhibition for carvone. Moreover, ɣ -terpinene and fenchone were found to behave as competitive inhibitors and carvone and camphor as non-competitive inhibitors on the inhibition of Electrophorus AChE (López *et al*., 2015b). In addition, Zarrad *et al*. (2015) evaluated the AChE inhibitory potency of *Citrus aurantium* essential oils on *B. tabaci*. Results showed that the oil and its major compound pure limonene exhibited a reversible non-competitive inhibition of this enzyme.

Several other studies have been carried out on the anticholinesterase activity of monoterpenes, especially oxygenated monoterpenes. Indeed, the two major constituents of Tea Tree oil, 1,8-cineole and terpinen-4-ol, were shown to inhibit acetylcholinesterase at IC_{50} values of 0.04 and 10.30 mM respectively (Mills *et al*., 2004). Besides, Abdelgaleil *et al*. (2009) indicated that 1,8-cineole was a potent inhibitor of AChE activity from *S. oryzae* and *T. castaneum*. Furthermore a straight relationship between the AChE inhibitory potency of essential oils and their high content in monoterpenoids was noted. Dohi *et al*. (2009) elucidated the

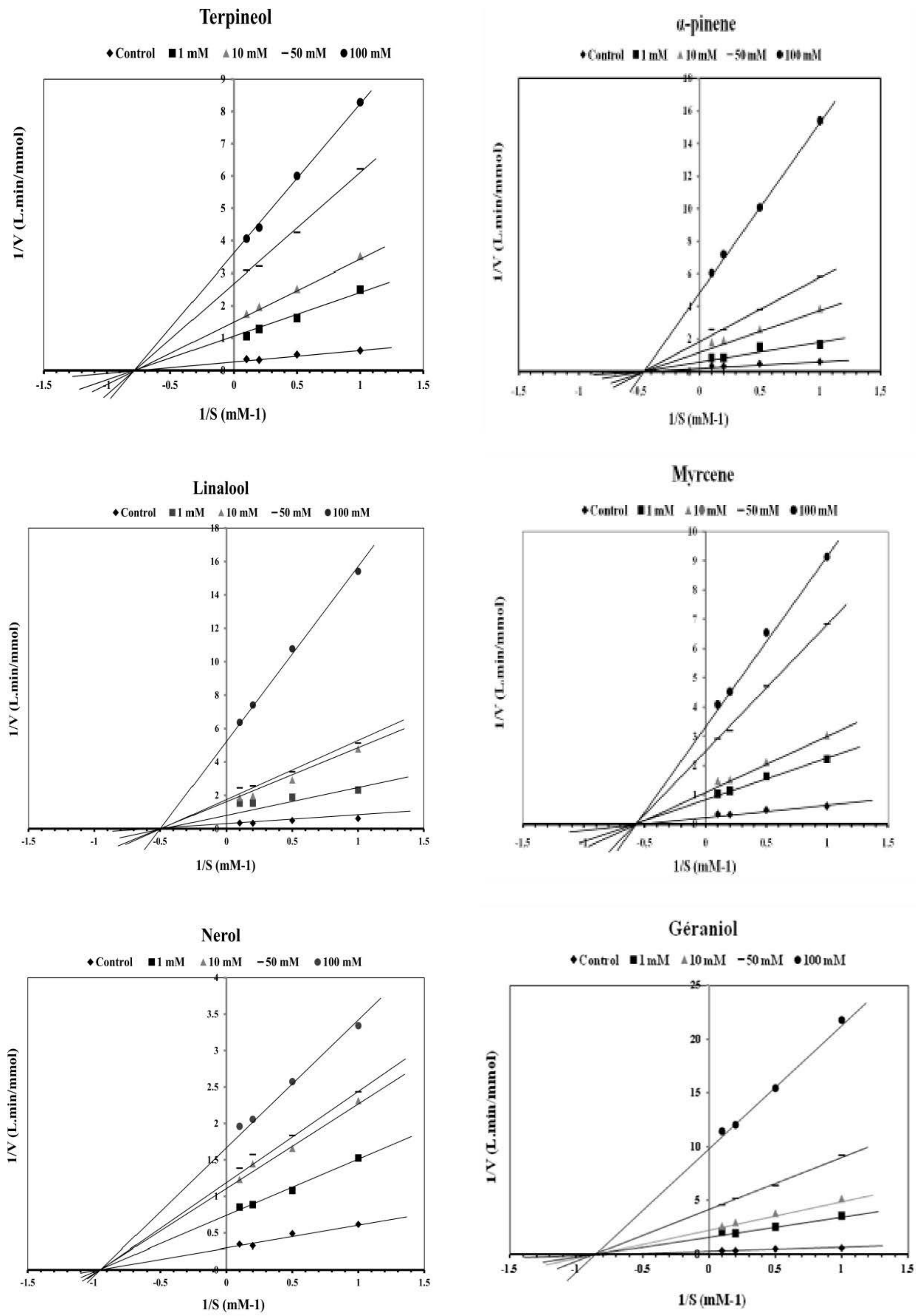

Figure 5. Lineweaver Burk plots of inhibition of AChE by six monoterpenoids (1, 2, 50, 100 mM and control).

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anticholinesterase activity of *O. sanctum* essential oils of Indian origin. Eugenol as main component of these oils showed the most potent inhibition with an IC_{50} value of 0.48 mg mL^{-1}. Additionally,1,8-cineole as main component of *Eucalyptus* oils could be potent inhibitor, producing an important inhibition of this enzyme with IC_{50} value of 6×10^{-3} M (Picollo *et al*., 2008) and it accounted for 25% of the observed inhibitory activity of the *L. officinalis* oils (Dohi *et al*., 2009).

Although, many of monoterpenoids showed important insecticidal potency on insect pests, their mechanisms of action have not been yet fully elucidated. In fact, the toxic action of these naturally occurring compounds could be seen through other modes of action such as GABA receptors (Priestley *et al*., 2003) and octopamine receptors (Kostyukovsky *et al*., 2002).

CONCLUSIONS

In conclusion, the present study reported first investigations on the mode of action of α-pinene, terpineol, linalool, ß-myrcene, nerol and geraniol on acetylcholinesterase activity from *B. tabaci*. These compounds, at relatively high concentrations, may act as weak acetylcholinesterase inhibitor and show potential to be a good alternative to conventional insecticides due to their relatively high toxicity to insect pests, low toxicity to non-target organisms, and biodegradability in the environment.

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REFERENCES

1. Abdelgaleil, S. A. M., Mohamed, M. I. E., Badawy, M. E. I and El-Arami, S. A. A. 2009. Fumigant and Contact Toxicities of Monoterpenes to *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) and Their Inhibitory Effects on Acetylcholinesterase Activity. *J. Chem. Ecol*., **35:** 518-525.
2. Alzogaray, R. A., Sfara, V., Moretti, A. N. and Zerba E. N. 2013. Behavioural and Toxicological Responses of *Blattella germanica* (Dictyoptera: Blattellidae) to Monoterpenes. *Eur. J. Entomol*., **2:** 247-252.
3. Benner, J. P. 1993. Pesticidal Compounds from Higher Plants. *Pestic. Sci*., **39:** 95-102.
4. Bhadra, S., Mukherjee, P. K., Kumar, N. S. and Bandyopadhyay, A. 2011. Anticholinesterase Activity of Standardized Extract of *Illicium verum* Hook. f. Fruits, *Fitoterapia*, **82:** 342–346.
5. Byrne, F. J. and Devonshire, A. L. 1997. Kinetics of Insensitive Acetylcholinesterases in Organophosphate- Resistant Tobacco Whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). *Pest Biochem. Physiol*., **58:** 119–124.
6. Chaubey, M. K. 2011. Fumigant Toxicity of Essential Oils against Rice Weevil *Sitophilus oryzae* L. (Coleoptera: Curculionidae). *J. Biol. Sci.* **11:** 411-416.
7. Chermiti, B., Braham, M., Cenis, J. L., Alonso, C. and Beitia, F. 1997. Sur la Présence en Tunisie des Biotypes "B" et non B" de *Bemisia tabaci* (Homoptera : Aleyrodidae) et de leurs Parasitoïdes Associés. *Bull. OILB/SROP*., **20:** 108-113.
8. Dohi, S., Terasaki, M. and Makino, M. 2009. Acetylcholinesterase Inhibitory Activity and Chemical Composition of Commercial Essential Oils. *J. Agric. Food Chem*., **57:** 4313-4318.
9. Dvir, H., Silman, I., Harel, M., Rosenberry, T. L. and Sussman, J. L. 2010. Acetylcholinesterase: From 3D Structure to Function. *Chem. Biol. Interact*., **187:** 10- 22.
10. Ellman, G. L., Courtney, K. D., Valentino, A. J. and Feathertone, R. M. 1961. A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity. *Biochem. Pharmacol*., **7:** 88–95.
11. Finney, D. L. 1971. *Probit Analysis*. 3rd Edition, Cambridge University Press, UK, 125 PP.
12. Gallardo, A., Picollo, M. I. and Mougabure-Cueto, G. 2015. Lethal Activity of Individual and Mixed Monoterpenoids of Geranium Essential Oil on Musa Domestica. *Parasitol. Res*., **114:** 229-1232.

13. Gonzalez-Zamora, J. E. and Moreno, R. 2011. Model Selection and Averaging in the Estimation of Population Parameters of *Bemisia tabaci* (Gennadius) from Stage Frequency Data in Sweet Pepper Plants. *J. Pest Sci*., **84:** 165–177.
14. Isman, M. B., Wan, A. J. and Passreiter, C. M. 2001. Insecticidal Activity of Essential Oils to the Tobacco Cutworm, *Spodoptera litura*. *Fitoterapia*, **72:** 65-8.
15. Isman, M. B. 2000. Plant Essential Oils for Pest and Disease Management. *Crop Prot*., **19:** 603-608.
16. Jukic, M., Politeo, O., Maksimovic, M., Milos, M. and Milos, M. 2007. *In Vitro* Acetylcholinesterase Inhibitory Properties of Thymol, Carvacrol and Their Derivatives Thymoquinone and Thymohydroquinone. *Phytother. Res*., **21:** 259–261.
17. Kim, S. W., Kang, J. and Park, I. K. 2013. Fumigant Toxicity of *Apiaceae* Essential Oils and Their Constituents against *Sitophilus oryzae* and Their Acetylcholinesterase Inhibitory Activity. *J. Asia Pac. Entomol*., **16:** 443-448.
18. Kostyukovsky, M., Rafaeli, A., Gileadi, C., Demchenko, N. and Shaaya, E. 2002. Activation of Octopaminergic Receptors by Essential Oil Constituents Isolated from Aromatic Plants: Possible Mode of Action against Insect Pests. *Pest Manag. Sci*., **58:** 1101–1106.
19. López, M. D. and Pascual-Villalobos, M. J. 2010. Mode of Inhibition of Acetylcholinesterase by Monoterpenoids and Implications for Pest Control. *Ind. Crop Prod*., **31:** 284–288.
20. López, M. D. and Pascual-Villalobos, M. J. 2015a. Are Monoterpenoids and Phenylpropanoids Efficient Inhibitors of Acetylcholinesterase from Stored Product Insect strains?. *Flav. Frag. J*., **30:** 108-112.
21. López, M. D., Campoy, F. J., Pascual-Villalobos, M. J., Muñoz-Delgado, E. and Vidal, C. J. 2015b. Acetylcholinesterase Activity of Electric Eel Is Increased or Decreased by Selected Monoterpenoids and Phenylpropanoids in a Concentration-Dependent Manner. *Chem. Biol. Interact*., **229:** 36-43.
22. Mills, C., Cleary, B. V., Walsh, J. J. and Gilmer, J. F. 2004. Inhibition of Acetylcholinesterase by Tea Tree Oil. *J. Pharm. Pharmacol*., **56:** 375–379.
23. Miyazawa, M., Watanabe, H., Umemoto, K. and Kameoka, H. 1998. Inhibition of Acetylcholinesterase Activity by Essential Oils of *Mentha* Species. *J. Agric. Food Chem*., **46:** 3431-3434.
24. Miyazawa, M. and Yamafuji, C. 2005. Inhibition of Acetylcholinesterase Activity by Tea Tree Oil and Constituent Terpenoids. *Flavour Frag. J*., **20:** 617-620.
25. Miyazawa, M. and Yamafuji, C. 2006. Inhibition of Acetylcholinesterase Activity by Tea Tree Oil and Constituent Terpenoids. *Flavour Frag. J*., **21:** 198–201.
26. Mukherjee, P. K., Kumar V., Mal M. and Houghton P. J. 2007. Acetylcholinesterase Inhibitors from Plants. *Phytomed*., **14:** 289–300.
27. Oliveira, M. R. V., Henneberry, T. J. and Anderson, P. 2001. History, Current Status, and Collaborative Research Projects for *Bemisia tabaci*. *Crop Prot*., **20:** 709–723.
28. Orhan I., Aslan, S., Kartal, M., Şener, B. and Hüsnü Can Başer, K. 2008. Inhibitory Effect of Turkish *Rosmarinus officinalis* L. on Acetylcholinesterase and Butyrylcholinesterase Enzymes. *Food Chem*., **108:** 663-668.
29. Orhan, I., Sener, B., Choudhary, M. I. and Khalid, A. 2004. Acetylcholinesterase and Butyrylcholinesterase Inhibitory Activity of Some Turkish Medicinal Plants. *J. Ethnopharmacol*., **91:** 57–60.
30. Paluch, G., Grodnitzky, J., Bartholomay, L. and Coats, J. 2009. Quantitative Structure-Activity Relationship of Botanical Sesquiterpenes: Spatial and Contact Repellency to the Yellow Fever Mosquito, *Aedes aegypti*. *J. Agric. Food Chem*., **57:** 7618-25.
31. Palumbo, J. C., Horowitz, A. R. and Prabhaker, N. 2001. Insecticidal Control and Resistance Management for *Bemisia tabaci*. *Crop Prot*., **20:** 739–765.
32. Park, B. S., Choi, W. S., Kim, K. H. and Lee, S. E. 2005. Monoterpenes from Thyme (*Thymus vulgaris*) as Potential Mosquito Repellents. *J. Am. Mosq Control Assoc*., **21:** 80-3.
33. Parrella, G., Scassillo, L. and Giorgini, M. 2012. Evidence for a New Genetic Variant in the *Bemisia tabaci* Species Complex and the Prevalence of the Biotype Q in Southern Italy. *J. Pest Sci*., **85:** 227–238.
34. Perry, N. S. L., Houghton, P. J., Jenner, K. A. and Perry, E. K. 2002. *Salvia lavandulaefolia*

Essential Oil Inhibits Cholinesterase *In Vivo*. *Phytomed.*, **9:** 48–51.

35. Picollo, M. I., Toloza, A. C., Cueto, G. M., Zygadlo, J. and Zerba, E. 2008. Anticholinesterase and Pediculicidal Activities of Monoterpenoids. *Fitoterapia*, **79:** 271-278.
36. Priestley, C. M., Williamson, E. M., Wafford, K. A. and Sattelle, D. B. 2003. Thymol, a Constituent of Thyme Essential Oil, Is a Positive Allosteric Modulator of Human GABAA Receptors and a Homo-Oligomeric GABA Receptor from *Drosophila melanogaster*. *Br. J. Pharmacol*., **140:** 1363-1372.
37. Roditakis, E., Grispou, M., Morou, E., Kristoffersen, J. B., Roditakis, N., Nauen, R., Vontas, J. and Tsagkarakou, A. 2009. Current Status of Insecticide Resistance in Q Biotype *Bemisia tabaci* Populations from Crete. *Pest Manage. Sci*., **65:** 313-322.
38. Ryan, M. F. and Bryan, O. 1988. Plant-insect Coevaluation and Inhibition of Acetylcholinesterase. *J. Chem. Ecol*., **14:** 965-1975.
39. Saleh, D., Laarif, A., Clouet, C. and Gauthier, N. 2012. Spatial and host-plant partitioning between coexisting *Bemisia tabaci* cryptic species in Tunisia. *Popul. Ecol*., **54:** 261–274.
40. Savelev, S., Okello, E., Perry, N. S. L., Wilkins, R. M. and Perry, E. K. 2003. Synergistic and Antagonistic Interactions of Anticholinesterase Terpenoids in *Salvia lavandulaefolia* Essential Oil. *Pharmacol. Biochem. Behav*., **75:** 661–668.
41. Wilson, M., Moshitzy, P., Laor, E., Ghanim, M., Horowtiz, A. R. and Morin, S. 2007. Reversal of Resistance to Pyriproxyfen in the Q Biotype of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Pest Manag. Sci*., **63:** 761-768.
42. Zarrad, K., Ben Hamouda, A., Chaieb, I., Laarif, A. and Mediouni-Ben Jemâa, J. 2015. Chemical Composition, Fumigant and Anti-Acetylcholinesterase Activity of the Tunisian *Citrus aurantium* L. Essential Oils. *Ind. Crop. Prod*., **76:** 121–127.

پتانسیل آنتی کولین استراز مونوترپنوئیدها بر سفید بالک *Bemisia tabaci* و مطالعات جنبشی آن

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چکیده

آفت سفید بالک بیوتیپ B آفتی مخرب در محصولات متنوعی مانند گیاهان زینتی، محصولات باغی و زراعی در سراسر جهان می باشد. کنترل این حشره به دلیل مقاومت آن به حشره کش های بازدارنده های استیل کولین استرازها مانند ارگانوفسفات ها و کاربامات ها بسیار سخت شده است. در مطالعه حاضر ما به بررسی فعالیت درون آزمایشگاهی (*in vitro*) و درون موجود زنده (*in vivo*) بازدارندگی استیل کولین استراز ۶ مونوترپنوئید به نام های α-pinene, terpineol, linalool, ß-myrcene, nerol و geraniol می پردازیم. بازدارندگی AChE در *B. tabaci* توسط روش colorimetric اندازه گیری شد. نتایج نشان داد که همه مونوترپنوئیدها بازدارنده فعالیت AChE با رنج IC50 از ۰.۹۶ تا ۲۶۸۵ تولید کردند. α-pinene قویترین فعالیت بازدارندگی را نشان داد (IC_{50} =0.96 mM). آنالیزهای جنبشی، بازدارندگی از نوع غیر رقابتی را نشان داد که بیانگر اتصال این اجزا هم به آنزیم به تنهایی و هم به سوبسترای آنزیم بود. نتایج، فعالیت بازدارندگی AChE را به عنوان نحوه عمل این مونوترپنوئیدها در غلظت های به نسبت زیاد را ثابت کرد. بنابراین این می تواند برای تحقیق روی ترکیبات حشره کش طبیعی دوست دار محیط زیست استفاده شود.

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