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RESEARCH ARTICLE

Effects of monoterpenes on mortality, growth, fecundity, and ovarian development of *Bactrocera zonata* (Saunders) (Diptera: Tephritidae)

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Abstract
The peach fruit fly (PFF), *Bactrocera zonata*, is a serious insect pest infesting fruits and vegetables. The insecticidal activity of three monoterpenes, namely, (*R*)-camphor, (*R*)-carvone, and (1*R*,2*S*,5*R*)-menthol, was evaluated on the second-instar larvae of *B. zonata*. In addition, the latent effects of monoterpenes on pupation, adult emergence, deformation, oviposition, adult longevity, and ovarian development were also examined. The three tested monoterpenes showed pronounced insecticidal activity against *B. zonata* larvae with (*R*)-carvone being the most potent toxicant. When the second-instar larvae of *B. zonata* were treated with monoterpenes at concentrations of 20, 50, and 70 mg/kg for 72 h, significant reduction in pupation and adult emergence was observed. The three monoterpenes caused complete suppression of adult emergence at 100 mg/kg. Moreover, monoterpenes induced complete inhibition of egg deposition at all tested concentrations. Some adult deformations were also noticed at 20, 50, and 70 mg/kg. However, (*R*)-carvone was more effective than (1*R*,2*S*,5*R*)-menthol and (*R*)-camphor on the examined biological parameters. On the other hand, histological examination of the ovaries of emerged females from larvae that fed on diet treated with (*R*)-carvone, (1*R*,2*S*,5*R*)-menthol, and (*R*)-camphor at 20 and 50 mg/L indicated that both concentrations caused retardation in the development of ovarioles. It is clear that all the egg chambers are empty; the germarium region is constricted at base due to the failure of oocyte formation. Many vacant spaces were present between ovarioles.

Keywords Monoterpenes · *Bactrocera zonata* · Insecticidal activity · Growth inhibition · Oviposition · Ovarian development

Introduction

The peach fruit fly (PFF), *Bactrocera zonata* (Saunders 1842) (Diptera: Tephritidae), is a serious polyphagous pest that attacks over 50 cultivated and wild plants, mainly those with fleshy fruits including guavas, mangoes, peach, apricots, figs, and citrus, in many parts of the world (EPPO 2005). The insect causes great economic loss in fruit crops through direct fruit damage, fruit drop, and loss of export markets due to quarantine restrictions. It originated in South and South-East Asian countries such as Pakistan, India, Nepal, Sri Lanka, Bangladesh, Myamar, Thailand, Laos, and Vietnam, and has been introduced into Mauritius, Reunion, Egypt, Oman, and Saudi Arabia (Agarwal et al. 1999; Carroll et al. 2006).

Egypt has been suffering from fruit flies associated problems ever since the beginning of the last century. In 1912, *B. zonata* was recorded for the first time in Egypt as a quarantine insect pest in Port-Said, Suez Canal region (Efflatoun 1924). In 1995, it was recorded attacking wide range of fruits but it was misidentified as *B. pallidus* (Perkins and May) (Aboul-Ela et al. 1998). As indicated by El-Minshawy et al. (1999), *B. zonata* was recorded as a new pest in Egypt in 1997 from infested guava fruits in Agamy and Sabahia districts near Alexandria, Egypt. Subsequently, it has been spread over the Nile Valley displacing the indigenous fruit fly *Ceratitis capitata* (Wiedemann). Current surveys in Egypt show that the insect is spreading in the whole Nile Delta and Valley, the Oases, and further east to the Sinai Peninsula (Draz et al. 2016).

Egyptian growers applied large quantities of broad-spectrum insecticides to protect their crops from the attack by

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B. zonata and other fruit fly pests. Therefore, the Egyptian Ministry of Agriculture has decided to target *B. zonata* through an emergency action plan by FAO/AIEA funded project for eradication using bait applied technique (BAT) and male annihilation techniques (MATs). Populations of *B. zonata* still threat orchards up to date, and farmers still control this pest with intensive use of synthetic insecticides. However, continuous use of synthetic insecticides has disrupted natural biological systems and accelerated the development of resistance. Moreover, the increasing public concerns about hazardous effects of conventional insecticides on public health and the environment have renewed the interest in developing new effective and ecofriendly insect control strategies. One of these strategies is the use of plant natural products that are known to be less harmful to human health and the environment.

Monoterpenes are the main constituents of many plant essential oils. In some aromatic plants, monoterpenes represent more than 90% of oil composition (De Sousa 2011). They provide specific flavors and odors to many plants because of their low boiling points and distinctive scents. Several hundred monoterpenes have been isolated from higher plants and their structures identified. They are biosynthesized from geranyl pyrophosphate, the ubiquitous acyclic C10 intermediate of the isoprenoid pathway (Windholz et al. 1983). Chemically, monoterpenes are classified into two major groups: monoterpene hydrocarbons and oxygenated monoterpenes. Both groups can be subdivided into acyclic, monocyclic, and bicyclic monoterpenes (Templeton 1969). Monoterpenes have a wide range of biological activities that are important in food chemistry, chemical ecology, and pharmaceutical industry (Schewe et al. 2011). They are involved in various ecological functions in plants, such as protection against herbivores and microbial diseases, attraction of pollinators, and in allelopathy (Langenheim 1994). Monoterpenes have been shown to possess remarkable biological activities, including insecticidal (Abdelgaleil et al. 2009; Kanda et al. 2017), herbicidal (Duke et al. 2002; Singh et al. 2002; Gouda et al. 2016), fungicidal (Marei et al. 2012; Zhang et al. 2016), and bactericidal (Cristani et al. 2007; Cantore et al. 2009; Silva et al. 2015) properties. These activities make monoterpenes useful as potential alternative insect control agents as well as good lead compounds for the development of safe, effective, and fully biodegradable insecticides (Isman et al. 2011; Salakhutdinov et al. 2017).

The toxic and growth inhibitory effects of the monoterpenes, (*R*)-camphor, (*R*)-carvone, and (1*R*,2*S*,5*R*)-menthol, against insects, have been demonstrated towards several insect species. For example, (*R*)-camphor has been reported to possess insecticidal activity against *Blattella germanica* (L.) (Jung et al. 2007) and *Tribolium castaneum* (Herbst) (Liska et al. 2010). (*R*)-carvone has been demonstrated to display insecticidal activity against several insect species, e.g., *Sitophilus zeamais* Motschulsky, *S. oryzae* (L.), *Rhyzopertha dominica* (Fabricius), *T. castaneum*, and *Cryptolestes pusillus* (Schönherr) (Fang et al. 2010; Lopez et al. 2010). Similarly, menthol exhibited insecticidal activity against *Triatoma infestans* (Klug) (Laurent et al. 1997) and mosquitoes (Samarasekera et al. 2008). In addition, in our previous studies, these three monoterpenes have been shown to display pronounced fumigant and contact toxicities against *T. castaneum* (Abdelgaleil et al. 2009), *Spodoptera littoralis* (Boisduval) (Abdelgaleil 2010), and *Culex pipiens* (L.) (Zahran and Abdelgaleil 2011). On the other hand, carvone and menthol were demonstrated to reduce pupation and adult emergence of *Ostrinia nubilalis* (Hübner) (Lee et al. 1999) and *C. pipiens* (Zahran and Abdelgaleil 2011).

However, to the best of our knowledge, there were no reported studies on the biological activities of monoterpenes towards *B. zonata*. Therefore, the present study was undertaken to investigate the insecticidal and growth inhibitory effects of (*R*)-camphor, (*R*)-carvone, and (1*R*,2*S*,5*R*)-menthol against *B. zonata*. The effects of these monoterpenes on oviposition and female reproductive system were also examined.

Materials and methods

Monoterpenes

Three monoterpenes, (*R*)-camphor (98%), (*R*)-carvone (98%), and (1*R*,2*S*,5*R*)-menthol (98%), were purchased from Sigma-Aldrich Chemical Co., Steinheim, Germany. Chemical structure of monoterpenes is shown in Fig. 1.

Insect culture and rearing

Adults of *B. zonata* (Saunders) (Diptera: Tephritidae) were obtained from infested guava, *Psidium guajava* L., fruits collected from house backyards in Agamy district at the Western North coast, Egypt. Insects were reared for three generations on guava fruits in the laboratory for adaptation, followed by rearing for six generations on semi-artificial diet on small scale in an insect rearing cabinet (2 × 3 × 3 m) (Rabab et al. 2016). The larvae were fed on diet containing soybean flour (15%), gelatin (12.6%), sugar (8.9%), corn oil (1.4%), nipagin (0.15%), sodium benzoate (0.15%), citric acid (1.7%), and water (60%), while the adults were fed on diet containing beef extract and sugar (3:1). The insects were kept under controlled conditions at 25 ± 2 °C, 70 ± 5% R.H., and a photoperiod of 14:10 (L:D).

Toxicity experiment

The second-instar larvae of *B. zonata* were used for evaluating the insecticidal activity of monoterpenes. The tested compounds were first prepared in acetone and mixed with semi-

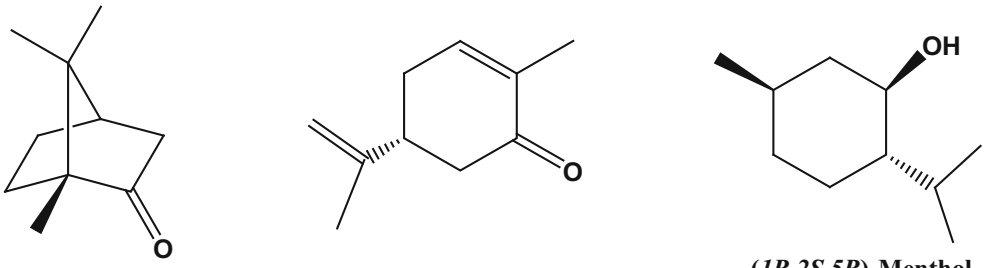

Fig. 1 The chemical structures of tested monoterpenes

(*R*)-Camphor (*R*)-Carvone (*1R,2S,5R*)-Menthol

artificial diet to give a series of concentrations that ranged between 5 and 200 mg/kg. The control diet was treated with acetone only. Ten larvae were placed in a Petri dish (9-cm diameter) containing 10 g of diet. All the treatments were replicated five times. The treated and untreated insects were maintained at same rearing conditions. The mortality percentages were recorded after 24, 48, and 72 h of treatment. The lethal concentration causing 50% mortality (LC_{50}) expressed as mg/kg was calculated from log-concentration mortality regression lines (Finney 1971).

Growth and development test

Effect of monoterpenes on the growth and development of *B. zonata* was evaluated on the second-instar larvae using no-choice bioassay. The solutions of monoterpenes in acetone were incorporated with diet to give final concentrations of 20, 50, 70, and 100 mg/kg. Twenty larvae were introduced to each Petri dish containing 20 g of treated diet. Control diet was prepared with a maximum volume of acetone alone used in the treated diet. Five replicates were carried out in each concentration. After 3 days of feeding on treated diet, the survived larvae were transferred to new Petri dishes and fed on untreated diet until pupation. The insects were allowed to complete their life cycle. The percentages of pupation, adult emergence and deformation, number of eggs/female, and adult longevity were recorded.

Histological studies on *B. zonata* adult ovary

Samples of adult females from both control and treatments were taken for histological examinations of ovaries. The insect ovaries were dissected out in 0.9% NaCl solution and fixed in aqueous Bouin's solution for 24 h. Then, ovaries were transferred to 70% ethyl alcohol. The individual ovarioles were separated and embedded in paraffin wax (58–60 °C). Longitudinal sections were cut at 7-μ thicknesses using rotary microtome. The sections were deparaffinized and stained in Heidenhain's hematoxylin and counterstained with eosin (Drury and Wallington 1980), and stained sections were examined (Hedaya 1990).

Statistical analysis

The mortality percentages were subjected to probit analysis (Finney 1971) to obtain the LC_{50} values, using SPSS 12.0 (SPSS, Chicago, IL, USA). The values of LC_{50} were considered significantly different if the 95% confidence limits did not overlap. Mortality, pupation, adult emergence, and deformation percentages were subjected to one-way analysis of variance followed by Student–Newman–Keuls test (Cohort Software Inc. 1985) to determine significant differences among mean values at the probability level of < 0.05.

Results

Insecticidal activity of monoterpenes on *B. zonata* larvae

The toxic effect of the three monoterpenes against the second-instar larvae of *B. zonata* is presented in Table 1. Based on LC_{50} values, the tested compounds showed pronounced insecticidal activity. The toxicity of compounds increased with increasing the exposure time. (−)-Carvone was significantly the most potent compounds after 24 h of treatment, followed by (*R*)-camphor and (1*R*,2*S*,5*R*)-menthol with LC_{50} values of 39.11, 64.46, and 71.20 mg/kg as the confidence limits of (−)-carvone were not overlapped with LC_{50} values of (*R*)-camphor and (1*R*,2*S*,5*R*)-menthol. Also, (−)-carvone had the highest insecticidal activity after 48 and 72 h treatment. (1*R*,2*S*,5*R*)-Menthol (LC_{50} = 53.79 mg/kg) was significantly more toxic than (*R*)-camphor (LC_{50} = 58.49 mg/kg) as their confidence limits were not overlapped with LC_{50} values.

Effect of monoterpenes on the growth and development of *B. zonata*

The effect of different concentrations (*R*)-camphor on growth and developmental parameters, such as larval mortality, pupation, adult emergence, adult deformation, laid eggs, and female longevity of *B. zonata*, is shown in Table 2. The results revealed that (*R*)-camphor was significantly high toxic to the larvae after 3 days of treatment, particularly at the higher

Table 1 Comparative toxicity of monoterpenes against second-instar larvae of *B. zonata* after 24, 48, and 72 h of treatment

Compound	Times (h)	LC_{50}[a] (mg/kg) (95% confidence limits)	Slope ± SE[b]	Intercept ± SE[c]	(χ^2)[d]	P[e]
(*R*)-Camphor	24	64.46 (56.91–72.94)	2.78 ± 0.41	− 5.04 ± 0.70	2.04	0.15
	48	58.49 (54.10–62.33)	5.90 ± 0.73	− 10.42 ± 1.32	1.54	0.22
	72	23.68 (21.10–26.21)	4.72 ± 0.54	− 6.49 ± 0.78	0.19	0.66
(1*R*,2*S*,5*R*)-Menthol	24	71.20 (67.62–75.03)	7.40 ± 0.74	− 13.7 ± 1.38	0.09	0.76
	48	53.79 (49.12–57.58)	5.09 ± 0.77	− 10.53 ± 1.39	1.80	0.18
	72	< 20	–	–	–	–
(*R*)-Carvone	24	39.11(34.82–43.66)	3.58 ± 0.34	− 5.71 ± 0.56	2.05	0.15
	48	< 20	–	–	–	–
	72	< 20	–	–	–	–

[a] The concentration causing 50% mortality

[b] Slope of the concentration-mortality regression line ± standard error

[c] Intercept of the regression line ± standard error

[d] Chi-squared value

[e] Probability value

concentrations of 50, 70, and 100 mg/kg ($P < 0.05$). Complete larval mortality was observed at 100 mg/kg. Similarly, (*R*)-camphor significantly decreased pupation and adult emergence percentages at 20 mg/kg ($P < 0.05$). There were no normal adults that emerged at the concentrations of 50, 70, and 100 mg/kg. Some of deformed adults were observed at the concentrations of 20, 50, and 70 mg/kg. The malformation appeared as shrinking wings or reduction in abdomen size (Fig. 2). All of tested concentrations completely inhibited egg deposition. In addition, the concentrations of 50 and 70 mg/kg significantly decreased female longevity to be 40.33 and 10.67 days compared with 68.33 days in control treatment ($P < 0.05$).

On the other hand, (1*R*,2*S*,5*R*)-menthol showed pronounced toxicity to *B. zonata* larvae with 70.0, 90.0, 93.33, and 100.0% mortality at 20, 50, 70, and 100 mg/kg, respectively (Table 3). In addition, pupation and adult emergence percentages were significantly reduced at the tested concentrations compared with control ($P < 0.05$). Female longevity was also decreased significantly at 50 and 70 mg/kg. Complete inhibition of egg deposition was observed at all tested concentrations.

The effect of (−)-carvone on larval mortality and developmental parameters of *B. zonata* is presented in Table 4. (−)-Carvone caused 73.33 and 93.33% mortality at 20 and 50 mg/kg and complete mortality (100%) at 70 and 100 mg/kg. The compound significantly decreased pupation and adult emergence percentages ($P < 0.05$). No eggs were laid by female at the tested concentrations. Female longevity was also decreased significantly at 20 mg/kg to be 9.33 days compared with control which is 68.33 days ($P < 0.05$).

Histological changes of the ovary of *B. zonata* females emerged from larvae fed on treated diet with monoterpenes

Treatment with monoterpenes disrupted the growth of ovary and caused retardation in the development of ovarioles (Fig. 3). Clear reduction in the numbers of oocytes per ovaries and the volume of the basal oocyte were also observed. The treatment of larvae with (1*R*,2*S*,5*R*)-menthol at concentration of 20 mg/kg caused advanced degeneration in ovarian contents. Most of the ovarioles appear empty. A small number of

Table 2 Effect of (*R*)-camphor on larval mortality and development of *B. zonata*

Conc. mg/Kg	Mortality after 72 h. (% ± SE)	Pupation (% ± SE)	Adult emergence (% ± SE)	Adult deformation (% ± SE)	No. of Eggs/female	Female longevity (days±SE)
Cont.	0.00 ± 0.0c	100.0 ± 0.0a	100.0 ± 0.0a	0.0 ± 0.0b	53.33 ± 3.34a	68.33 ± 0.32a
20	36.67 ± 3.34b	63.30 ± 5.13b	33.33 ± 1.54b	3.73 ± 1.30ab	0.0 ± 0.0b	65.67 ± 0.67a
50	93.33 ± 6.67a	6.67 ± 3.33c	6.67 ± 3.33c	6.67 ± 1.66a	0.0 ± 0.0b	40.33 ± 0.58b
70	93.33 ± 6.67a	6.67 ± 3.33c	6.67 ± 3.33c	6.67 ± 1.66a	0.0 ± 0.0b	10.67 ± 1.34c
100	100.0 ± 0.0a	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0d

Mean values with different letters are significantly different ($P < 0.05$)

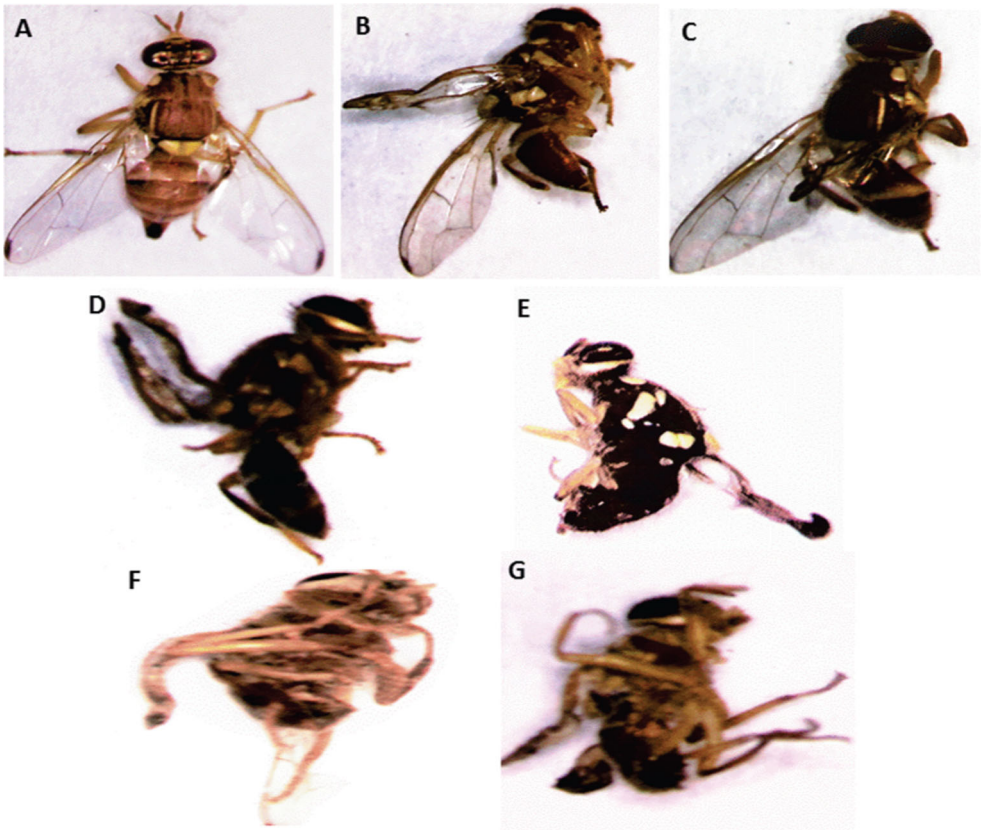

Fig. 2 Malformation of *B. zonata* adults emerged from larvae treated with monoterpenes. **a** Untreated larvae. **b** Treated with 20 mg/kg of (*R*)-camphor. **c** Treated with 50 mg/kg of (*R*)-camphor. **d** Treated with 20 mg/kg of (1*R*,2*S*,5*R*)-menthol. **e** Treated with 50 mg/kg of (1*R*,2*S*,5*R*)-menthol. **f** Treated with 20 mg/kg of (*R*)-carvone. **g** Treated with 50 mg/kg of (*R*)-carvone

oocytes were observed, and the nurse cells were not clear. The treatment with (1*R*,2*S*,5*R*)-menthol at 50 mg/mL caused abnormal ovarioles full of nurse cells but without any oocytes. Also, the larval treatment with (*R*)-camphor at 20 or 50 mg/kg showed abnormality indicated by dark stained masses (d.m.), vacuated spaces (v.s.) between ovarioles, and complete disappearance of oocytes. Similarly, treatment with (−)-carvone caused the maximum destructive effect on the ovaries. At 20 mg/kg, the degeneration of all the ovariol contents represented by only few irregular egg chambers and vacant spaces was observed (Fig. 4). At 50 mg/kg, all ovarioles reduced to few dwarfed oocytes.

Discussion

In this study, the tested monoterpenes, (*R*)-camphor, (*R*)-carvone, and (1*R*,2*S*,5*R*)-menthol, exhibited pronounced insecticidal activity against the second-instar larvae of *B. zonata*. To the best of our knowledge, there were no reported studies on the insecticidal activity of monoterpenes against *B. zonata*. However, the tested monoterpenes have shown to possess insecticidal activity against *S. oryzae* (Lee et al. 2003), *T. castaneum* (Abdelgaleil et al. 2009), *S. littoralis* (Abdelgaleil 2010), and *C. pipiens* (Zahran and Abdelgaleil 2011). In the present study, (*R*)-carvone displayed higher insecticidal activity than (*R*)-camphor and

Table 3 Effect of (1*R*,2*S*,5*R*)-menthol on larval mortality and development of *B. zonata*

Conc. mg/Kg	Mortality after 72 h. (% ± SE)	Pupation (% ± SE)	Adult emergence (% ± SE)	Adult deformation (% ± SE)	No. of Eggs/female	Female longevity (days±SE)
Cont.	0.0 ± 0.0c	100.0 ± 0.0a	100.0 ± 0.0a	0.0 ± 0.0a	53.33 ± 3.34a	68.33 ± 0.32a
20	70.0 ± 5.7b	30.0 ± 5.7b	30.0 ± 5.78b	0.0 ± 0.0a	0.0 ± 0.0b	53.0 ± 0.67b
50	90.0 ± 5.78a	10.0 ± 5.78c	6.67 ± 3.34c	3.70 ± 1.88a	0.0 ± 0.0b	49.33 ± 1.20c
70	93.33 ± 6.67a	6.67 ± 3.34c	6.67 ± 3.34c	6.67 ± 3.34a	0.0 ± 0.0b	26.67 ± 0.66d
100	100.0 ± 0.0a	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0a	0.0 ± 0.0b	0.0 ± 0.0e

Mean values with different letters are significantly different ($P < 0.05$)

Table 4 Effect of (*R*)-carvone on larval mortality and development of *B. zonata*

Concentration (mg/kg)	Mortality after 72 h (% ± SE)	Pupation (% ± SE)	Adult emergence (% ± SE)	Adult deformation (% ± SE)	No. of eggs/female	Female longevity (days ± SE)
Control	0.0 ± 0.0 c	100.0 ± 0.0 a	100.0 ± 0.0 a	0.0 ± 0.0 b	53.33 ± 3.34 a	68.33 ± 0.32 a
20	73.33 ± 3.34 b	26.67 ± 6.67 b	16.67 ± 3.34 b	10.0 ± 0.0 a	0.0 ± 0.0 b	9.33 ± 0.67 b
50	93.33 ± 6.67 a	6.67 ± 6.67 c	6.67 ± 3.34 c	6.67 ± 3.34 a	0.0 ± 0.0 b	0.0 ± 0.0 c
70	100 ± 0.0 a	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 c
100	100 ± 0.0 a	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 c

Mean values with different letters are significantly different ($P < 0.05$)

(1*R*,2*S*,5*R*)-menthol against *B. zonata* larvae. In consistent with these results, (*R*)-carvone has been reported to be more toxic than (*R*)-camphor and (1*R*,2*S*,5*R*)-menthol against *S. oryzae*, *T. castaneum*, *C. pipiens*, and *S. littoralis*. The results also indicated that the ketones ((*R*)-carvone and (*R*)-camphor) had higher toxicity than an alcohol ((1*R*,2*S*,5*R*)-menthol). Similarly, Rice and Coats (1994) found that some ketones were more effective toxicants than alcohols. The toxicity of monoterpenes improved with increasing the time of exposure.

Despite numerous researches on the insecticidal activity of monoterpenes, the mechanisms of action of monoterpenes are not fully understood. Monoterpenes are volatile and lipophilic compounds which are able to penetrate rapidly inside insects and interfere with their physiological functions (Lee et al. 2003). It has been reported that monoterpenes were inhibitors of acetylcholinesterases (AChEs) from insects and other organisms (Ryan and Byrne 1988; Miyazawa et al. 1997; Picollo et al. 2008; Abdelgaleil et al. 2009). It has been also found that monoterpenes had binding affinity for octopamine receptors and GABA-gated chloride ion channel (Höld et al. 2000; Enan 2001). Other effects of monoterpenes on the hormone and pheromone systems and cytochrome P_{450} monooxygenase have been described (De-Oliveira et al. 1999; Garcia et al. 2005). Therefore, toxic effects of tested monoterpenes on *B. zonata* may be attributed to one or more of these possible modes of action.

The results of the present study demonstrated that the three tested monoterpenes caused significant reduction in pupation and adult emergence percentages of *B. zonata*. These results are in agreement with our previous study, in which (*R*)-carvone caused significant reduction in pupation and adult emergence of *C. pipiens* (Zahran and Abdelgaleil 2011). Lee et al. (1999) reported that carvone and menthol had remarkable reduction in pupation and adult emergence of European corn borer, *O. nubilalis*. Harwood et al. (1990) reported that menthol deleteriously affected growth, feeding, and pupation of *Peridroma saucia* (Hübner) larvae. In addition, Jilani et al. (2006) found that ethanol extracts and petroleum ether extract of *Valariana officianalis* (L.) at 500 and 1000 mg/L significantly reduced pupal production and adult emergence of *B. zonata.*

On the other hand, the tested monoterpenes caused complete oviposition inhibition of *B. zonata*. In accordance with these results, the oviposition deterrent effects of plant extracts on *B. zonata* have been demonstrated (Siddiqi et al. 2006, 2011; Khattak et al. 2006; Ur-Rehman et al. 2009). Moreover, the effects of monoterpenes on oviposition of other insect species were documented. Chaubey (2012) found that two monoterpenes, α-pinene and β-caryophyllene, significantly reduced oviposition potential and inhibited pupation and adult emergence of *T. castaneum*. Stamopoulos et al. (2007) reported that monoterpenes (terpinen-4-ol, 1,8-cineole, linalool, R-(+)-limonene, and geraniol) induced lower fecundity and egg hatchability of *T. confusum* (Jacquelin du Val).

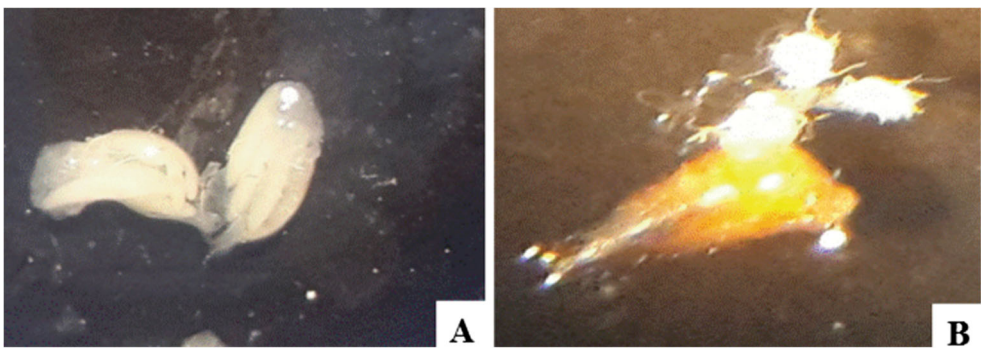

Fig. 3 Dissected ovaries of *B. zonata*. **a** Ovaries of adult emerged from untreated larva. **b** Ovaries of adult emerged from larva treated with 20 mg/kg of (*R*)-carvone

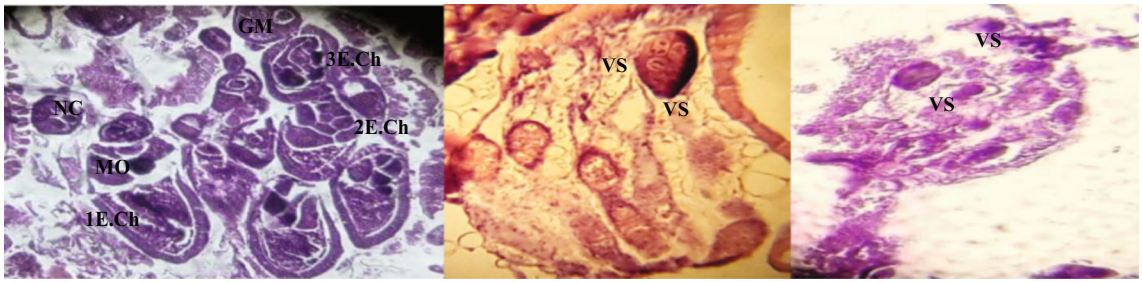

Fig. 4 Effect of (*R*)-carvone on *B. zonata* ovary. *left* Longitudinal section in ovary of female emerged from untreated larva. *center* Longitudinal section in ovary of female emerged from larva treated with 20 mg/kg. *right* Longitudinal section in ovary of female emerged from larva treated with 50 mg/kg. *GM* germarium, *MO* mature ovum, *NC* nurse cell, *VS* vacant spaces, *ECh* egg chamber

Also, the monoterpenoids revealed insect growth regulator properties and produced deformed adults. In addition, terpinen-4-ol and 1,8-cineole were described to reduce fecundity and egg hatchability of *T. castaneum* and *T. confusum* (Amos et al. 1974). Carvone also completely suppressed the egg hatching of *T. castaneum* at 7.22-mg/cm^2 surface treatment (Tripathi et al. 2003).

The results of the present study indicated that the tested monoterpenes caused adult malformation and decreased female longevity of *B. zonata*. These results indicated that the tested monoterpenes affected the hormonal balance of *B. zonata*. Earlier studies confirmed these findings and clearly indicated that terpenes can cause adult deformation, similar to growth-regulating hormones, such as juvenile hormone (Amos et al. 1974). Geraniol and camphene are among common terpenes that cause deformities in adult insects (Stamopoulos et al. 2007; Sharaby and EL-Dosary 2016). In addition, eugenol was reported to cause adult deformation and reduce female longevity of *Agrotis ipsilon* (Hufnagel) (Abd El-Aziz et al. 2007).

The tested monoterpenes caused destructive effects on ovary and ovarioles of *B. zonata* adult females. These effects led to complete inhibition of oviposition. The results indicated that the monoterpenes can be used to produce sterilize insects and can be used as chemical sterilants in sterile insect release programs which is very effective control method. Few studies have been reported on the effects of natural products on insect ovary and ovarioles. For example, the treatment with caraway oil affected the basophilic affinity of the nuclei of germarium cells and the follicular epithelium of developing oocytes of *Trogoderma granarium* Everts (Osman et al. 2016). The treatment with garlic, mint, and eucalyptus oils inhibited oogenesis and egg laying of *Heteracris littoralis* (Rambur) (Sharaby et al. 2012). Combinations of cedarwood oil with eucalyptus, peppermint, and camphor oils showed potent fecundity inhibition of *Corcyra cephalonica* (Stainton) as evident by appearance of numerous empty spaces within the ovarioles. The oils also disturbed distribution and arrangement of oocytes/ova with two or more ova coalescing and fusing to produce a lumpy mass within the ovarioles (Jacob and Qamar 2013). The treatment with forskolin (a diterpene extracted from the roots of *Plectranthus barbatus* Andrews) affected ovarian development of *T. confusum*. The ovaries showed variation in the length and size of the ovarioles, oocyte degeneration, resorption, and inability of the mature oocytes to oviposite (Lingampally et al. 2012). It has been reported that thymol, a monoterpene, interfered with the development of oocytes of *Rhipicephalus sanguineus* (Latreille) and caused degeneration signs (Matos et al. 2014).

Conclusions

In conclusion, the tested monoterpenes, (*R*)-camphor, (*R*)-carvone, and (1*R*,2*S*,5*R*)-menthol, showed promising biological activities on *B. zonata* including insecticidal, growth regulatory, sterilizing, and ovarian destructive effects. These results indicated that the tested monoterpenes have multiple modes of action. Accordingly, the tested monoterpenes could be useful in different insect management programs with less probability of developing resistance and polluting environment. Therefore, the tested monoterpenes could provide suitable components in integrated pest management (IPM) of *B. zonata*.

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