Article

Chemical composition and insecticidal efficacy of essential oil of *Echinophora platiloba* DC (Apiaceae) from Zagros foothills, Iran

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Abstract
Essential oil of *Echinophora platyloba* was screened for its chemical composition and possible fumigant and contact toxicity effects against *Tribolium castaneum* (Herbst), *Callosobruchus maculatus* (F.) and *Rhyzopertha dominica* (F.). Aerial parts were subjected to hydrodistillation and obtained oil chemical composition was analyzed by GC-MS. (Z)-β-ocimene (33.06%), p-cymene (10.98%) and Limonene (5.77 %) were major constituents. Fumigation tests were performed for 24, 48 and 72 h, while contact toxicity of essential oil was evaluated in 24h. Experimental units were located in 25±2 °C and darkness condition. In contact toxicity evaluation tests *T. castaneum* (LC_{50}= 14.712 µl/39cm^2) was more tolerant and *R. dominica* (LC_{50}= 9.712 µl/39cm^2) was more susceptible species. After 24 h, *T. castaneum* (LC_{50}= 39.658 µl/250 ml air) and *C. maculatus* (LC_{50}= 3.835 µl/250 ml air) were more tolerant and susceptible species in fumigation bioassays, respectively. In general, mortality increased as the doses of essential oil and exposure time increased.

Keywords *Echinophora platyloba*; chemical composition; insecticidal efficacy; contact toxicity; fumigant toxicity; stored products beetles.

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1 Introduction

Stored products of agricultural and animal origin were attacked by more than 600 species of beetle pests causing quantitative and qualitative losses (Rajendran, 2002). Fumigation plays a major role in insect pest elimination in stored products. Chemical control of stored products' pests with current chemical pesticides may cause special problems on stored products (Collins et al., 2002). These problems have highlighted the need for the development of new types of selective insect-control alternatives with fumigant action (Negahban et al., 2006). Plant essential oils do not leave toxic residues in the environment and have medicinal properties for humans (Isman, 2006). It is believed that they have the advantage over conventional fumigants in terms of low mammalian toxicity, rapid degradation and local availability (Rajendran and Sriranjini, 2008).

In some cases it is alleged that the Apiaceae family oils are alternatives to conventional chemical insecticides (Ebadollahi, 2013). Coumarins, polyacetylenes, flavonoids, sesquiterpenes, and phthalides are among the important chemical constituents of Apiaceae family (Christensen and Brandt, 2006; Iranshahy and Iranshahi, 2011; Nazari and Iranshahi, 2011; Sajjadi et al., 2009; Shokoohinia et al., 2010). Iran's climate is suitable for the growth of apiaceous plants and 114 wild genera with 12 endemic genera are growing in it (Mozaffarian, 1996).

Some Apiaceae species (for example *Azilia eryngioides* (Cham. & Schltdl.), *Carum copticum* (L.), *Carum carvi* (L.), *Cuminum cyminum* (L.), *Coriandrum sativum* (L.) *Ferula gummosa* (L.), *Foeniculum vulgare* (Mill.), *Heracleum persicum* (Desf. ex Fisch.) and *Prangos acaulis* (DC.), *Cominum cyminum* (L.) etc.) showed repellent, antifeedant and insecticidal activities against many species of stored products pests (*Sitophilus granaries*; *T. castaneum*; *Plodia interpunctella*; *Oryzaphilus surinamensis*; *Rhyzopertha dominica*; *Tribolium confusum*; *Callosobruchus maculatus*; etc.) in Iran (Ebadollahi and Mahboubi, 2011; Sahaf et al., 2007; Shojaaddini et al., 2008; Rafiei-Karahroodi et al,. 2009; Shokri-Habashi et al., 2011). *Echinophora* genus is endemic for Iran and there are no published documents about insecticidal activity of *E. platyloba*.

Echinophora platyloba (Apiaceae, subfamily Apioideae, tribe Echinophoreae) is an aromatic, mid-summer plant that wildly grows and mainly used for imparting flavor and taste to the food in Iran (Avijgan et al., 2010). Hydroalcholic and aqueous extracts of *E. platyloba* have antioxidant and antibacterial effects and could inhibit oxidation by scavenging free radicals as a natural food preservative (Sharafati-chaleshtori et al., 2012).

In this study, we investigated the chemical composition of the essential oil of *E. platiloba*. In addition, we evaluated its efficacy as a contact and fumigant toxic agent in the management of the red flour beetle *Tribolium castaneum* (Herbst), cowpea weevil *Callosobruchus maculatus* (F.), and lesser grain borer *Rhyzopertha dominica* (F.) that are of most important beetle pests of stored products all over the world.

2 Materials and Methods

2.1 Plant material and extract preparation

The aerial parts of the plant were collected from Zagros foothills (Charmahal o Bakhtiary province [latitude: 32° 16', longitude: 50° 59'; altitude: 2101 m]), Iran. Plant parts were picked up in May-June 2013 and dried away from direct sunlight. Shade-dried parts were crushed using a pestle and their extract obtained by hydrodistillation using Clevenger apparatus (Sharifian et al., 2013).

2.2 GC-MS analysis

The constituents of *E. platyloba* essential oil were analyzed by gas chromatography mass spectrometry (GC-MS) (Thermo-UFM). The GS conditions were as follows: capillary column 1-ph (30 m x 0.25 mm, film thickness 0.25 pm); helium as a carrier gas (0.5 ml/min); oven temperature program, initially 40°C rising to 250°C (80°C/ min, 3 min); injector and detector temperature of 250°C. The identification of individual compounds was based on comparison of their relative retention times with those of authentic samples on a capillary column, and by matching their mass spectra of peaks with those obtained from authentic samples and published data.

2.3 Bioassay

In contact toxicity evaluation, 7 cm-diameter Petri dishes were applied as experimental vials. A 2cm hole was embedded on top of each Petri dish for ventilation and removing the fumigant effects of essential oil. Essential oil was poured on filter papers that covered the vials floor after dilution in 0.5 ml acetone. Twenty adult insects were located on filter papers in each treatment after 2-3 minutes (time of acetone evaporation). 0.5 ml acetone was poured on filter paper in control with similar conditions. Experimental units were located in 25±2 °C, 65±15% relative humidity and darkness condition for 24 h.

For investigation of fumigant toxicity, each concentration was applied to filter paper strips (Whatman No. 1, cut into 4 × 5 cm paper strip). Treated filter papers were placed at the bottom of 250 ml glass jars. Twenty adult of insects (1-7 days old) were placed in small plastic tubes (3.5 cm diameter and 5 cm height) with open ends covered with cloth mesh. Tubes were hung at the geometrical centre of the glass jars and then sealed with air-tight lids. Thus, there was no direct contact between the oil and insects (Sharifian et al., 2012). In the control jars, oil was not applied on the filter papers. Experimental units were located in 25±2 °C and darkness condition. Mortality was determined after 24, 48, and 72 h of exposure's beginning.

To determine the mortalities at each contact and fumigant toxicity tests, insects were removed from the jar and checked with a fine brush. Insects were considered as dead when no leg or antennal movements were observed. Each experiment was replicated five times for each concentration and time (in case of fumigant toxicity).

2.4 Data analysis

In order to calculate significant differences in toxicity between concentrations and times of exposure, a one-way analysis of variance (ANOVA) was used at the $P<0.05$ (SPSS 18.0) (Darvishzadeh et al., 2014). Probit analysis was used to evaluate the LC_{50} values (Abbott 1925). Poloplus 2.0 software was used to calculate LC_{50} values.

3 Results

The essential oils isolated by hydro-distillation from the parts of *E. platyloba* species were found to be light yellowish liquids. The oil yield was 0.8±0.1% (V/W, oil/dried plant). A total of 15 compounds have been identified representing around 94.5% of the total oil. (Z)-β-ocimene (33.06%), p-cymene (10.98%), Limonene (5.77 %) were major constituents in the essential oil of *E. platyloba* (Table 1).

Table 1 Major constituents of *E. platyloba* essential oil analyzed by GC-MS.

No.	Constituent	Percent	Retention Index (RI)
1	α –Pinene	4.45	0858
2	Myrcene	3.87	0918
3	α –phellandrene	3.21	0982
4	Δ-3-carene	4.43	1003
5	p-cymene	10.98	1021
6	Limonene	5.77	1028
7	(Z)- β-ocimene	33.06	1142
8	Linalool	1.93	1194
9	Methyleugenol	2.52	1271
10	γ-Decalactone	1.84	1325
11	4-decanolide	3.82	1383
12	Myristicin	5.21	1395
13	Cis-3-hexylbenzoate	5.03	1420
14	Spathunenol	5.89	1439
15	δ –dodecalactone	2.49	1558
	Total	94.5	

The contact toxicity was varied with different concentrations of the essential oil. Data analysis showed that more resistant and more susceptible species to contact toxicity of the *E. platyloba* oil were *T. castaneum* and *R. dominica*, respectively (Fig. 1).

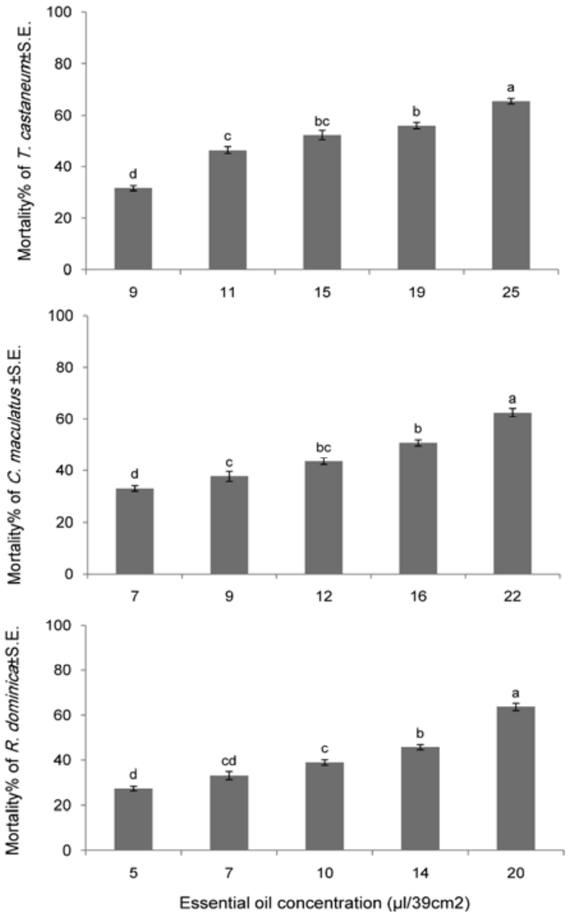

Fig. 1 Mean mortality (%) of beetle species due to contact toxicity of *E. platyloba* essential oil in different concentrations. Different letters over columns indicate significant differences according to Tukey test at α=0.01. Columns with the same letter are not significantly different.

Calculated parameters of bioassay and contact LC_{50} of *E. platyloba* oil against three beetles are shown in Table 2.

Table 2 Contact LC_{50} of *E. platyloba* against three stored product beetles.

Insects	LC_{50}^{*} (Lower-Upper)	Slope±S.E.	χ^2	*P*
T. castaneum	14.712 (10.519-19.610)	5.17±1.02	0.383	0.184
C. maculates	13.793 (10.254-17.984)	2.995±0.85	0.37	0.831
R. dominica	9.712 (8.519-10.61)	4.17±1.3	0.483	0.183

* µl/39cm^2

E. platyloba essential oil showed strong fumigant toxicity against the beetle species at several concentrations and exposure times. The lethal concentration of 50% (LC_{50}) for *T. castaneum*, *C. maculatus* and *R. dominica* at 24 h exposure time were 39.658, 3.835 and 5.66 µl/250 ml air, respectively. Probit analysis showed that at exposure time of 24 h, *C. maculatus* was more susceptible than the other species to fumigant toxicity of the oil, while *T. castaneum* was more resistant one. Furthermore, with the increase of exposure time to 72 h, mortality increased and LC_{50} values decreased in all three species (Table 3).

Table 3 LC_{50} and parameters of probit analysis of *E. platyloba* essential oil fumigant toxicity against three stored product beetles.

Insect species	Exposure time	LC_{50}*	χ^2 (df=3)	Slope±S.E.	*P*
T. castaneum	24	39.658	4.294	2.23±0.78	0.231
	48	29.418	3.017	2.817±0.96	0.39
	72	26.772	3.502	2.984±0.79	0.32
C. maculates	24	3.835	1.616	1.444±0.12	0.656
	48	1.511	4.335	1.773±0.67	0.227
	72	1.345	6.480	1.892±0.85	0.090
R. dominica	24	5.66	0.372	1.426±0.35	0.496
	48	4.275	4.025	1.983±0.42	0.259
	72	2.36	8.032	1.719±0.51	0.08

* (µl/250 ml air)

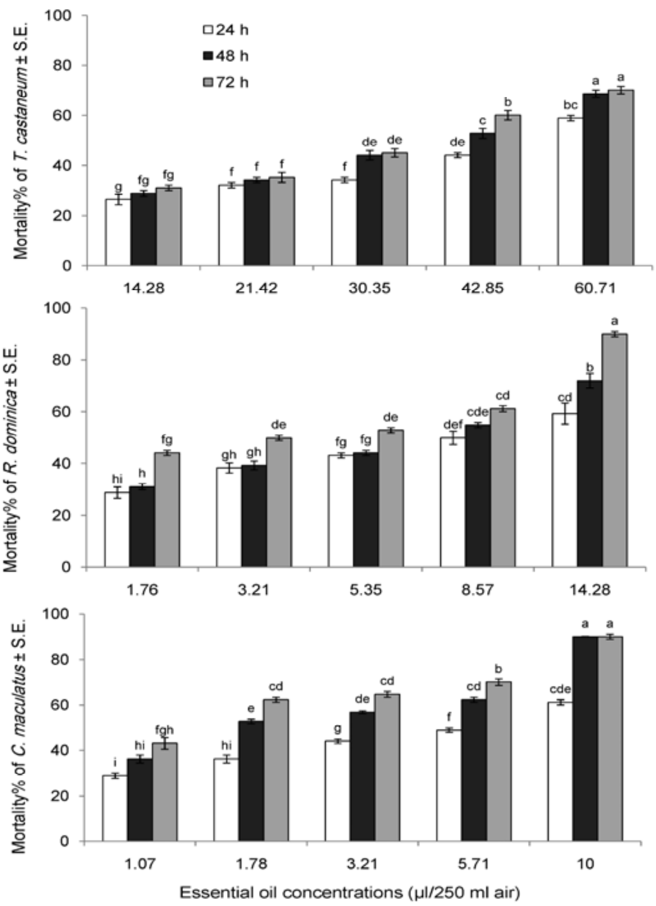

Fig. 2 Mean mortality (%) of *T. castaneum*, R. dominica and *C. maculatus* adults exposed to different concentrations of *E. platyloba* essential oil fumigation. Different letters over columns indicate significant differences according to Tukey test at α= 0.01. Columns with the same letter are not significantly different.

Mortality percentages of five different essential oil concentrations used three times on three mentioned adult beetles displayed in Fig. 2. Comparison of the means (Using Tukey test; α= 0.01) showed that there were significant differences in the mortalities exposed to different essential oil concentrations for 24, 48, and 72 h (Fig. 2).

On the other hand, there was an increased susceptibility of the insects associated with an increase in the different concentrations of oil and time of exposures. For example, the LC_{50} value *E. platyloba* essential oil decreased from 39. 658 at 24 h exposure time to 26.772 µl/250 ml air after 72 h (Table 3).

4 Discussion

E. platyloba essential oil yield was 0.8±0.1% (V/W) in current research that is relatively high in comparison with other studies (0.7 % (Rahimi-Nasrabadi *et al.* 2010) and 0.55±0.1% (Hassanpouraghdam et al., 2009).

Main constituents of the *E. platyloba* essential oil were found to be (Z)-β-ocimene (26.71%), Δ-3-carene (16.16%), Limonene (6.59%) (Rahimi-Nasrabadi *et al.* 2010). In another study the major constituents were identified as trans-β-ocimene (67.9%), 2-furanone (6.2%), myrcene (6.0%), linalool (3.1%), and cis-β-ocimene (2.3%) (Asghari et al., 2003). Hassanpouraghdam *et al.* (2009) identified (Z)-β-ocimene (38.9%) and α-phellandrene (24.2%) as principle monoterpene hydrocarbon constituents of essential oil of *E. platyloba* using GC/MS analysis method. Differences between essential oils components could be due to differences in their growth area, season of collecting and the plant phenology. Various components in essential oils are the source of variety in their biological activities (Isman, 2006).

Results of current and earlier studies indicate that essential oil of *E. platyloba* is a source of biologically active vapors which may potentially be an insecticide (Avijgan et al., 2010; Shokoohinia et al., 2010; Nazari and Iranshahi, 2011; Sharafati-chaleshtori et al., 2012). The use of herbal factors with insecticidal properties, particularly essential oils, was considered by many researchers and so many studies have been done in this subject. In these researches the most frequent insects for evaluating of essential oils fumigant toxicity were *Tribolium castaneum* Herbst., *Sitophilus oryzae* (L.), *Sitophilus zeamais* Motschulsky. and *Rhyzopertha dominica* (F.) (Rajendran and Sriranjini, 2008).

It is the first to show that *E. platyloba* essential oil can act as insecticide against insect species especially on stored product beetles. Due to the lack of insecticidal activity research on *Echinophora* sp. essential oil, we compare its effectiveness with other Apiaceae plants essential oils on similar insect pests.

Sahhaf et al. (2007) investigated the fumigant toxicity of *C. copticum* essential oil from Apiaceae family against adults of *T. castaneum*, and their obtained LC_{50} was 33.14 µl/L. Their calculated LC_{50} in comparison with our results showed that *Carum copticum* essential oil is more effective than *E. platyloba*. However the effects of experimental conditions and insect population resistance level should not be ignored. Also Ebadollahi and Mahboubi (2011) evaluated the 24 h LC_{50} of *Azilia eryngioides* oil on *T. castaneum* and their result was 46.48 µl/L that is more efficient than our calculated value in similar exposure time (39.66 µl/250 ml). However different plant essential oils have different insecticidal activity that is related to their chemical components and their oil extraction method. Islam et al. (2009) in a research on toxicity of *Coriandrum sativum* (Apiaceae) showed that essential oil LC_{50} on *T. castaneum* adults in 24h is less than 2 µg/ml. experimental conditions and different plant species could be the reason of difference between their results in comparison with ours. Heydarzade and Moravvej (2012) also worked on insecticidal activity of another species of Apiaceae family. They showed that contact toxicity of *Foeniculum vulgare* on *C. maculatus* is different between male (LC_{50}= 390.38 µl/m^2) and female (513.46 µl/m^2).

In general, the essential oil of *E. platyloba* possesses a potential for use in the management of *T. castaneum, R. dominica,* and especially *C. maculatus*. For the practical application of this essential oil as insecticides, further studies on the development of formulations are necessary to improve efficacy and stability, and to reduce cost.

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