International Journal of Tropical Insect Science (2020) 40:637–643
https://doi.org/10.1007/s42690-020-00113-y

ORIGINAL RESEARCH ARTICLE

Insecticidal effects of some essential oils against *Tribolium confusum* (du Val.) and *Acanthoscelides obtectus* (Say), (Coleoptera: Tenebrionidae and Bruchidae) adults

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Received: 22 October 2019 / Accepted: 15 January 2020 / Published online: 18 January 2020

Abstract

In this study, insecticidal effects of the essential oils obtained from plants *Ocimum basilicum* L., *Rosmarinus officinalis* L. and *Artemisia dracunculus* L. on confused flour beetle (*Tribolium confusum* du Val., 1863 (Coleoptera: Tenebrionidae)) and bean weevil (*Acanthoscelides obtectus* (Say), 1831 (Coleoptera: Bruchidae)) adults were tested in laboratory conditions. In this context, *T. confusum* and *A. obtectus* adults were exposed to essential oils at 10 and 20 μL/petri doses for 24, 48, 72 and 96 h. All of the essential oils used in the study caused mortalities at different rates in two application doses but end of the 96 h all mortality rates were obtained similar (*O. basilicum* 98.3%, *R. officinalis* 98.3%, *A. dracunculus* 93.3% against *T. confusum* adults; *O. basilicum* 100%, *R. officinalis* 100%, *A. dracunculus* 100% against *A. obtectus* adults). It was determined that the effects of essential oils on *A. obtectus* adults were greater than *T. confusum* adults. Especially when the dosage was 20 μL, the death rate increased up to over 95% after 96 h for all types. The mortality rates increased with increasing exposure period at the 48, 72 and 96 h. in all applications. The results of the study suggest that essential oils from *O.basilicum* and *R.officinalis* could have a potential as control agents against *A. obtectus* and *T.confusum* adults under storage conditions.

Keywords *Tribolium confusum* · *Acanthoscelides obtectus* · Essential oil · Insecticidal effect

Introduction

Quantitative and qualitative losses appear in stored products that are attacked by microorganisms, rodents, mites, birds and insects (Franzolin et al. 1999). It has been estimated that the economic losses caused by stored anti-crop agents vary between 1,25–2,5 billion Dollars in the United States of America (Flinn et al. 2007). There are more than 600 bugs insects that harm the stored agricultural products. These insects lead to damages approximately between 10% and 40% on the stored agricultural products in the World (Tripathi et al. 2001).

Tribolium confusum (du Val., 1863) (Coleoptera: Tenebrionidae) and *Acanthoscelides obtectus* (Say, 1831) (Coleoptera: Bruchidae) are two important pest species in the order Coleoptera that damage stored products (Thakur 2012). Many control methods involving physical, chemical, biological fights were used to harmful insects on the stored products (Isman 2006). The most frequently used chemicals worldwide to control the anti-crop agents that act in storage facilities are the synthetic insecticides and fumigants (Wasala et al. 2016). The synthetic insecticides have negative effects like the damage to the environment, the excessive cost of application, the resistance they form in the insects, and killing the non-targeted living organisms (Isman 2000, 2006). Because of these negative effects of the synthetic insecticides, alternative fighting methods have been taken into consideration (Athanassiou et al. 2008).

In recent years, vegetational products have been being used against anti-crop agents, and considerable studies have been being conducted in this field. There are many studies claiming

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that essential oils may be sued for this purpose (Isman 2000; Isman and Machial 2006; Saharkhiz et al. 2011). The essential oils obtained from plants contain many compounds that cause acute toxicity, repellent effect, preventing of nutrition and limitations in development and reproduction on insects (Isman 2006; Regnault-Roger and Hamraoui 1995; Papachristos and Stamopoulos 2002). Some of the advantages of essential oils are their low costs, easy availability and using, lack of negative effects on human health and the environment (Ivbijaro 2012).

In many studies conducted so far, the extracts and components of hundreds of plant families have been investigated in terms of fumigant toxicity and it has been reported that they might be alternative in protecting stored crops (Negahban et al. 2007; Rajendran and Sriranjini 2008). It has been also reported that the essential oils obtained from the plants of the Lamiaceae and Asteraceae families have repellent effects against many harmful Coleopter species (Nerio et al. 2009).

There are many different studies on the insecticidal effects of essential oils, Stamopoulos et al. (2007) investigated the fumigant effects of essential oils against *T. confusum* and reported positive results. Theou et al. (2013) applied the essential oils obtained from *L. hybrida, L. nobilis*, *T. orientalis, C. sinensis* and *C. limon* plants against the adults of *T. confusum* and found the toxic effects to be extremely influential. Demirel et al. (2009) determined that the essential oils of *O. vulgare*, *O. onites* and *O. minutiflorum* were effective against *T. confusum* adults. In applications in which the essential oils of *Eucalyptus camaldulensis* Dehnh. and *Callistemon viminalis* G. Don were used against the adults of *T. confusum*, successful results were reported (Hamzavi and Moharramipour 2017). Karaborklu et al. (2010) used the essential oils obtained from Lamiaceae, Myrtaceae, Chenopodiaceae, Asteraceae, Rutaceae families against *Tribolium castaneum* and *Acanthoscelides obtectus* adults and obtained successful results. Papachristos and Stamopoulos (2004) recorded strong insecticidal effects of the essential oils obtained from *L. hybrida* (Labiatae), *R. officinalis* (Labiatae) and *E. globulus* (Myrtaceae) on *A. obtectus* adults. Ndomo et al. (2008) tried the essential oils obtained from the leaves of *C. anisata* (Rutaceae) on *A. obtectus* adults; and observed that there were deaths in the insects at a significant level. Similarly, Bittner et al. (2008) determined that the essential oils of *G. keule* (Gomortegaceae), *L. sempervirens* (Monimiaceae), *O. vulgare* (Labiatae), *E. globulus* (Myrtaceae) and *T. vulgaris* (Labiatae) plants had the fumigant toxicity at an extremely high level on *A. obtectus* adults.

In another study, the essential oils obtained from *R. officinalis, L. hybrida, M. microphylla* and *M. viridis* plants were reported to be the ones that had the highest toxic effects on *A. obtectus* adults (Papachristos and Stamopoulos 2002). Çetin et al. (2014), tested the fumigant effects of the essential oils obtained from 18 plants belonged to the Lauraceae, Apiaceae, Lamiaceae, Araceae and Asteraceae families on *A. obtectus*. Regnault-Roge and Hamraoui (1993) reported that 22 medical and aromatic plants especially from *Origanum marjorana* and *Thymus serpyllum*, which are form the Labiatae family, had high fumigant effects on the adults of *A. obtectus*.

The aim of this study was to determine the insecticidal effects of the essential oils obtained from the *Ocimum basilicum* L., *Rosmarinus officinalis* L. and *Artemisia dracunculus* L. plants in vitro conditions against *Tribolium confusum* du Val. and *Acanthoscelides obtectus* (Say) adults.

Materials and methods

Insect cultures

Tribolium confusum and *Acanthoscelides obtectus* adults used as test insects were obtained from a laboratory culture maintained at the Plant Protection Department, Agricultural Faculty, Ataturk University, Erzurum, Turkey, which were initially collected from hard wheat and bean seeds (cv. Seval in grain storage) in 2017 and were reared on cracked wheat and bean grains. The adults were kept in cracked wheat grains and bean seeds under laboratory conditions in cloth mesh covered plastic pots (15 cm diameter, 20 cm high) until used in the experiments as newly emerged adults with mixed sex. Each experiment was conducted with three replicates and 33 adults were used for each replicate. The adults were fed with wheat grains and bean seeds in plastic Petri dishes (9 cm) during laboratory bioassay of essential oils. The adults were subjected to experiments in the laboratory at 25 ± 1 °C, at 64 ± 5% relative humidity, and at lighting conditions of 16:8 h (light, dark).

Plant material and isolation of essential oils

The plants used in the study, *Artemisia dracunculus* L. (Asteraceae), *Rosmarinus officinalis* L. (Lamiaceae), *Ocimum basilicum* L. (Lamiaceae), were collected at the flowering stage from different localities of Turkey between June and August of 2016 and 2017. Voucher specimens have been deposited in the herbarium of Ataturk University, Faculty of Agriculture, the Department of Plant Protection, Erzurum, Turkey. Aerial parts of the plants were dried in shade and ground in a grinder. The dried plant samples (500 g) were subjected to hydro distillation for 4 h using a Clevenger-type apparatus. The oil yields of *A. dracunculus, R. officinalis* and *O. basilicum* were 1, 1.46 and 1.50% (*w*/w, dry weight basis), respectively. The yield was based on dry materials of plant samples. The essential oils were stored in a freezer at 4 °C for further tests.

Bioassays using essential oils

In order to test the toxicity of the essential oils from three different plants, 33 individuals of *Tribolium confusum* and *Acanthoscelides obtectus* adults were used. Enough amounts (20 g) bean seeds and cracked wheat were placed in each of glass petri dishes to feed tested adult insects (9 cm × 1.5 cm). Adults of *T. confusum* and *A. obtectus* in the Petri dishes were exposed separately essential oils of *A. dracunculus, R. officinalis* and *O. basilicum.* The amounts of essential oils were applied at rates of 10 and 20 μl corresponding to 76.92 and 153.84 μl/L air oils impregnated into Whatman no. 1 filter paper, which was stuck onto the inner top of the Petri dishes. A filter paper was placed at the bottom of each Petri dish (9 cm × 1.5 cm deep) and 33 adults of *T. confusum* and *A. obtectus* were placed onto filter paper containing 20 g cracked wheat and bean seeds. This prevented direct contact between the oils *T. confusum* and *A. obtectus* individuals. The Petri dishes were covered with the lid and transferred to an incubator, and then kept under standard conditions of 25 ± 2 °C, 65 ± 5 r.h. and in the darkness for 2 days. Mortalities of the adults were then counted at 24, 48, 72 and 96 h. Dichlorvos ® (10 and 20 μL/petri) was used as a positive control in the study. A Petri dish treated with only sterile water was used as control. Each assay was repeated three times for each dose and exposure time combination and insecticidal activities of the oils were expressed as percent mean mortality of the adults.

Major constituents of the essential oils of test plants has been previously reported by, Kordali et al. (2005), Gachkar et al. (2007), Sajadi (2006). A list of the constituents and grouped components of this essential oils are presented in Table 1.

Statistical analysis

The differences among the insecticidal activities of tested essential oils were determined according to analysis of variance (ANOVA) test contained in SPSS 17.0 software package. Differences between means were tested through Duncan tests and values with $p < 0.05$ were considered significantly different.

Period of study

This study was carried out in April–December in 2017.

Results and discussion

As a result of in vitro studies, it was reported that the essential oils obtained from *O. basilicum*, *R. officinalis, A. dracunculus* plants had insecticide effects at different rates and there were statistically significant differences among them. The insecticidal effects of essential oils at different concentrations on the adults of *T.confusum* and *A. obtectus* are given in Figs. 1 and 2.

Although there were not complete differences among the types at the same dosage of the essential oils applied to *T. confusum* after 24 h, this difference became clear after 48 h. In the controls performed 48 h after the application, the best death rates were determined in the essential oil of *R. officinalis* and the lowest death rate was determined in the essential oil of *O. basilicum*. After 72 h from the application, the highest death rate was determined for *R. officinalis* oil again. At 96th of the application, the lowest death rates were determined in both dosages for *A. dracunculus* oil and no significant differences were detected as a result of the application of the essential oils of *O. basilicum* and *R. officinalis.*

With the increase in the dosage, an increase was also detected in the death rate only when *R. officinalis* oil was used

Table 1 Major constituents of the essential oils of test plants

Test plants	Major constituents	Relative percent (%)	Literature
A. dracunculus	(*Z*)-anethole	81.0	Kordali et.al., [27]
	(*Z*)-β-ocimene	6.5	
	(*E*)-β-ocimene	3.1	
	limonene	3.1	
	methyl eugenol	1.8	
R. officinalis	α- Pinene	14.9	Gachkar et.al., [28]
	1,8-Cineole	7.43	
	Linalool	14.9	
O. basilicum	methyl chavicol	52.4	Sajadi, [29]
	linalool	20.1	
	epi-α-cadinol	5.9	
	trans-α-bergamotene	5.2	

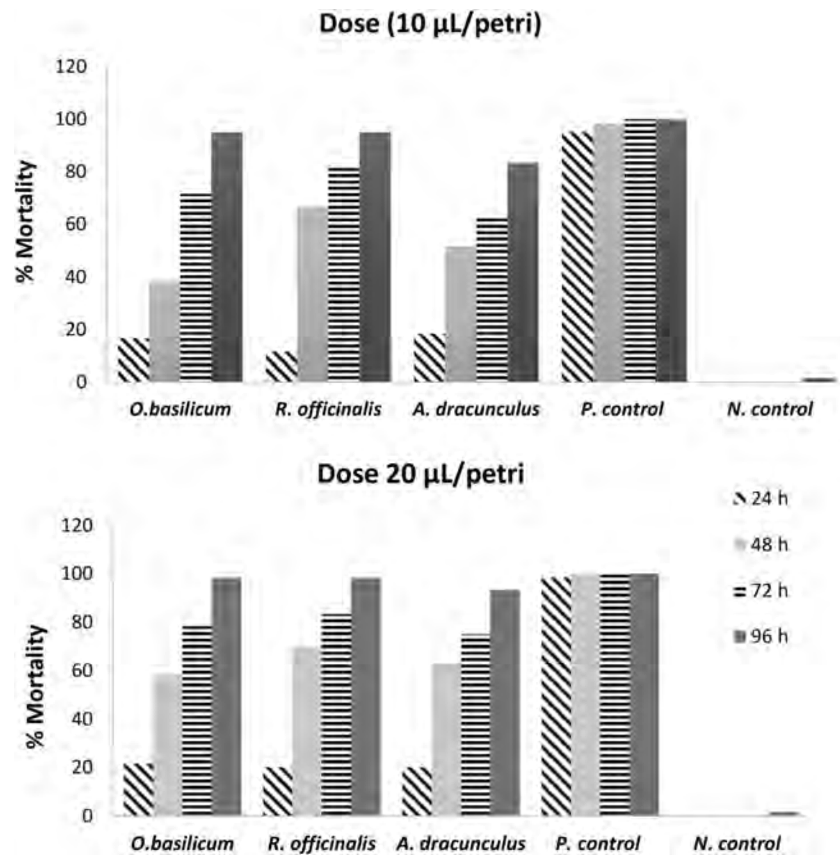

Fig. 1 Percent mortality of adults of *Tribolium confusum* after treatment with 10, 20 μL/petri doses essential oils and treatment times

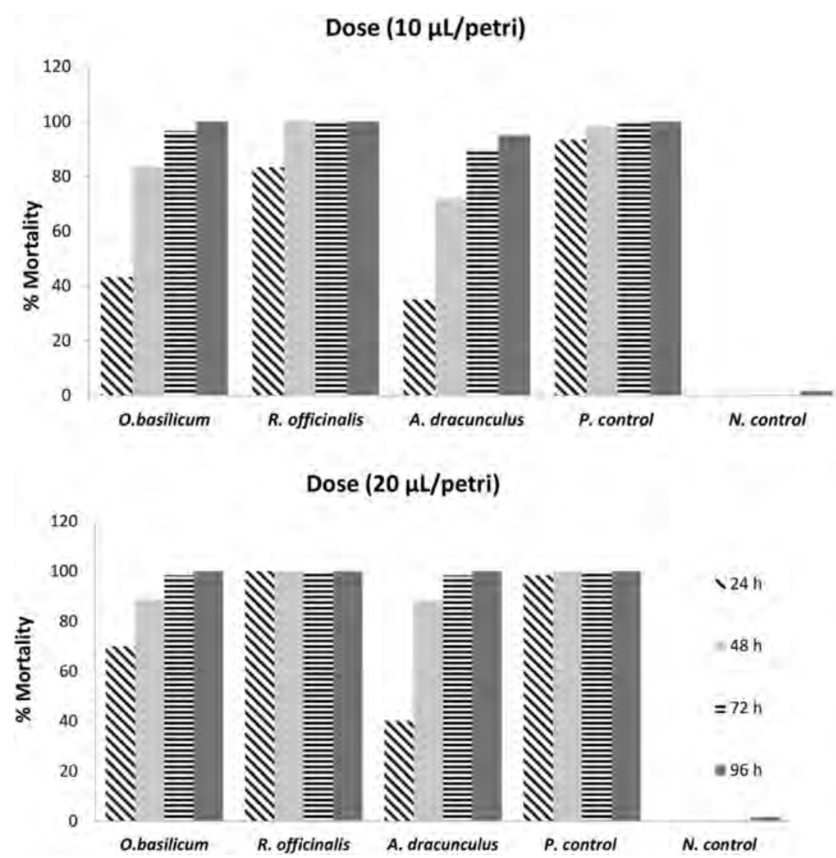

Fig. 2 Percent mortality of adults of *Acanthoscelides obtectus* after treatment with 10, 20 μL/petri doses essential oils and treatment times

Table 2 Toxicity of three plant essential oils on adults of *T.confusum* after 24, 48, 72 and 96 h

treatment Essential oils	Dose (μL/petri)	Exposure time (h) – Mortality (%)			
		24 h	48 h	72 h	96 h
O. basilicium	10	16.6 ± 1.7 bc	38.3 ± 4.4 b	71.6 ± 3.3 c	95.0 ± 2.9 cd
	20	21.6 ± 3.3 c	58.3 ± 1.7 d	78.3 ± 1.7 de	98.3 ± 1.3 cd
R. officinalis	10	11.6 ± 1.7 b b	66.6 ± 1.4 ef	81.6 ± 1.7 ef	95.0 ± 2.9 cd
	20	20.0 ± 2.9 c	70.0 ± 2.9 f	83.3 ± 1.7 f	98.3 ± 1.3 cd
A.dracunculus	10	18.3 ± 1.7 c	51.6 ± 1.7 c	63.3 ± 1.7 b	83.3 ± 1.7 b
	20	20.0 ± 2.9 c	63.3 ± 3.3 de	75.0 ± 2.9 cd	93.3 ± 3.3 c
Pozitive Control	10	95.0 ± 2.9 d	98.3 ± 1.7 g	100 ± 0.0 g	100 ± 0.0 d
(Dichlorvos)	20	98.3 ± 1.7 d	100 ± 0.0 g	100 ± 0.0 g	100 ± 0.0 d
Negative Control (Ethanol+Sterile water mix)		0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	1.66 ± 1.4 a

Values followed by different letters in the same column differ significantly at $P \leq 0.05$ according to Duncan Multiple test

Mean ± SE of three replicates, each set up with 33 adults

after 24 h and an increase was also detected in the *O. basilicum* and *A. dracunculus* oils after 48 h. After 72 h, when the dosage was increased, it is possible to claim that the application of essential oils of *O. basilicum* and *A. dracunculus* oils were influential (Table 2).

In the results of the essential oil application to *A. obtectus*, the best and significant death rates were obtained with the same dosage (10 μL) among the types with the essential oil of *R.officinalis* after 24 h, this superiority increased with the 20 μL dosage. After the other exposure durations, *R. officinalis* oil had a superiority with 100% death rate. After 72 h, again the highest death rate was obtained for *R. officinalis* oil and after 96 h, the lowest death rate was obtained for *A. dracunculus* oil with 10 μL dosage and no significant differences were determined in the others (Table 3).

Especially when the dosage was 20 μL, the death rate increased up to over 95% after 96 h for all types. At 10 μL dosage of *A. dracunculus* oil, the death rate was 83% at the highest even after 96 h. Dichlorvos® was used as the positive control in essential oil trials against the tested insect adults and it was determined that 93.3% death rate was determined at 10 μL/ petri dose at 24th h, 98.3% death rate was determined at 20 μL/petri dose, and this rate was 100% at 48th h.

In this study, in which three different essential oils were used, the death rates for *T. confusum* was between 83.3 and 100%. Boussaadai et al. (2008) investigated the insecticidal effects of 16 different aromatic plant essential oils from Asteraceae family against *T. confusum* and reported that these plant essential oils were effective at rates of between 77 and 83%. Rahdari and Hamzei (2017) investigated the effect of the essential oil obtained from *R. officinalis* on *T. confusum* and

Table 3 Toxicity of three plant essential oils on adults of *A. obtectus* after 24, 48, 72 and 96 h

Treatment essential oils	Dose (μL/petri)	Exposure time (h) – Mortality (%)			
		24 h	48 h	72 h	96 h
O. basilicium	10	43.3 ± 13.3 b	83.3 ± 4.4 c	96.6 ± 3.3 c	100 ± 0.0 c
	20	70.0 ± 2.9 c	88.8 ± 1.7 cd	98.3 ± 1.7 c	100 ± 0.0 c
R. officinalis	10	83.3 ± 4.4 d	100 ± 0.0 e	100 ± 0.0 c	100 ± 0.0 c
	20	100 ± 0.0 e	100 ± 0.0 e	100 ± 0.0 c	100 ± 0.0 c
A.dracunculus	10	35.0 ± 2.9 b	71.6 ± 11.6 b	90.0 ± 2.9 b	95.0 ± 2.9 b
	20	40.0 ± 5.8 b	88.3 ± 1.7 cd	98.3 ± 1.7 c	100 ± 0.0 c
Pozitive Control	10	93.3 ± 1.7 de	98.3 ± 1.7 de	100 ± 0.0 c	100 ± 0.0 c
(Dichlorvos)	20	98.3 ± 1.7 e	100 ± 0.0 e	100 ± 0.0 c	100 ± 0.0 c
Negative Control (Ethanol+Sterile water mix)		0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	1.7 ± 1.4 a

Values followed by different letters in the same column differ significantly at $P \leq 0.05$

Mean ± SE of three replicates, each set up with 33 adults observed that there was death at a rate of 86,22% after 24 h. Khani and Rahdari (2012) investigated the insecticidal effect of the essential oil extracted from *Coriandrum sativum* L. on *T.confusum* and the death rate was found to be over 90%. Hamzavi and Moharramipour (2017) investigated the effects of the essential oils of *Eucalyptus camaldulensis* Dehnh. and *Callisteman viminalis* L. on *T.confusum*; and observed that all the individuals died after 72 h.

In the present study, the death rates of three different essential oils were between 95 and 100% for *A. obtectus*. Çetin et al. (2014) investigated the fumigant effects of the essential oils obtained from 18 plants of Lauraceae, Lamiaceae, Araceae and Asteraceae families on *A. obtectus,* and determined that 100% death rates were achieved after 24 h with *R. officinalis* oil. Carlos et al. (2016) tested the effects of *Ocimum basilicum* L. essential oil on *A. obtectus*; and observed that there were at different rates deaths after 48 h. Harvet et al. (2012) researched the effect of the essential oil of *Zanthoxylum xanthoxloides* DC. on *A. obtectus* adults. In their study, the mortality rates of *A. obtectus* adults were observed up to 100%. Similarly, Regnault-Roge and Hamraoui 1993tried to determine the insecticidal effects of the essential oils obtained from aromatic and medical 22 different plants and determined that among them, *Origanium majorama* L. and *Thymus serpyllum* L. essential oils were more effective. Ayvaz et al. (2010) studied the efficiency of the essential oils extracted from 10 aromatic plants on *A. obtectus* and found successful results.

Conclusions

It has already been known that plant-based insecticides do not have or have little effects on the organisms in the environment and on the non-targeted organisms and affect insects from different types in different ways. As a result of the present study, the effects of the essential oils extracted from three different plants on the adults of *T. confusum* and *A. obtectus*, which are important pests on the stored products were established. It is possible to claim that all the essential oils used in the present study are toxic for the adults of *T. confusum* and *A. obtectus* and show a satisfying activity. However, using the essential oil of *R.officinalis* at 20 μL dosage for 24 h or 10 μL dosage after 48 h will be more accurate because these dosages cause 100% death in the insects.

Compliance with ethical standards

Conflict of interest Authors; Temel Gokturk, Saban Korali, Kibar Ak, Memis Kesdek and Ayse Usanmaz Bozhuyuk declares that they have no conflict of interest.

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