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Fumigant and repellent activity of Rutaceae and Lamiaceae essential oils against *Acanthoscelides obtectus* Say

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Acanthoscelides obtectus **Say, a principal pest of kidney beans** ***Phaseolus vulgaris*** **L. in the Mediterranean area, is a multivoltine and oligophagous bruchid that damages not only host plant (kidney bean) but also other** ***Leguminosae*** **species. Essential oils of Rutaceae:** ***Citrus reticulata*** **L. and** ***Citrus limonum*** **L. and of Lamiaceae** ***Mentha piperita*** **L. and** ***Lavandula angustifolia*** **L. were evaluated by fumigation upon** ***A. obtectus*** **adults using concentrations of 13.33, 40, 80 and 106.66 µl/L air. Their vapours have a toxic increasing effect depending upon concentrations and time exposure. Lamiaceae essential oils exhibited the higher fumigant activity inducing mortality of about 37.5 to 100% and 45.25 to 95% for, respectively lavender and peppermint essential oils after 24 h only. Mortality percentages of 85 and 72.5%, respectively for** ***C. limonum*** **and** ***C. reticulata*** **were recorded after exposure time of 96 h at the highest dose. Repellency activity studied at 0.105, 0.315, 0.631 and 0.842 µl/cm^2 concentrations showed a higher repellency percentage of 71.25% for peppermint essential oil on** ***A. obtectus*** **adults. Our results suggest that Lamiaceae essential oils more than Rutaceae ones may be useful as a seed protecting tool with fumigation effects against** ***A. obtectus*** **and could replace synthetic insecticides which are harmful to the environment.**

Key words: *Acanthoscelides obtectus, Phaseolus vulgaris,* essential oils, repellency, fumigant toxicity.

INTRODUCTION

The bean beetle *Acanthoscelides obtectus* Say (Coleoptera, Chrysomelidae, Bruchinae) is a cosmopolitan and polyvoltin insect which attacks its host plant *Phaseolus vulgaris* L. and other Fabaceae such as those in the genus *Vigna* (Leroi and Jarry, 1981; Dobie et al., 1984). As in several other bruchids, *A. obtectus* can complete its development both in maturing seeds and in stored ones (Thiery et al., 1994). Its life cycle on stored beans can occur without returning to the field (Labeyrie, 1962) causing crop losses of about 80% after six to

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seven months of storage (Idi, 1994). Control of this stored product pest is of great importance especially in developing countries where Leguminosae seeds represent a fundamental food resource (Abate and Ampofo, 1996). The most frequently used method and economical tool for managing stored grain insect pests is a chemical one often through fumigation (Mueller, 1990). Since this method pose human health risks, there is actually claim to natural products (Isman, 2000; Huignard et al., 2011). Essential oils are produced in 17500 aromatic species of higher plants belonging mostly to a few families, including the Myrtaceae, Lauraceae, Lamiaceae and Asteraceae which hide a real insecticide arsenal capable of crop protection (Regnault-Roger et al., 2012).

Essential oils of several aromatic plants have been studied in Algeria, demonstrating their efficiency upon stored product pests *Callosobruchus maculatus* (F.) (Kellouche and Soltani, 2004; Kellouche, 2005), *Bruchus rufimanus* (Boh.) (Medjdoub-Bensaad et al., 2007) and *A. obtectus* (Bouchikhi et al., 2008, 2010).

The present study aims to evaluate fumigant and repellent insecticidal activity of essential oils of lemon (*Citrus reticulata* L.), tangerine (*Citrus limonum* L.) (Rutaceae); peppermint (*Mentha piperita* L.) and lavender (*Lavandula angustifolia* L.) (Lamiaceae) as control alternatives against a stored product beetle *A. obtectus.*

MATERIALS AND METHODS

Insects

Acanthoscelides obtectus strain came from a sample collected in Tlemcen (34° 52' 41"N 1° 18' 53"W) (Algeria) and was reared in a dark incubator under controlled conditions (30 ± 1°C and 70 ± 5% relative humidity) on *P. vulgaris* dry beans (Rognon blanc cultivar). Insects were maintained into glass flasks (1 L capacity) closed at the top with a fine mesh nylon screen to allow aeration. Every two months, unsexed adults, new emerged, were transferred on fresh beans for rearing. Adult insects, of 0 to 24 h old, were removed daily from the livestock insect and used in each bioassay.

Essential oils

The plants from which essential oils were extracted were lemon (*C. reticulata* L.), tangerine (*C. limonum* L.), peppermint (*M. piperita* L.) and lavender (*L. angustifolia* L.) collected from Tizi Ouzou (36° 42' 42"N 4° 02' 45''E) (Algeria). Essential oils were obtained by hydrodistillation of fresh peel for Rutaceae and from peppermint leaves and lavender flowered tops for Lamiaceae.

Fumigation bioassay

Fumigation bioassays were carried out with 10 adults (5 males and 5 females of 0 to 24 h old) exposed in Plexiglas bottles (750 ml capacity). Filter papers (Whatman number 1) were attached to inner side of the bottle screw cap and impregnated with different oil doses: 10, 30, 60 and 80 µL, giving calculated fumigant concentrations corresponding, respectively to 13.33, 40, 80 and 106.66 µl/L air. Four replications were made for each concentration and control. Mortality was recorded after 24, 48, 72 and 96 h until total death of individuals.

Repellency bioassay

Repellent effects of essential oils on *A. obtectus* adults were assessed using preferential zone method on filter paper described by Mc Donalds et al*.* (1970). Whatman filter papers (diameter 11 cm) were cut in equal halves. Then 10, 30, 60 and 80 µL corresponding to 0.105, 0.315, 0.631 and 0.842 µl/cm^2 of essential oils were dissolved in 0.5 ml acetone and applied uniformly to a half filter disc; while the other half received 0.5 ml acetone alone as control. After complete evaporation of a solvent (15 mn), discs were reconstituted then placed in Petri dishes (diameter 11 cm). Ten adults beetles (5 males and 5 females of 0 to 24 h old) were introduced in the filter paper center of each Petri dish sealed immediately using four replications for each treatment. After 30 min of treatment at 25°C, number of insects present on the treated (Nt) and non treated (Nc) halves was recorded. Repellency percentage (RP) was calculated as follows: RP (%) = [(Nc-Nt) / (Nc+Nt)] ×100. Mean repellency percentages were calculated and compared with Mc Donald et al*.* (1970) classification which proposed five classes: class 0 (RP<0.1%), class I (0.1% ≥RP≤20%), class II (20.1%≥RP≤40%), class III (40.1%≥RP≤60%), class IV (60.1% ≥RP≤80%) and class V (RP≥80.1%).

Data analysis

The data obtained were submitted to one way analysis of variance using Statbox version 6.3 (Grimmersoft, 2004). Newman and Keuls test was performed to compare means ($P < 0.05$) (Dagnelie, 1975).

RESULTS

Fumigant toxicity

Fumigant activity of essential oils is shown in Table 1. It was highly dependent upon oil concentration and exposure time. Essential oils from Lamiaceae compared to Rutaceae showed the highest fumigant effect against *A. obtectus.* At the lowest concentration, 13.33 µl/L air, lavender and peppermint essential oils showed comparable percentage mortality of 37.5 ± 28.7% and 45 ± 25.16 after 24 h, 90 ± 14.14% and 95 ± 5.77% after 48 h and 100% after 72 h. The maximum activity (100% mortality) was recorded after only 24 h of time exposure to a highest concentration of 106.66 µl/L air. Rutaceae essential oils vapours were less toxic to *A. obtectus*. Percentage mortalities of 85 ± 5.77% and 72.5 ± 5% were observed, respectively for lemon and tangerine essential oils after time exposure of 96 h while only 17.5 ± 9.57% and 12.5 ± 5% of beetles died at the lowest concentration (13.33 µl/L air).

Repellent activity

Repellent activity percentages evaluated for the 4 essential oils are given in Table 2. One way variance analysis showed highly significant differences for all

Table 1. Mortality percentage (Mean±SD) of *Acanthoscelides obtectus* adults exposed to *C. limonum*, *C. reticulata, L. angustifolia* and *M. piperita* essential oils vapours.

Essential oils	Doses µl/L air	24 h	48 h	72 h	96 h
	0	0±0[c]	0±0[d]	0±0[d]	0±0[d]
	13.33	10±0[b]	10±0[c]	10±0[c]	17.5±9.57[c]
C. limonum	40	7.5±5[b]	15±5.77[bc]	17.5±9.5[bc]	17.5±9.5[c]
	80	12.5±5[b]	20±8.16[b]	25±5.77[b]	35±5.77[b]
	106.66	70±0[a]	72.5±5[a]	85±5.77[a]	85±5.77[a]
	0	0±0[b]	0±0[d]	0±0[d]	0±0[d]
	13.33	7.5±5[b]	12.5±5[c]	12.5±5[c]	12.5±5[c]
C. reticulata	40	10±0[b]	17.5±9.5[c]	22.5±5[b]	27.5±5[b]
	80	10±8.16[b]	27.5±5[b]	30±8.16[b]	32.5±9.57[b]
	106.66	35±5.77[a]	45±5.7[a]	57.5±5[a]	72.5±5[a]
	0	0±0[c]	0±0[b]	0±0[b]	0±0[b]
	13.33	37.5±28.7[b]	90±14.14[a]	100±0[a]	100±0[a]
L. angustifolia	40	80±24.49[a]	95±5.77[a]	100±0[a]	100±0[a]
	80	100±0[a]	100±0[a]	100±0[a]	100±0[a]
	106.66	100±0[a]	100±0[a]	100±0[a]	100±0[a]
	0	0±0[c]	0±0[b]	0±0[b]	0±0[b]
	13.33	45±25.16[b]	95±5.77[a]	100±0[a]	100± 0[a]
M. piperita	40	90±8.16[a]	100±0[a]	100±0[a]	100±0[a]
	80	92.5±9.57[a]	100±0[a]	100±0[a]	100±0[a]
	106.66	95±5.77[a]	100±0[a]	100±0[a]	100±0[a]

All experiments were replicated four times. P-value: significant differences determined by a one way ANOVA. Numbers followed by the same letter in each table row are not significantly different at 0.05 level (Newman and Keuls test).

Table 2. Repulsion means percentages of *M. piperita, L. angustifolia, C. reticulata* and *C. limonum* essential oils against *Acanthoscelides obtectus* adults classified according to Mc Donalds et al. (1970).

Doses µl/cm^2 essential oils	0.105	0.315	0.631	0.842	Repulsion Mean %	Class
M. piperita	50±16.32	70±19.14	80±18.3	85±16.34	71.25	Repulsive (IV)
L. angustifolia	30±11.5[c]	60±16.3[b]	75±10[ab]	90±11.5[a]	63.75	Repulsive (IV)
C. reticulata	40±8.16[c]	35±5.77[c]	80±8.1[b]	100±00[a]	63.75	Repulsive (IV)
C. limonum	10±4.08[d]	45±5.77[c]	55±5.7[b]	65±5.77[a]	43.75	Moderately repulsive (III)

All experiments were replicated four times. Significant differences determined by a one way ANOVA. Numbers followed by the same letter in each table row are not significantly different at 0.05 level (Newman and Keuls test). Classes are given according to Mc Donalds et al. (1970).

essential oils except peppermint essential oil. The latter is the more strongly repulsive since at the lowest dose (0.105 µl/cm^2), it induced repulsion rate of 50 ± 16.32% showing consequently the highest mean repulsion rate of 71.25% calculated according to Mc Donalds et al. (1970). Repulsion activity evaluated after a time exposure of 30 min is doses dependent. Significant differences were observed between treatments as the doses increase with a maximum of repulsion recorded at the highest concentration (0.842 µl/cm^2); it was of about 85 ± 16.34, 90 ± 11.54, 100 ± 00 and 65 ± 5.77%, respectively for peppermint, lavender, tangerine and lemon essential oils.

DISCUSSION

The four essential oils screened for fumigant activity demonstrated toxicity against *A. obtectus* adults. The insecticidal activity varied with oil concentrations and time exposure. Results showed higher efficiency of Lamiaceae essential oils compared to Rutaceae.

Fumigant toxicity of essential oils and their major compounds, volatile monoterpenoids was largely described especially for Mediterranean species (Regnault-Roger et al*.,* 1993; Regnault-Roger and Hamraoui, 1995; Regnault-Roger and Hamraoui, 1994). According to Regnault-Roger and Hamraoui (1993), essential oils extracted by hydrodistillation from Lamiaceae, Myrtaceae, Lauraceae and Gramineae present fumigant toxicity against *A*. *obtectus* adults especially, *Thymus serpyllum*, *Thymus vulgaris* and *Lavandula angustifolia* essential oils which induce a mortality of 95 to 100% after 24 and 48 h when exposed, respectively to 160, 136.1 and 145 mg/dm^3.

Essential oils from Rutaceae seem to exert a low activity against *A. obtectus* adults. Our results corroborate with those obtained by Papachristos and Stamopoulos (2002) who showed that essential oils of *Citrus sinensis* have a low action on *A. obtectus* with LC_{50} of 11.4 µl/L air for males and 19.54 µl/L air for females while *Lavandula hybrida* LC_{50} was 1.64 µl/L air for males and 2.34 µl/L air for females in comparison to Lamiaceae (basil, peppermint and lavender) and Myrtaceae (eucalyptus) essential oils. The same authors screened 13 essential oils for their fumigant activity against *A. obtectus* adults and reported that Lamiaceae essential oils were more strongly toxic to males than females and noted LC_{50} of about 1.1, 1.2, 1.6 and 2.1 µl/L air for *Mentha microphylla*, *Mentha viridis*, *L. hybrida* and *Rosmarinus officinalis* essential oils, respectively. Ayvaz et al*.* (2010) investigated insecticidal activity of oregano (*Origanum onites* L.), savory (*Satureja thymbra* L.) (Lamiaceae) and myrtle (*Myrtus communis* L.) (Myrtaceae) against three stored product insects. They reported that *A. obtectus* was the most tolerant to essential oils with the most toxic action attributed to *M. communis* (linalool) while oregano and savory essential oils were highly efficient against the Mediterranean flour moth (*Ephestia kuehniella* Zeller) and the Indian meal moth (*Plodia interpunctella* Hûbner) (Lepidoptera: Pyralidae) inducing 100% mortality after 24 h exposure at 9 and 25 µl/L air for *P. interpunctella* and *E. kuehniella,* respectively. Raja et al*.* (2001) reported that oils derived from *Mentha arvensis*, *M. piperita*, *M. spicata* and *Cymbopogon nardus* influenced significantly adult mortality, oviposition and adult emergence of cowpea beetle (*Callosobruchus maculatus* (F.)).

Similar effects of volatile components of essential oils of laurel, rosemary and lavender were described by Shaaya et al. (1997) for *A. obtectus* and for other stored products pests like *Oryzaephilus surinamensis* (L.), *Sitophilus oryzae* (F.) and *Rhyzoperta dominica* (L.). These authors recorded a mortality of 85 to 100% after 4 days exposure to a dose of 70 µl/L air. However, LC_{50} values were higher than those recorded on *A. obtectus* adults. Rossi et al*.* (2012) studied insecticidal properties of *Citrus bergamia* L. essential oil vapours on *Sitophilus oryzae* adults and showed significant mortality after 96 h at 10 $\mu l\ cm^{-2}$ dose; they also observed that a dose of 0.75 $\mu l\ insect^{-1}$ induced a relatively high mortality of about 65 and 83% after 24 and 96 h, respectively showing LD_{50} of 0.36 $\mu L\ insect^{-1}$ after 96 h.

In addition to the fumigant toxicity, a studied essential oils exhibited repulsion activity against *A. obtectus* adults. Peppermint, lavender and tangerine essential oils were classed in the repulsive class according to Mc Donalds et al*.* (1970) and the Lamiaceae essential oils again were highly effective against *A. obtectus* adults. Essential oils showed significant variations in their repulsion activity depending upon several factors especially their chemical composition. Although the chemical analysis was not performed for the studied essential oils, the superiority of the insecticidal potential of peppermint and lavender can be attributed to their major components, menthol and menthone (Kumar et al., 2011) for the first and linalool and linalyle acetate (Rossi et al., 2012) for the second. Papachristos and Stamopoulos (2002) reported that *M. viridis*, *E. globulus*, *M. microphylla*, *R. officinalis* and *L. hybrida* essential oils showed high repulsive effects compared to *Thuja orientalis*, *Citrus sinensis* and *Pistacia terebinthus* essential oils concluding, as in our study, that the most toxic essential oils exhibited in the same time high repulsive effects and inhibited beetle reproduction. Aggarwal et al. (2001) evaluated L-menthol and seven of its acyl derivates for repellent activity against *C. maculatus*, *T. castaneum*, *S. oryzae* and *R. dominica* and found the repellent activity of 100, 82, 78 and 72%, respectively at the concentration of 20 µg in 1 ml acetone.

Conclusion

The results of fumigant and repellent activity of peppermint, lavender, tangerine and lemon essential oils against *A. obtectus*, a serious pest in the stocks, permitted to propose natural products of plants from Mediterranean area as alternative to chemical control. As it was studied against several stored grain pests and vectors for its fumigant and repellent activity (Kumar et al., 2011), *Mentha piperita* essential oil seem to be the most strongly effective. More studies should be undertaken using other doses and other indigenous plants so that a range of organic insecticides could be produced in order to preserve nutritional quality of kidney beans in the stocks, especially in developing countries where this commodity constitute a fundamental food resource.

Conflict of interests

The authors have not declared any conflict of interest.

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