Antifeedant effect of Mediterranean plant essential oils upon *Acanthoscelides obtectus* Say (Coleoptera), bruchid of kidney beans, *Phaseolus vulgaris* L.

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Abstract

The insecticidal effect of 23 essential oils from mediterranean plants (Labiateae, Umbellifereae, Lauraceae, Rutaceae, Myrtaceae, Gramineae and Myristicaceae) was tested on *Acanthoscelides obtectus* Say, one of the most damaging pests of stored legumes. The oils exhibited fumigant effect on adults and decreased both oviposition and larval growth. Reduced larval penetration and development inside artificial seeds made from cotyledon flour of *Phaseolus vulgaris* were due to antifeedant activity. Slight activity was produced by *Laurus nobilis*, *Eucalyptus globulus* and *Mentha piperata* and the strongest activity was produced by *Anethum graveolens*, *Ocimum basilicum*, *Myristica fragrans* and *Cuminum cyminum*.

Introduction

The use of traditional protectants of crops is an old and common practice (Golob and Webley 1980) and, recently, insecticidal and repellent properties of plants have been underlined (Anjana et al. 1988; Saxena 1989; Rajapakse 1990; Morallo-Rejesus et al. 1990; Regnault-Roger and Hamraoui 1993). Among the most efficient upon *Acanthoscelides obtectus* Say (Coleoptera), the bruchid of kidney bean, *Phaseolus vulgaris* L., the Labiateae family was identified. These plants are well known to provide essential oils and we observed that the hydrodistillated extract presented two kinds of toxicity upon the beetle: a strong and rapid effect induced by vapours but also, at lower concentrations, an inhibition of reproduction by decreasing oviposition or by producing ovicidal and larvicidal effects (Regnault-Roger et al. 1993; Regnault-Roger and Hamraoui 1994).

In this work, we intend to complete the previous observations and to study if essential oils have an antifeedant effect upon *Acanthoscelides obtectus* Say. This activity will increase interest in using essential oils for management of stored-product pests.

Material and Methods

Biological

Insects (*A. obtectus* Say) and beans (*Phaseolus vulgaris* L.) were laboratory reared. Fertilised eggs were chosen for this experiment. Beetles were kept in a room at 27°C with a moisture level of 65–75% and photoperiod 12 hours light/12 hours dark.

Botanicals

Leguminoseae seeds were hulled and cotyledons reduced to flour which was riddled (mesh 0.5mm). Aromatic plants used in the experiment (Table 1) were obtained either from the Institute fields or the local market. Essential oils were extracted by steam distillation (Guenther 1972). Two samples of *Thymus vulgaris* were experimented because of the numerous chemotypes.

Bioassay

Each essential oil was incorporated into the seed flour. Two series were done: respectively 99.5% and 99% of flour for

Table 1. Botanical classification of tested essential oils (according to G. Bonnier 1990).

Botanical species	Family	Sample number in experiments
Anethum graveolens L.	Umbellifereae	20
Apium graveolens Houtt	Umbellifereae	18
Cinnamomum verum Presl	Lauraceae	15
Citrus limon (L) Brum F.	Rutaceae	21
Coriandum sativum L.	Umbellifereae	23
Cuminum cyminum L.	Umbellifereae	17
Cymbopogon nardis Wats	Gramineae	11
Eucalyptus globulus Labill.	Myrtaceae	6
Laurus nobilis L.	Lauraceae	7
Lavandula angusifolia P. Miller	Labiateae	5
Mentha piperata L.	Labiateae	2
Myristica fragrans L.	Myristicaceae	19
Ocimum basilicum L.	Labiateae	14
Origanum majorana L.	Labiateae	13
Origanum vulgare L.	Labiateae	10
Pertoselinum sativum L.	Umbellifereae	16
Rosmarinus officinalis L.	Labiateae	3
Salvia officinalis L.	Labiateae	12
Satureia hortensis L.	Labiateae	4
Thymus serpyllum L.	Labiateae	1
Thymus vulgaris L.	Labiateae	8 and 9
Verbena officinalis L.	Labiateae	22

N.B.: sample n°24 is control (no essential oils)

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0.50% and 1% of essential oils (w:w). The mix was homogeneous and dry (laboratory temperature) before being included in artificial seeds (gelatine capsule). The reproduction of the beetle was previously studied with this material (Hamraoui and Regnault-Roger, 1994). Each series included ten pierced artificial seeds (ten holes by capsule). Each seed was put with ten fertilised eggs, oviposited on the same day. The penetration of larvae inside the capsules and the adult emergence counted. Each series plus a control (*Phaseolus vulgaris* L. flour without essential oil) were replicated ten times.

Statistical analysis

Statistical studies performed a one factor variance analysis ANOVA test (Sokal and Rohlf 1981) followed by a rank of the averages by Newman-Keuls (N.K.) test (Dagnelie 1973) and a t-test.

Results

Essential oils did not appear to decrease significantly the larval penetration of the beetle. N.K. test distinguished four groups for the lower concentration of essential oils and two for the higher amount but groups including the control greatly overlapped (Table 2). The influence of the doses could be noticed for some essential oils. Conversely, a strong inhibition of emergence was observed (Table 3). Several essential oils completely depressed the emergence even at lower concentration (*R. officinalis, S. officinalis, O. majorana, C. nardus, O. majorana, O. basilicum, C. verum, C. cyminum, M. fragrans, Anethum graveolens*). This effect was enhanced with the dose, in particular for *C. limon* and *T. vulgaris*. The weak effect was induced by *L. nobilis*, *M. piperata* and *E. globulus* in both cases but the larvicide effect increased with the higher concentration and no more than 45% fertilised eggs could be transformed into imagos.

Table 2. Influence of essential oils incorporated into artificial seeds upon the larvae penetration of *Acanthoscelides obtectus* Say (n = 10 ± SD).

Series No.	Essential oils (E.O.)	Average for		t-test
		0.5% E.O.	0.5 % E.O.	
1	*T. serpyllum*	3.8 ± 2.8	3.1 ± 2.9	
2	*M. piperata*	7.0 ± 2.3	5.2 ± 2.6	
3	*R. officinalis*	3.5 ± 2.7	2.9 ± 3.2	
4	*S. hortensis*	4.9 ± 2.5	2.9 ± 2.4	
5	*L. angustifolia*	4.9 ± 2.2	4.2 ± 2.4	
6	*E. globulus*	6.0 ± 2.6	6.5 ± 2.1	
7	*L. nobilis*	7.9 ± 2.8	4.9 ± 2.9	*
8	*T. vulgaris 1*	5.0 ± 2.5	3.7 ± 2.3	
9	*T. vulgaris 2*	5.6 ± 2.2	4.9 ± 3.2	
10	*O. vulgare*	4.2 ± 2.5	3.8 ± 1.3	
11	*C. nardus*	4.9 ± 3.4	6.2 ± 2.6	
12	*S. officinalis*	7.0 ± 2.9	4.5 ± 2.6	*
13	*O. majorana*	5.1 ± 1.7	4.3 ± 1.8	
14	*O. basilicum*	4.9 ± 1.9	5.9 ± 2.1	
15	*C. verum*	2.8 ± 2.0	3.1 ± 2.2	
16	*P. sativum*	5.2 ± 2.8	3.3 ± 2.8	
17	*C. cyminum*	4.0 ± 1.4	2.5 ± 1.9	
18	*Apium graveolens*	3.0 ± 2.0	2.6 ± 2.0	
19	*M. fragrans*	4.0 ± 1.9	5.5 ± 2.5	
20	*Anethum graveolens*	4.6 ± 2.1	5.6 ± 2.7	
21	*C. limon*	7.8 ± 2.2	4.9 ± 2.5	*
22	*V. officinalis*	7.2 ± 2.9	4.5 ± 2.1	*
23	*C. sativum*	4.2 ± 1.3	3.1 ± 1.7	
24	Control	7.8 ± 1.8	7.8 ± 1.8	
	Variance analysis	$F = 4.13$ $P<10^{-4}$	$F = 3.23$ $P<10^{-4}$	
	N–K test	-7.21.24.22.12.2.6.9.16. 13.8.5.14.11.4.20.10.23 -21.24.22.12.2.6.9.16. 13.8.5.14.11.4.20.10. 23.19.17 -22.12.2.6.9.16.13.8.5. 14.11.4.20.10.23.19. 17.1.3. -6.9.16.13.8.5.14.11.4.20 10.23.19.17.1.3.18.15	-24.6.11.14.20.19.2.7.21. 9.22.12.13.5 -6.11.14.20.19.2.7.21.9. 22.12.13.5.10.8.16.23. 15.1.3.4.18.17	

Table 3. Influence of essential oils incorporated into artificial seeds upon the emergence of *Acanthoscelides obtectus* Say (n = 10 ± SD).

Series No.	Essential oils	Average for		t-test
		0.5% E.O.	1% E.O.	
1	*T. serpyllum*	0.00 ± 0.00	0.00 ± 0.00	
2	*M. piperata*	5.80 ± 2.46	5.00 ± 2.44	
3	*R. officinalis*	0.00 ± 0.00	0.00 ± 0.00	
4	*S. hortensis*	0.00 ± 0.00	0.00 ± 0.00	
5	*L. angustifolia*	0.50 ± 1.26	0.00 ± 0.00	
6	*E. globulus*	4.70 ± 2.20	4.70 ± 1.88	
7	*L. nobilis*	6.50 ± 1.50	4.80 ±3.08	
8	*T. vulgaris 1*	2.70 ± 2.00	0.00 ± 0.00	*
9	*T. vulgaris 2*	1.90 ± 1.66	0.00 ± 0.00	*
10	*O. vulgare*	0.00 ± 0.00	0.00 ± 0.00	
11	*C. nardus*	0.00 ± 0.00	0.00 ± 0.00	
12	*S. officinalis*	1.10 ± 2.02	0.00 ± 0.00	
13	*O. majorana*	0.00 ± 0.00	0.00 ± 0.00	
14	*O. basilicum*	0.00 ± 0.00	0.00 ± 0.00	
15	*C. verum*	0.00 ± 0.00	0.00 ± 0.00	
16	*P. sativum*	0.00 ± 0.00	0.00 ± 0.00	
17	*C. cyminum*	0.00 ± 0.00	0.00 ± 0.00	
18	*Apium graveolens*	0.60 ± 1.26	0.00 ± 0.00	
19	*M. fragrans*	0.00 ± 0.00	0.00 ± 0.00	
20	*Anethum graveolens*	0.00 ± 0.00	0.00 ± 0.00	
21	*C. limon*	2.90 ± 1.59	0.40 ± 0.96	*
22	*V. officinalis*	1.20 ± 1.22	0.00 ± 0.00	*
23	*C. sativum*	1.00 ± 1.05	0.10 ± 0.31	
24	Control	7.80 ± 1.81	7.80 ± 1.81	
	Variance analysis	$F=34.73$ $P<10^{-4}$	$F=44.88$ $P<10^{-4}$	
Analysis	N–K test	-24.7.2 –6 –21.89 9.22.12.23.18.5.3 1.16.13.11.10.15.4.17.19.14.20	–24 –2.7.6 –21.23.9.5.16.3.1. 10.20.11.17.7.4 14.13.19.15.12.22.18	

Discussion

Essential oils incorporated into the flour of *Phaseolus vulgaris* clearly decreased the emergence of the beetle. As the penetration of larvae was not significantly affected, the toxicity of the extracts appears to be an antifeedant effect: the larvae could not develop inside the artificial seeds.

Although it is now well established that the species sensitivity for a same essential oil might be quite different and that the physiological response ought to be noted for each insect species, some inhibiting effects on reproduction were previously described. A toxic effect of lavender oils was mentioned on *Trialeurodes vaporariorum* larvae (Mateeva and Karov 1983) and more recently the activity of some essential oils extracted from Greek aromatic plants on eggs hatching and larvae of the fly *D. auraria* was detected: dead larvae and pupae presenting several kinds of malformations (Konstantopoulou et al. 1992).

However, the effects of essential oils depend on chemical patterns of these extracts. Most chemical compounds of essential oils are closely involved in the secondary metabolism, and often precursors and transformation compounds are found together. The amounts depend on the maturity of the plants at the crop period, the season (temperature, photoperiod, hygrometry) and the geographical and pedological conditions.

Gas chromatography has determined the main isoprenoid constituents of the tested essential oils (Regnault-Roger et al. 1993) and a widespread diversity of the compounds was noticed. Moreover, the larvicidal activity of fatty compounds (Lalonde et al. 1979; Hill and Schoonhoven 1981; Hwang and Mulla 1979; Kabara 1987) was previously reported.

Thus, the antifeedant effect of essential oils might be the result of the different kinds of toxicities of several compounds which could very probably work together with antagonistic or synergistic effects.

Conclusion

Beside a toxicity acting on adults by vapours and a delayed effect decreasing the reproduction of the beetle, another kind of toxicity of essential oils upon the beetle *Acanthoscelides obtectus* Say would be noticed: an antifeedant toxicity. The effect inhibits the larvae development inside the seeds. The toxic effect of essential oils upon the beetles appear to be the result of several kinds of activities.

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