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Insecticidal Activities of Four *Citrus* Essential Oils on the Bean Weevil Longevity, *Acanthoscelides obtectus* Say. (Coleoptera: Bruchidae)

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ABSTRACT

The study focuses on the evaluation of the insecticidal potential of Citrus limonum, C. sinensis, C. paradisi and C. aurantium as bioinsecticides to reduce the damage caused by the pest Acanthoscelides obtectus on white bean crops. The toxicity of essential oils extracted from 04 species of Citrus was tested under laboratory conditions at 30°C and 70% RH by three modes of action contact, inhalation and repulsion on the A. obtectus adults longevity. The results obtained for the parameter studied indicate that the essential oils extracted from C. limonum, C. sinensis and C. paradisi exert a more or less significant toxicity towards the insect pest for all the tests carried out, however the oil extracted from C. aurantium is the most effective since the A. obtectus longevity decreases from the lowest dose of 2µl and is 100% at 6µl. Regarding the inhalation test, the fumigation effect is very important from the lowest doses achieved with C. aurantium essential oil, on the other hand the other oils have only shown significant effectiveness at the highest doses. The results of the repellency test revealed that C. aurantium essential oil is the most repellant compared to the other essential oils tested.

KEYWORDS: *Acanthoscelides obtectus, Bean, Citrus, Essential Oils, Toxicity, Longevity.*

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INTRODUCTION

The bean weevil, *A. obtectus* Say, is a potentially ubiquitous cosmopolitan insect that can infest its host plant *Phaseolus vulgaris* both in the field and in storage. It is a polyvoltine species whose dispersal is closely linked to human societies (Hossaert-McKey et Alvarez, 2003). The damage is due to the larvae which penetrate the seed of *P. vulgaris* and consume the reserves contained in the cotyledons (Stamopoulos et Huignard, 1980). According to Regnault-Roger et Hamraoui (1997), this insect infests other legumes, which are food crops of great economic importance for developing countries such as cowpea (*Vigna unguiculata*), broad bean (*Vicia faba*) and chickpea (*Cicer arietinum*). Stored products are generally protected by the application of synthetic insecticides, but the presence of toxic residues in foodstuffs and the appearance of strains of insects resistant to these products are becoming more and more frequent. The plants have been the subject of numerous studies with a view to reducing the losses caused by insect pests of stored seeds by their insecticidal effects, particularly against Bruchiidae. According to Rajapakse *et al.* (1998); Tunç *et al.* (2000); Keita *et al.* (2001), foods such as citrus fruits (*C. limonum*, *C. sinensis*, *C. aurantium*, *C. paradisi*, etc.) have great resistance to insect attacks and could contain substances effective against pests. The present study aims to assess the impact of essential oils extracted from four species of *Citrus* on the adults of *A. obtectus* longevity. obtained by contact, inhalation and repulsion.

MATERIAL AND METHODS

Laboratory equipment

Several tools necessary to approach our experimental work such as the oven equipped with a thermometer and a humidifier, adjusted to have the most favorable conditions to ensure rapid development of the bean weevil, namely a temperature of 30 ±1°C and a relative humidity of 70 ±5%. The small laboratory equipment includes glass jars for the mass rearing of bruchids as well as for the inhalation test, glass Petri dishes for carrying out the contact and repulsion tests, a binocular magnifying glass for determine the sex of *A. obtectus* individuals, a micropipette for the dosage of essential oils used.

Biological material

The essential oils of four aromatic plants belonging to the genus *Citrus*, whose extraction is carried out in the laboratory, are used for the study, *C. limonum* (lemon), *C. sinensis* (sweet orange), *C. paradisi* (grapefruit) and *C. aurantium* (bitter orange).

The species studied is *A. obtectus* which is obtained from mass rearing carried out in the laboratory. Mass rearing is a method that consists of bringing bruchids male and female of indeterminate ages into contact with bean seeds not treated with insecticides, in glass jars in order to produce a large and sufficient number of individuals. Aged between 0 and 24 hours required for the various experimental tests. For the duration of the experiments, the jars were kept in the dark at a temperature of 30 ± 1°C and the humidity of 70 ± 5% RH in a conditioned oven.

Oil contact test: 25g masses of beans are placed in glass Petri dishes and then treated with the four *Citrus* oils (Lemon, sweet orange, grapefruit and bitter orange) at different doses for each (2, 4, 6, 8 and 10µl) using a micropipette. These doses are dispersed homogeneously in the seeds. A batch of 5 pairs of *A. obtectus* less than 24 hours old is introduced into each box while control batches are made with untreated seeds. Four repetitions are carried out for each essential oil, each dose and each control batch.

Inhalation test: In jars of one liter volume, small masses of cotton are suspended using a thread attached to the inside of the lid. Doses of 4µl, 8µl, 12µl, 16µl and 20µl of each *Citrus* essential oil were introduced into the cotton using a micropipette. Five pairs of adult weevils less than 24 hours old are placed in jars whose closure is perfectly sealed. Four repetitions were carried out for each treatment and in parallel a control sample was carried out. The count of dead individuals is carried out for each dose after 24 hours, 48 hours, 72 hours and 96 hours of exposure.

Repulsion test: 11cm diameter filter paper discs were divided into two equal parts; four doses of 10, 20, 30 and 40µl of each essential oil used were prepared by dilution in 0.5ml of acetone. One half of the paper is treated with oil plus acetone and the other half is treated with acetone only. After evaporation of the solvent, the disc was reconstituted using an adhesive strip and then placed in a Petri dish in the center of which are placed 5 pairs of *A. obtectus*. Four repetitions were carried out for each dose of essential oil used. After half an hour of exposure, the individuals are counted on each part of the disc. The percentage of repulsion is calculated by the following formula:

$$PR (\%) = [(Nac - Nsh) / (Nac + Nsh)] \times 100$$

- Nac is the number of individuals present on the part treated only with acetone.

- Nsh is the number of individuals present on the part treated with the oily solution.

Statistical analysis: The results obtained are subjected to an analysis of variance with one or two classification criteria using the Stat Box software, version 6.3 to determine the action of essential oils against bruchid of the bean and to analyze the biological parameter studied. When this analysis shows significant differences, it is supplemented by the Newman and Keuls test.

RESULTS

The results obtained show that the longevity of *A. obtectus* adults is inversely proportional to the dose of the essential oils tested, it is on average 11.56 ± 1.74 days in the control batches (Table 1). It should be noted that the 4 essential oils used express toxicity towards adults of *A. obtectus*. A slight decrease in longevity is recorded from the lowest dose of 2µl. It is 8, 9, 8.75, 3.5 days, values corresponding respectively to the essential oils of lemon, orange, grapefruit and bitter orange. The latter induces 100% mortality at a dose of 6 µl before 24 hours of exposure, while the others cause 100% mortality at a dose of 10 µl before 12, 30 and 18 hours of exposure respectively for the oils of the lemon, orange and grapefruit.

Table 1: Mean longevity in days (± standard deviation) of *A. obtectus* adults according to the dose and type of essential oil used.

Dose / Essential oils	0µl	2 µl	4 µl	6 µl	8 µl	10 µl
Lemon	12.00±2.08	8.00±2.21	6.75±1.70	4.00±1.41	1.75±0.81	0.50±0.50
Orange	11.50±0.57	9.00±0.81	7.25±0.50	5.50±0.57	2.25±0.95	1.25±0.50
Grapefruit	11.00±0.81	8.75±0.95	7.25±0.95	5.75±0.50	2.00±0.81	0.75±0.95
Bitter orange	11.75± 0.5	3.5±0.57	1.75±0.5	0.00	0.00	0.00

The oil extracted from the bitter orange tree is the most toxic; because the longevity undergoes a significant reduction (3.5 days) from the lowest dose and is canceled at the dose of 6 µl.

The analysis of variance with two classification criteria reveals a very highly significant difference for the essential oil (P = 0.0016) and dose (P = 0.0000) factors (Table 2).

Table 2: Analysis of variance at the 5% threshold for the longevity parameter of *A. obtectus* adults treated with the four *Citrus* essential oils.

	S.C.E	DDL	C.M.	Test F	Proba	E.T.	C.V.
Var. Total	393,9766	23	17,1294				
Var. Factor 1	42,0912	3	14,0304	8,4226	0,00169		
Var. Factor 2	326,8985	5	65,3797	39,2483	0		
Residual Var. 1	24,9869	15	1,6658			1,2907	25,34%

The Newman and Keuls test, at the 5% significance level, classifies the four essential oils used in two homogeneous groups (Table 3).

Table 3: Result of the Newman and Keuls test concerning the effect of the essential oil factor on the longevity of *A. obtectus* adults.

F1	Essential oils libels	Average longevity	Homogeneous groups	
2.0	Orange	6,125	A	
3.0	Grapefruit	5,9167	A	
1.0	Lemon	5,5	A	
4.0	bitter orange	2,8333		B

The Newman and Keuls test, at the 5% significance level, classifies the 6 doses used in 4 homogeneous groups (Table 4).

Table 4: Result of the Newman and Keuls test concerning the effect of the oil dose factor on the longevity of *A. obtectus* adults.

F2	Essential Oils libels	Average longevity	Homogeneous groups			
1.0	0ul	11,5625	A			
2.0	2ul	7,3125		B		
3.0	4ul	5,75		B	C	
4.0	6ul	3,8125			C	
5.0	8ul	1,5				D
6.0	10ul	0,625				D

For the inhalation test, the results show that the adult mortality rates of *A. obtectus* are proportional to the two dose and time factors for the four essential oils used (Tab.5). The lowest percentage of mortality is recorded for the essential oil of *C. sinensis* with an average of 18.33% over all the doses and durations of exposure, while the highest is recorded for the *C. aurantium* essential oil with an average of 79.27% over all doses and durations of treatment and after 24 hours of exposure and at a dose of 16 µl the mortality is 100% therefore, this one has the greatest inhalation effect on adults of *A. obtectus*.

Table 5: Average mortality rate (%) of adults of *A. obtectus* tested with *Citrus* oils according to doses and duration of treatment.

Essential oils	***C. limonum***				***C. sinensis***				***C. parasidi***				***C. autrantium***			
Duration Doses	24H	48H	72H	96H	24H	48H	72H	96H	24H	48H	72H	96H	24H	48H	72H	96H
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	7.5	20	27.5	0	0	0	0	0	0	0	7.5	47.5	92.5	100	100
8	7.5	12.5	32.5	40	0	0	12.5	27.5	0	7.5	20	30	72.5	100	100	100
12	12.5	30	37.5	57.5	0	10	17.5	30	7.5	12.5	30	40	90	100	100	100
16	27.5	37.5	50	75	10	22.5	40	60	22.5	30	42.5	62.5	100	100	100	100
20	67.5	70	82.5	92.5	22.5	50	60	77.5	30	57.5	70	82.5	100	100	100	100

The analysis of variance with three classification criteria reveals that there is a very highly significant difference for both the essential oil factor (P=0.0000), the dose factor (P=0.0000) and the time factor (P=0.0000), however the interactions between the factors do not show significant differences; their probabilities are 0.1891 and 0.0695 respectively for the essential oil-dose and dose-time interaction. The statistical test of Newman and Keuls classifies the 4 species of *Citrus* essential oils in 3 homogeneous groups for the parameter mortality rate of *A. obtectus* adults by inhalation effect (Tab. 6). The Newman and Keuls test, at the 5% significance level, classifies the 6 doses of essential oils in 6 homogeneous groups (Table 7).

Table 6: Result of the Newman and Keuls test concerning the effect of the essential oil factor on the mortality of *A. obtectus* individuals tested by inhalation.

F1	**Essential oil libels**	**Mean Mortality**	**Homogeneous Groups**		
4.0	***C. autrantium***	79,2708	A		
1.0	***C. limonum***	33,1875		B	
3.0	***C.parasidi***	23,0208			C
2.0	***C. sinensis***	18,3333			C

Table 7: Result of the Newman and Keuls test concerning the effect of oils on the mortality of *A. obtectus* adults tested by inhalation.

F2	**Essential oil libels**	**Mean Mortality**	**Homogeneous Groups**					
6.0	20ul	72,6563	A					
5.0	16ul	55		B				
4.0	12ul	43,3125			C			
3.0	8ul	34,5938				D		
2.0	4ul	25,1563					E	
1.0	0ul	0						F

The analysis of variance with three classification criteria reveals that there is a very highly significant difference for both the essential oil factor (P=0.0000), the dose factor (P=0.0000) and the time factor (P=0.0000), however the interactions between the factors do not show significant differences; their probabilities are 0.1891 and 0.0695 respectively for the essential oil-dose and dose-time interaction. The statistical test of Newman and Keuls classifies the essential oils of the 4 species of *Citrus* in 3 homogeneous groups for the parameter mortality rate of *A. obtectus* adults by inhalation effect (Tab.8). The Newman and Keuls test, at the 5% significance level, classifies the 6 doses of essential oils in 6 homogeneous groups (Table 9).

Table 8: Result of the Newman and Keuls test concerning the effect of the oil factor on the mortality of *A. obtectus* individuals tested by inhalation.

F1	**Essential oil libels**	**Mean Mortality**	**Homogeneous Groups**		
4.0	***C. autrantium***	79,2708	A		
1.0	***C. limonum***	33,1875		B	
3.0	***C.parasidi***	23,0208			C
2.0	***C. sinensis***	18,3333			C

Table 9: Result of the Newman and Keuls test concerning the effect of oils on the mortality of *A. obtectus* adults tested by inhalation.

F2	Essential oil libels	Mean Mortality	Homogeneous Groups					
6.0	20ul	72,6563	A					
5.0	16ul	55		B				
4.0	12ul	43,3125			C			
3.0	8ul	34,5938				D		
2.0	4ul	25,1563					E	
1.0	0ul	0						F

Concerning the test by repulsion, we observe that the number of bruchids decreases in the treated parts with the increase in the dose of essential oil used (Table 10), however the most considerable effects are recorded at the dose of 40µl with percentages repellent of 75%, 35%, 65% and 85% respectively for lemon, orange, grapefruit and bitter orange essential oils.

Table 10: Average number of bruchids identified in the filter paper at different doses of essential oils used.

Essential oils	Doses (µl)	Number of bruchids present		Repulsion (%)
		Treated part	Untreated part	
C. limonum	10	3.75	6.25	25
	20	3.00	7.00	40
	30	2.00	8.00	60
	40	1.25	8.75	75
C. sinensis	10	4.75	5.25	5
	20	4.75	5.25	5
	30	3.75	6.25	25
	40	3.25	6.75	35
C. paradisi	10	4.50	5.50	10
	20	3.25	6.75	35
	30	2.00	8.00	60
	40	1.75	8.25	65
C. aurantium	10	3.00	7.00	40
	20	1.25	8.75	75
	30	1.00	9.00	80
	40	0.75	9.25	85

The results present in table 11 shows that the lowest repellency rate is recorded with *C. sinensis* essential oil with an average of 17.5%, while the highest is recorded with *C. aurantium* essential oil with an average of 70%. For the other two essential oils we noted repulsion rates of 50% and 42.5% respectively for *C. limonum* and *C. paradisi* essential oil.

According to McDonald and *al.* (1970), the essential oils used are divided into the following repellent classes (Table 11).

Table 11: Classification of essential oils according to their repellent properties

Essential oils	*C. limonum*	*C. sinensis*	*C. paradisi.*	*C. aurantium*
Repulsion %	50%	17.5%	42.5%	70%
Repellent class	III	I	III	IV
Effect	Moderately repellent	Very weakly repellent	Moderately repellent	Repellent

DISCUSSION

The study carried out on the insecticidal action of four *Citrus* essential oils on the longevity of adult bean weevil *A. obtectus* shows that the essential oils tested express toxicity and induce significant mortality from the lowest doses. Our results are consistent with the work of several authors who have highlighted the action of essential oils on the longevity of stored food pests. Regnault-Roger and Hamraoui (1995) found a toxic effect of monoterpenes on bruchid *A.obtectus*. These authors report linalool being the most toxic and estragole being the least. This may explain the results of our experimentation which showed that the essential oil extracted from bitter orange is the most toxic, since it is richer in linalool and linalyl acetate. Indeed, Haubruge *et al.* (1989) tested the toxicity of five *Citrus* essential oils against three beetles, the results of the test by contact of the treated grains indicated that the essential oil extracted from the sour orange tree is the most effective simultaneously against *Sitophilus zeamais*, (Coleoptera: Curculionidae), *Prostephanus truncatus* (Coleoptera: Bostrychidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). These authors also found that *S. zamais* is the most sensitive to these essential oils since they noted for this insect a mortality of 96% at a dose of 5µl of bitter orange oil after 7 days of exposure. For the test by topical application, the work of these authors has shown that the longevity of *P. truncatus* is one day at a dose of 2µl of bitter orange essential oil. Indeed, mortalities of 28%, 98%, 34% and 24% of *S. zamais* adults were recorded at a dose of 2µl after 24 hours, respectively for oils of sweet orange, bitter orange, lemon and grapefruit. Hamani-Aoudjit (2019) indicates that on adults of diapausing *Bruchus rufimanus* the essential oils extracted from two plants of the Lamiaceae family *Origanum vulgare* (LD50=3.23µl/cm2 and 1.88µl/cm2 for females and males respectively) and *Salvia officinalis* (LD50=3.68 µl/cm2 and 3.02 µl/cm2 for females and males respectively) show a significant insecticidal effect by contact. Bouchikhi Tani *et al.* (2011) demonstrated the insecticidal effect of the essential oil extracted from *Origanum glandulusum* on the adults of three bruchids *A. obtectus*, *B. rufimanus* and *Callosobruchus maculatus*. They showed that this oil is very toxic on *A. obtectus* (DL50=1.44µl/30g of seeds, presents a slightly variable toxicity on *C. maculatus* (DL50=2.60µl/30g of seed, and less toxic on *B. rufimanus* with LD50=7.72µl/30 g of seeds. They explained this reduction by the richness of the oil of *O. glandulosum* by insecticidal components, this is the case of Thymol, p-cynene, γ-Terpinene, limonene, α-pinene, linalool and carvacrol Weaver and *al.* (1991) found that linalool extracted from a plant of the Lamiaceae family, namely *Ocimum canum*, has a very significant effect on the longevity of adults of *A. obtectus*, *Zabrotes subfasciatus* (Coleoptera: Bruchidae), *Rhyzopertha dominica* (Coleoptera: Bostrichidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae), in fact, a very marked effect is recorded before a treatment period of 48 hours. Bittner et *al.* (2008) tested the toxicity of essential oils from five aromatic plants on *A. obtectus* and *S. zeamais*, their results reveal that the oils extracted from *Eucalyptus globulus* (Myrtaceae) and *Thymus vulgaris* (Lamiaceae) are the most toxic on *S. zeamais*, while the essential oils of *Gomortega keule* (Gomortegaceae) and *Laurelia sempervirens* (Monimiaceae) are the most toxic on *A. obtectus*. In addition, our results corroborate those of the work of several researchers who have highlighted the toxicity of essential oils by way of inhalation or fumigation with regard to the pests of stored foodstuffs. According to Haubruge *et al.* (1989), the effect of five essential oils of *Citrus* applied by inhalation on the sheets of filter paper, reveals that the essential oil of the orange tree is the most toxic with regard to both

Sitophilus zeamais, *Prostephanus truncatus* and *Tribolium castaneum*. According to Don Pedro (1996), the essential oil extracted from lemon zest applied by way of fumigation on *C.maculatus* and *Dermestes maculatus*, respectively at doses of 7.8 and 21.5ul/l of air causes a mortality of 50% for the eggs of these two insects. Hedjal-Chebheb *et al.* (2022) noted that the two essential oils of *E. cinerea* and *E. maidenii* caused 100% mortality in *Sitophylus oryzae* and *Callosobruchus maculatus* adults, at a dose of 12.5 µl/l, for 24 and 72h exposure, respectively. Several authors have also noted a difference in the mortality of pests depending on the duration of exposure to essential oils. Thus, Kim *et al.* (2003) obtained a 90% mortality of *S. oryzae* adults treated with the essential oil of *Brassica juncea*, *Cinnamonum cassia* and *Cocholeria Arocaria*, with a dose of 3.5mg/cm², after one day exposure; whereas with the other essential oils *Acarus calamus*, *Acarus gramineus* and *Agastache rugosa*, the mortality rate is 100% after 3 days of exposure. It appears that the mode of action of essential oils against insects is attributed largely to the penetration of the terpene compounds in the respiratory system. Kim *et al.* (2003), who have studied the fumigation of essential oils on *S. oryzae* and *C. chinensis*, obtained results which show that toxicity depends on the insect species, the plant and the time of exposure to the essential oil. Temet and *al.* 2020 indicates that the essential oil of *R. officinalis* causes high mortality from 10 µl. Kotan *et al*. (2010) report that *Salvia hydrangena* has insecticidal activity by fumigation on adults of *T. confusum* with mortality varying from 68.3 and 75%. Moreover, the repellent effect of certain essential oils has been observed by many researchers, thus Ndomo *et al.* (2009) report that after two hours of exposure, the different doses of the essential oil from the leaves of *Callistemon viminalis* (from 0.031 to 0.25 µl/cm2) caused repulsion, the rate of which varies from 36.6 to 80% against adults of *A. obtectus* adults. This clearly shows that the results of our study for the test by repulsions are in conformity with the work of these authors who reveal that the repulsion increases according to the dose of the essential oil used. Taleb *et al.* (2014), the essential oil of *E. globulus* has been shown to be highly repulsive at a dose of 12.5µl/l against *C. maculatus*. Thus, according to the results obtained, the oils tested can be used to fight against stored grain insects in general and *A. obtectus* in particular, representing an effective alternative that is more respectful of the environment.

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