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Bioefficacy of Essential Oils Extracted from the Leaves of *Rosmarinus officinalis* and *Artemisia herba-alba* towards the Bruche Bean *Acanthoscelides obtectus* (Coleoptera: Bruchidae)

Z. Bouchikhi Tani, F. Hassani and M.A. Khelil

Laboratory of Ecology and Management of the Natural Ecosystems - Tlemcen, Algeria.

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The present study was designed to evaluate the effect of essential oils extracted from *Rosmarinus officinalis* and *Artemisia herba-alba* on *A. obtectus* (Coleoptera: Bruchidae). Compounds known for their insecticidal properties are α-pinene and camphor.

The bioassays on bruchids have been made in laboratory conditions (temperature and relative humidity respectively maintained at 27°C and 75%). The essential oils were extracted by hydrodistillation (yield of 0.6% for *Rosmarinus officinalis* and 0.83% for *Artemisia herba-alba*). The doses used were 1 to 5 µL/ 30g seed for the essential oil of each plant. The results show that the two tested essential oils are very toxic for the adults *A.obtectus*, as they also cause a significant reduction in fertility by bruchids. The LD50 calculates after 48 hours of exposure, shows that the essential oils toxicity are partly variable. The oil extracted from *Rosmarinus officinalis* is the most toxic to adults with LD50 = 0.59 µL/30g seed, against LD50 = 1.69 µ L/30g seeds for *Artemisia herba-alba*.

Key words: *Acanthoscelides obtectus, Rosmarinus officinalis, Artemisia herba-alba*, essential oils, insecticidal activity.

The losses are enormous by insects in countries where modern technology have not yet been introduced. The Bruchidae beetle, whose larvae eat and not occurring only in the seeds (Caswell, 1960), was one of the very few families which have colonized the seeds mature legumes, the Bruche bean *Acanthoscelides obtectus,* a potentially ubiquitous cosmopolitan insect, which can infect its host plant *Phaseolus vulgaris* in both scope and stock. Insecticides are one of the control methods prevalent against the pests. But there are strains of insects resistant to these insecticides, *A. obtectus* possess high resistance to conventional insecticides (Regnault-Roger and Hamraoui, 1997). The risks that have on the health of consumers (Champ and Dyte, 1976; Subramanyam and Hagstrum, 1995; Wite and Leesch, 1995), as well as the sky rocketting prices of these pesticides encouraged to seek alternative methods of struggle.

* To whom all correspondence should be addressed.

The plant kingdom can present many possibilities, the use of plants equipped with insecticidal properties in some developing countries represents an alternative to chemical control for crop protection (Menn and Hall, 1999). Indeed, we have proposed in this study is testing a bioefficiency of essential oils extracted from aromatic plants, two very abundant in our region on the bruche *A. obtectus* bean.

MATERIAL AND METHODS

Breeding bruchids

Raising mass of bruche *A. obtectus* was carried out in glass jars 15.5 cm high and 8 cm in diameter, on grain bean *Phaseolus vulgaris*, with a weight of 500 g in each jar incubated in an oven at 27°C and a relative humidity of 75%.

The strain of origin of bruchids comes from a warehouse in the area of Ouled Mimoun.

The purpose of the breeding is to obtain imagos of *Acanthoscelides obtectus* useful in our experiments conducted on the grain bean *Phaseolus vulgaris*.

Harvest and preparation of plant material

The leaves of two plants tested were collected in March 2007, in the region of Méchria (180 Km south of Tlemcen). The plant material was dried for a period of ten days at 25°C to extract essential oils by hydrodistillation for 5 hours.

Yields of essential oils were calculated by the formula

$$R = \frac{m1}{m2} \times 100$$

R: Return in essential oil expressed in %
m1: mass of essential oil in gram
m2: mass of sample in gram

In the present study, we used white variety of average bean seeds. Seeds which were not treated by any insecticidal agent were collected from a warehouse in the region of Tlemcen.

Livestock witness

We mixed 1ml acetone with 30g of bean seeds in Petri box. After evaporation of the solvent, we introduced five (5) pairs of *Acanthoscelides obtectus* (aged 0 to 48 hours), with three replicates for each breeding.

Doses and salaries

For each test, 1ml of acetone solution containing each of the essential oils at 1, 2, 3, 4, 5 µL/ ml of acetone was added to 30g seeds contained in a box of plastic Petrie, and then

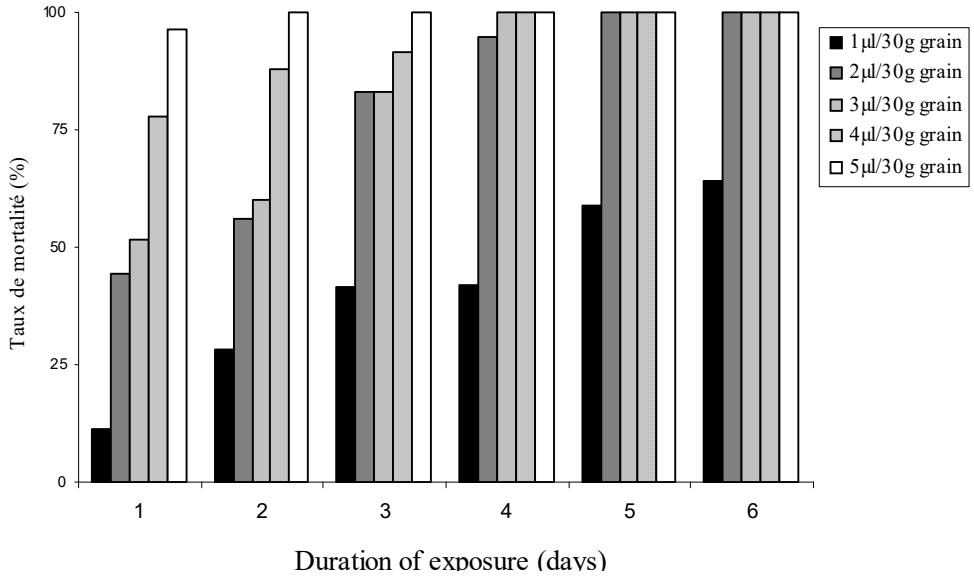

Fig. 1. Evolution of the mortality of *A. obtectus* adults with respect to the duration of exposure to dose of leaf oils of *Artemisia herba-alba*

all had been properly mixed. All boxes were not infested couple 5 *A. obtectus* (aged 0 to 48 hours).

The tests were repeated 3 times for each dose. The counting of the dead insects were made 24hours each for a period of 6 days. Mortalities recorded by the treated seeds were expressed after correction by the formula used by Abbott (1925).

$$Pc = \frac{P_0 - Pt}{100 - pt} \times 100$$

where
Pc: corrected% mortality,
Pt: mortality observed in the witness
Po: mortality observed in the test.

LD50 values were calculated for comparison of the toxicity of the two essential oils tested.

The percentages of deaths have been transformed into probit, reducing the log dose depending probit mortalities identified the lethal dose for 50% of the insect population for each essential oil to be tested (Finney, 1971).

Statistical analysis of data

The results were tested in the analysis of variance to two criteria for classification (Anova 2), useful for the study of the action of two factors (Dagnelie, 1970).

We used this type of analysis to test the effect of the dose and duration of exposure of essential oils on the mortality rate of bruchids.

And the study of the effect of essential oils extracted from the two plants tested and the doses used in fertility bruchids.

RESULTS

Effect of essential oils on the mortality of bruchids, the mortality rate among adults of *A. obtectus* rose significantly by using essential oils extracted from the two aromatic plants tested.

According to the doses of essential oils, analysis of variance revealed a highly significant difference with F = 29.96 for P = 3.42, so there is a variation on the mortality rate of bruchids which depends on the dose used of essential oil of *A. herba-alba.*

Depending on the duration of exposure factor, there is also a variation with F = 10.63 for P = 4.29.

Regarding the dose factor in essential oils, There is a variation in mortality with F = 17.10 for P = 3.18.

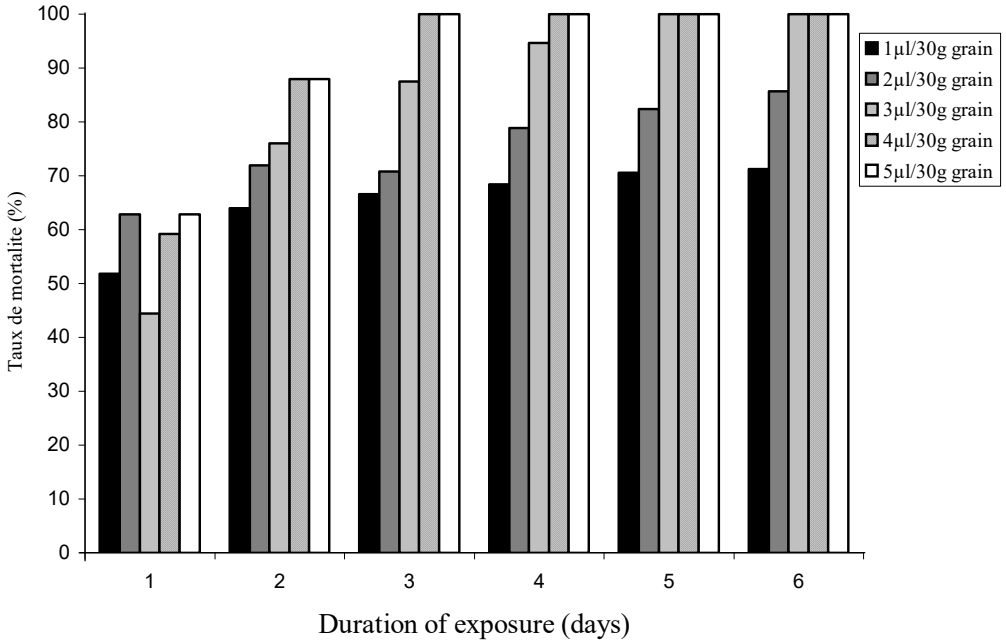

Fig. 2. Evolution of the mortality of *A. obtectus* adults with respect to the duration of exposure to dose of leaf oils of *Rosmarinus officinalis*

Regarding factor lasted exposure, the statistical analysis showed a difference between the mortality rate of bruche with F = 20.35 for P = 3.18.

Comparison of the toxicity of the two essential oils

The transformation of percentages of mortality after two days of exposure and probit regression of the data according to the log dose of essential oils has resulted in the following equations:

Y = 4,37708 x + 3,99689 (R^2 = 72,6%)
Artemisia herba-alba
Y = 1,25178 x + 5,28171 (R^2 = 88,4%)
Rosmarinus officinalis

The LD50 determined from these equations were 1.69 µ L/30g seeds for the leaves oil of *A. herba-alba* µ L/30 and 0.59 g for *R. officinalis*. These results indicate that oil of *R. officinalis* is more toxic than *A. herba-alba.*

Effects of essential oils on fertility of bruchids

Concerning the factor measure in essential oil, the statistical study confirms a significant variation, enter the averages of fertility with F = 89,36 for P = 5,05, thus the fertility of bruches changes according to the dose used.

Concerning the factor plant, the statistical analysis showed q' there is no significant difference between the averages with F = 0,31 for P = 0,59mm, thus both essential oil extracted both studied plants present an identical effect on the fertility of bruches.

DISCUSSION

The results of the bioefficiency essential oils on mortality of bruche *A.obtectus* show that essential oils extracted from the two plants tested had an effect on the insecticide bruche *A.obtectus*, which varies depending on the dose used, and duration of exposure which is confirmed by the statistics. As for the effectiveness of the two essential oils studied on the fertility bruchids, the statistical study confirmed the same effect, fertility bruche varies depending on the dose used, the dose 5 µL/30g seed seems to be the most efficient (Fertility is zero on the seeds treated with the two essential oils).

LD50 values after 48 hours of exposure showed that the essential oil extracted from *R. officinalis* is the most toxic to the adults of *A. obtectus* by comparison has the *A. herba-alba*.

According Atik BEKKARA (2007), the components of essential oils extracted from *R. officinalis* in the region of Tlemcen are α-pinene (23.1%), β-pinene (12%), camphor (14.5%), cineole (5%) and α-terpineol (1.1%).

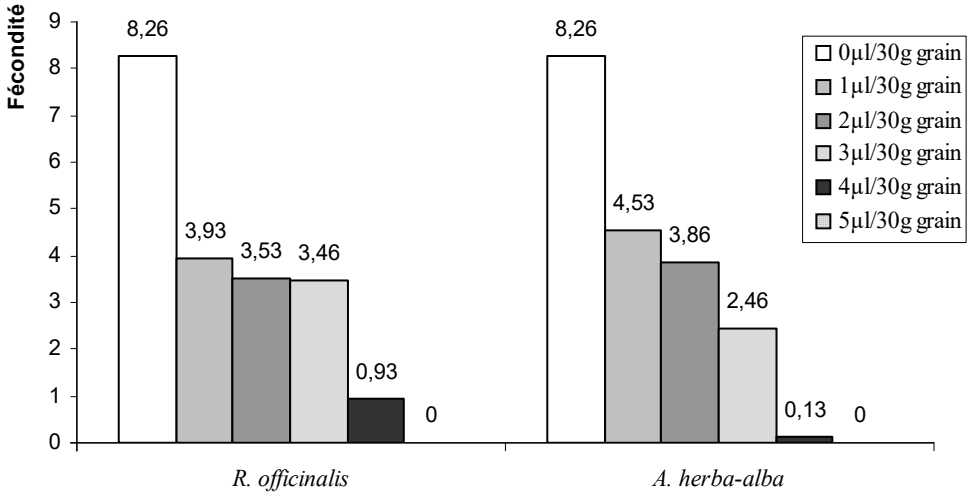

Fig. 3. Fertility of bruchids on seed treated with essential oils

According Benmansour (1999), the components of the essential oil extracted from *A. herba-alba* are α-pinene (3.21%), α-thyyone (12.80%), β-thyyone (29.43%) camphor (13.71%), and α-terpineol (0.48%).

Both studied essential oils contain substances known for their insecticidal properties, in the case of α-pinene, α-pinene, camphor, cineole and α-terpineol.

According Ojimelukwe (1999), α-pinene revealed an interesting effect insecticide against *Tribulium confusum*, and similar effects were also observed with α-terpineol, and the cineole limonene (PRATE *et al,* 1998).

Several authors (Klocke *et al*. 1985; Haubruge *et al*. 1989; Weaver *et al*. 1991; Konstantopoulou *et al.,* 1992) reported that essential oils were found to be toxic to many insects.

Essential oils also reduce fertility bruchids, according to Kellouche and Soltani (2004); Kellouche (2005) on chickpea seeds, powders leaves of four plants rich in essential oils (the fig, olive, lemon and eucalyptus) reduces fertility of females *Callosobruchus maculatus*, while essential oils extracted from the clove completely inhibit spawning.

CONCLUSION

The study of bioefficiency of essential oils extracted from the leaves of two aromatic plants *viz. Rosmarinus officinalis* and *Artemisia herba-alba* on the biology of *A. obtectus,* we take these essential oils that are effective in protecting the seeds of *P. vulgaris* against attacks from bruche *A. obtectus*. Indeed, it directly affects the mortality rate and laying insects, this is illustrated by a high mortality of bruchids seeds grown on previously treated with essential oils, and a reduction in fertility.

Statistical analyses showed that the dose factor essential oils to a highly significant impact on mortality and fertility, pest, the dose 5 µL/30g seeds, the dose being the most effective.

This repulsive power of essential oil extracted from the sheets of *Rosmarinus officinalis* and *Artemisia herba-alba* is essentially due to their chemical compositions.

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