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Toxicity and repellency of three Algerian medicinal plants against pests of stored product: *Ryzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae)

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Abstract. In order to find alternatives to chemical synthetic insecticides in the fight against pest of stored products, we evaluate the insecticidal and repellent activities of three essential oils by contact on the adults of *R. dominica.* Oils were extracted by hydrodistillation technique for 4 h using a Clevenger from the indigenous medicinal plants then the identification of the constituents was carried out on the basis of gas chromatography coupled with the GCMS spectrometry type Shimadzu TQ8030 coupled with a spectrometer. The different plants are *S. molle, M. rotundifolia* and *S. calamintha.* Our results of the contact–toxicity test of oils on substrate showed that the essential oil of *M. rotundifoli*a has a highly significant insecticidal effect with a 100 % mortality at a concentration of 3 μL from the first h of treatment, Similarly the essential oil of *S. calamintha* has a highly significant biocidal effect with a 100 % mortality at a concentration of 5 μL even the first h of treatment also, the essential oil of *S. molle* that has an insecticidal effect with a mortality of 100 % at a concentration of 25 μL after 96 h of the treatment. The results of the study of the repulsive effect of the oils tested against the insect showed that the three essential oils possess a remarkable repelling effect just after 2 h of exposure with a PR (The percentage of repulsion) of 50 % for *S. molle*, 100 % for *S. calamintha* and *M. rotundifolia*. The toxicity test of dry powders against the adult beetle showed that the powders exerted their biocidal effect (1.2 g) with a mortality rate of 96, 90 and 75 % respectively for *S. calamintha, M. rotundifolia* and *S. molle* after 96 h of treatment. Biotechnological control through the use of essential oils extracted from these plants is tested as a bio insecticide has proved very effective in the protection of cereals against such insects that degrade quality and reduce yield and in addition threaten health of the consumer.
Keyword: *Schinus molle, Mentha rotundifolia, Satureja calamintha, Ryzopertha dominica,* cereals, toxicity.

Introduction

Cereals have always been the main food resources in the World. In Algeria, cereal products occupy a strategic role in the food system and in the national economy. According to the Minister of Agriculture in 2016, national production of cereals (barley, soft and hard wheat) fell to 3.3 million tonnes during the last season reported to previous years.

Stored commodities are constantly threatened by several deterioration agents that are responsible for qualitative and quantitative losses. It is estimated that about 44 % of the losses are due directly or indirectly to insect activity, which is the main danger to dry seeds and grains in developing countries And in Africa in particular, because of climatic conditions favorable to their development [AGNES FLOREN *et al.*, 2009].

In Algeria, many species of insect's attack and depreciate the grains of storage and conservation. These insects include beetles such as *Calosobruchus chinensis, Ryzopertha dominica, Tribolium ssp, Stegobium paniceum* and Lepidoptera *such as Sitotroga cerealella, Ephestia* ssp and many others.

As part of the chemical control of these harmful agents, Contamination of our foodstuffs is cause for concern. Insecticides can induce chronic consumer intoxication, resistance to pests, and have a negative impact on the environment [NGAMO *et al.*, 2006]. As a result, several other methods of integrated control have developed among others, biological control by using non–polluting natural active substances for less harmful control.

The use of plants with insecticidal properties in some developing countries is an alternative to chemical control for crop protection.

Essential oils from plants have received considerable attention for control of stored products insects, because of their relative safety to the non–target organisms its use can be beneficial for human health and environment safety [BUTU, *et al.,* 2014c, SAMFIRA *et al.,* 2015].

Thus, are examples of contributions, they studied the oils and powders of the different medicinal plants as part of the biological control against such aggressors [MURRAY 2000, BOUDA *et al.,* 2001, ISMAN 2006, REGNAULT–ROGER *et al.,* 2008, GLITHO *et al.,* 2008, ARNASON *et al.,* 2008, CHIASSON *et al.,* 2008, YOVO 2010, RIGHI *et al.,* 2010].

Algeria has important potentialities in aromatic and medicinal plants due to the spontaneous flora, which is particularly rich in useful plants.

This is why our contribution through this study is based on the use of essential oils and powders of three medicinal plants of Algerian ecotype tested for the first time*: Mentha rotundifolia* (Lamiaceae), *Schinus molle* (Anacardiaceae) and *Satureja calamintha* (Lamiaceae) at different doses (3, 5, 10, 15, 20 and 25 µL) for oils and 0.6, 0.8, 1 and 1.2 g [ZHAOHUI and FANGQIANG, 2001, CAN *et al.,* 2004, BUTU, *et al.,* 2014b, BUTNARIU, *et al.,* 2015] for powders as bio insecticides against *Ryzopertha dominica* (F.) which is the most widely encountered insect causing serious damages to stored products.

Material and methods

Plants material. The plants of *S. molle* (Anacardiaceae)*, M. rotundifolia* and *S. calamintha* (Lamiaceae) were collected from Mascara region (West Algeria) in March 2016 and then dried at room temperature (24 ± 1 °C) for 7 days in the laboratory where the extraction of Essential oils, preparation of powders for bioassays have taken place.

Extraction of essential oils. The essential oils were extracted separately from the dry leaves of the different plants tested by the hydrodistillation technique for 4 h using a Clevenger and then dehydrated with anhydrous sodium sulfate. The calculated yield is 0.38 %, 0.35 % and 0.32 %, respectively, for *S. molle, S. calamintha* and *M. rotundifolia* these oils were stored in the refrigerator at + 4 °C until used.

Analysis of volatile compound. The identification of the constituents was carried out on the basis of gas chromatography coupled with the GCMS spectrometry type Shimadzu TQ8030 coupled with a spectrometer.

The fragmentation was carried out by electron impact under a field of 70 eV. The column used is a capillary column RTX–1 (30 m x 0.25 mm), the film thickness is 0.25 mm. The temperature of the column was programmed. at a rate of 5 °C/mm from 40 °C to 150 °C and 15 °C/min from 150 °C to 300 °C.

The vector was the helium whose flow rate is set at 40 mL / Mm. Injection mode is split less leakage ratio: 30. The identification of the components was based on comparison of their mass spectra with those of Wiley and Nist Tutore Libraries [ADAMS ,2001].

Powders Preparation. Dry leaves of *S. molle, S. calamintha and M. rotundifolia* (300 g) each were ground to a powder with an electrical blender and then sieved to obtain a fine powder of homogeneous particle size.

Insect rearing. A mass rearing of the adult insects harvested from the infested stocks of some farmers was carried out in order to obtain a permanent rearing and sufficient number of insects to initiate the various biological tests.

The identification of the pest was confirmed by Dr. Righi (entomologist of the Department of Agronomic Sciences of the University of Mascara).

This rearing was carried out on wheat seeds contained in glass jars (18 cm high by 11 cm in diameter). The device is placed in incubator at a temperature of 27 ± 1 °C and a relative humidity of 85 ± 5 % according to the method used by [TAPONDJOU *et al.,* 2002].

Bioassays. The bioassays were carried out in the entomology laboratory in Petri dishes 5.5 cm in diameter, at an average temperature of 27 ± 1 °C and a relative humidity of 85 ± 5 %.

Contact toxicity of essential oils. After several tests on the effective doses, six doses were tested: (3,5, 10, 15, 20 and 25 µL / mL) of acetone solution of essential oil extracted from the three selected plants. One mL of each dose was added to 10 g of semolina in Petri dishes and the whole was homogenized so that all substrate was uniformly coated. In the control Petri dishes, the substrate was treated only with acetone.

Five replicates were performed for each dose and each plant, then disch was infested with a lot of 10 non–sexed adult insects, aged two days. The counting of dead insects thus daily.

The mortalities recorded in the treated Petrie dishes (Mo) were expressed using the Abbott formula (1925) in corrected mortality (Mc), taking into account natural mortality observed in the control disch (Mt) according to the following formula:

$$Mc=\frac{Mo-Mt}{100-Mt}100$$

Repellent effect of essential oils on filter paper. The repellent effect of the essential oil with regard *to R. dominica* adults was evaluated using the preferential zone method on filter paper described by [MCDONALD et al., 1970], Thus, the filter paper discs of 7 cm diameter used for this purpose were cut into two equal parts each having the same surface area.

The different doses of essential oil were prepared by dilution in acetone.

Then, 1 mL of each of the prepared solutions was evenly spread on one half of the disc while the other half received only 1 mL of acetone.

After twenty minutes, time required for complete evaporation of the dilution solvent, a lot of 10 non–sexed adult insects, aged two days, was placed in the center of each disc.

Five replicates were performed for each dose and each plant. After two h, the number of insects present on the parts of filter paper treated with the various essential oils (Nt) and the number of those present on the parts treated with acetone (Nc) alone were recorded.

The percentage of repulsion (PR) was calculated using the following formula:

$$PR=\frac{Nc-Nt}{Nc+Nt}100$$

The mean percentage of repulsion for the various essential oils was calculated and assigned according to the classification of [McDONALD et al., 1970, BUTNARIU, 2012, PETRACHE, *et al.*, 2014] to one of the different repulsive classes varying from 0 to V: Class 0 (PR<0.1 %), Class I (PR=0.1–20 %), Class II (PR=20.1–40 %), class III (PR=40.1–60 %), class IV (PR=60.1–80 %) and class V (PR=80.1–100 %).

Powders contact toxicity of test plants. The powders obtained from the dried leaves of the various tested plants were impregnated with 1 g of semolina contained in Petri dishes at doses of 0.6,0.8,1 and 1.2 g). After stirring the mixture thoroughly (powder, substrate), a lot of ten unsexed adult insects aged 48 h was introduced into each of the Petrie dishes.

Statistical analysis. All the results obtained were subjected to the analysis of the variance (ANOVA) to a classification criterion using software Static, version 5 for all the studied parameters. These analyzes allowed us to compare the effect of several treatments studied taking into account parameters such as doses, plants and time.

Results and discussion

Chemical composition of essential oils. The main chemical constituents of three different essential oils of Mascara ecotyp (Algeria) were given in Table 1 and illustrated by Figure 1, 2 and 3.

The essential oil of *S. calamintha* contains 62 compounds, 58 of which are identified, characterized by the presence of six major components mainly of pulegone (35 %), borneol (32.1 %), isomenthone (28.2 %), thymol (25.6 %), oxide peperitone (19.7 %) and chysanthemic acid (15.3 %), in addition to other constituents with relatively low contents such as terpineol (0.89 %), spathulenol (1.45 %), germacrene (1.8 %), and (–) pirene.

Table 1.

The main Chemical constituent of the essential oils obtained from three plants.

S. mole (% composition)		*S. calamintha* (% composition)		*M. rotundifolia* (% composition)	
Compound	%	Compound	%	Compound	%
Alpha terpineol	16.05	Thymol	25.6	Piperitenone Oxide	49.96
Terpinen	15.84	Pulegone	35	Silane, tetra–1–propynyl	14.68
Eucalyptol	9.09	Menthone	11.1	Grindelene	6.5
Limalool	8.60	Isomenthone	28.2	Myrtene acide chloride	5.31
Alpha terpineolacetate	11.26	Oxyde piperitone	19.07	Iso borneol	3.49
Beta pinene	2.73	Camphene	1.4	Z–jasmone	2.64
3–carene	2.40	Myrcene	2.5	Caryophyllene oxide	2.06
Epoxylinalol	1.48	A–campholenic aldehyd	21.21	Cubenol	1.10
2,3–pinanediol	7.27	Borneol	32.1	Thujanol	1.17
Beta–Phellandrene	1.30	Acidechrysanthemique	15.3	Borneol	1.21
Beta–Myrcene	1.05	Isophorone	7.6	Bicyclo(4.1.0)	1.18
Beta–Eudesmol	1.57	Germacrened	1.8	Oxabicyclo(4.1.0)heptane–2,1–6methyl–3	1.81
Methyl eugenol	1.09	D– limonene	10.9	Benzaldehyde4–(1–methylethyl)	2.03
Eugenol	1.68	Cedren–13–ol	5.35	Cis–beta terpineol	2.91
Beta–Myrcene	1.05	Trans–hydrate de sabinene	2.3	Carene	1.90
ND	1.12			Retinol acetate	1.33

The chemical analysis of the essential oil of *M. rotundifolia* revealed the presence of more than 70 chemical compounds all identified with dominance of piperitenone oxide (49.96 %), silane, tetra–1–propynyl (14.68 %), followed by grindelene (6.59 %), myrtene acid chloride (5.31 %), isoborneol (3.49 %), Z–jasmone (2.64 %), caryophyllene oxide (2.06 %), cubenol (1.10 %), bicyclo (4.1.0)(1.18 %), borneol (1.21 %), thujanol (1.17 %) and other low–grade compounds such as spathulenol (0.47 %), caryophyllene (0.59 %), carene (0.47 %) santoline triene (0.27 %), alpha terpinene (0.42 %) and many others.

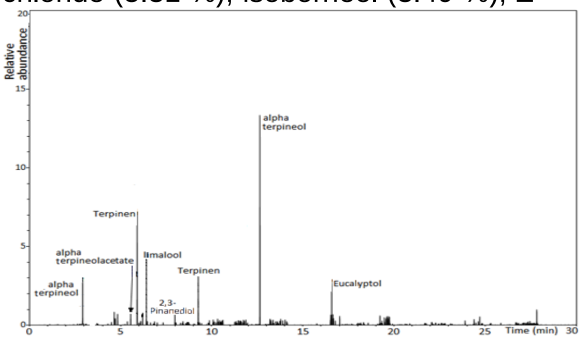

Figure 1. Chromatogram of Essential oil of *S. molle*

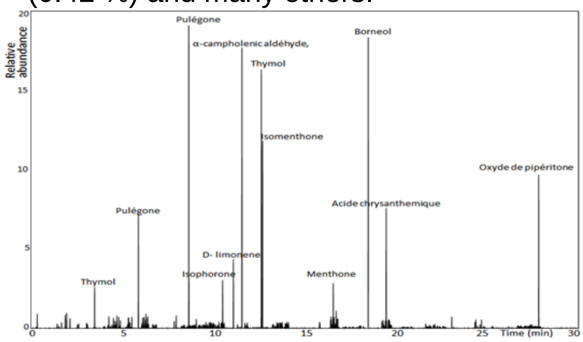

Figure 2. Chromatogram of Essential oil of *S. calamintha*

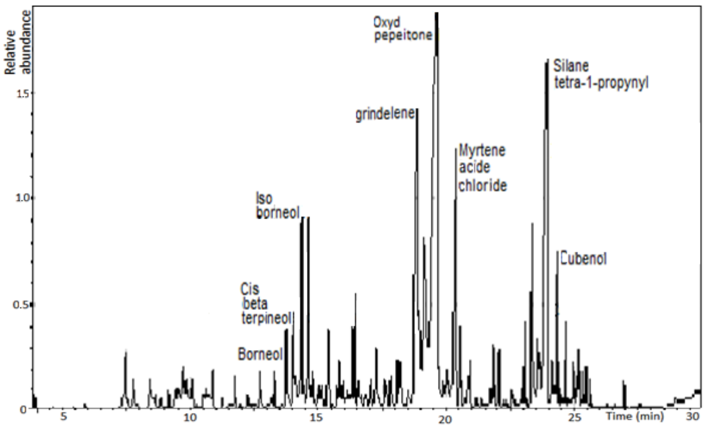

Figure 3. Chromatogram of essential oil *of M. rotundifolia*

For the oil of *S. molle*, we identified 74 compounds of which 50 metabolites are identified this oil was characterized by the presence of 4 major compounds who's most interesting is alpha terpineol (16.05 %), followed by terpinen (15.84 %) eucalyptol (9.09 %) limalool (8.60 %) as it contains other appreciable quantities such as; alpha terpineol acetate (5.93 %), beta pinene (2.73 %) and carene (2.40 %).

In the current study, the chromatographic analysis of the three plants tested showed the presence of important quantities of chemicals compounds. The toxic effects of *Mentha* could be attributed to major constituent such as oxide of piperitenone (49.96 %). This oxygenated monoterpene has very interesting biological effects [DAMIEN *et al.*, 2003, BUTNARIU, and BOSTAN, 2011, VARDANIAN, *et al.*, 2018].

Piperitone oxide has been reported as a major constituent of the essential oil of *M. rotundifolia* from Greece [KOKKINI and PAPAGEORGIOU, 1988] and from Germany [LORENZO *et al.*, 2002]. Concerning the essential oil of *Satureja* the lethal effect on *ryzopertha* is due to the important toxic effect of the major compounds pulegone (35 %) followed by borneol (32.1 %), isomenthone (28.2 %) and tymol (25.6 %). Our results are similaire to [KITIC et al., 2005, BUTU, *et al.*, 2015, BUTNARIU, *et al.*, 2016], who noted that the chemical composition of the essential oil of the species of Serbian origin contains predominantly pulegone (75.5 %) by comparison with that of Morocco, [ECH–CHAHAD et al., 2013] noted the dominance of borneol (34.52 %). *Schinus* is composed mainly of alpha terpineol (16.5 %) followed by terpinen (15.8 %) this metabolite can clearly explain the insecticidal effect against this beetle.

Contact toxicity of Essential Oils. As shown in figure 4, the essential oils of *S. calamintha and M. rotundifollia* at doses of 3 and 5 µL exert a remarkable biocidal effect with acute toxicity on *R. dominica* adults during only 24 h of exposure. A mortality of 100 % was observed in individuals treated with *M. rotundifollia* at 3 µL and in individuals treated with 5 µL of *S calamintha.*

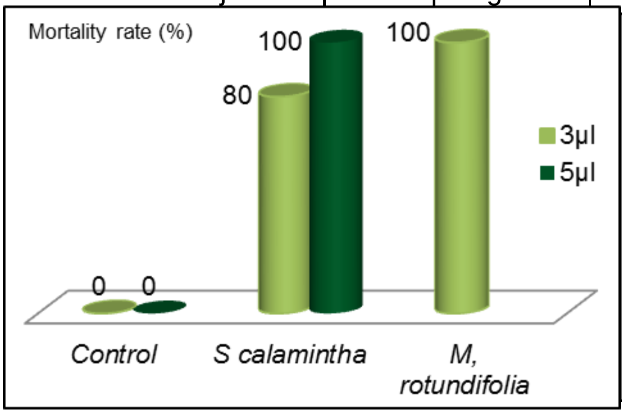

Figure 4. Insecticidal effect of essential oils of *S. calamintha and M. rotundifolia* on *R. dominica* after 24h

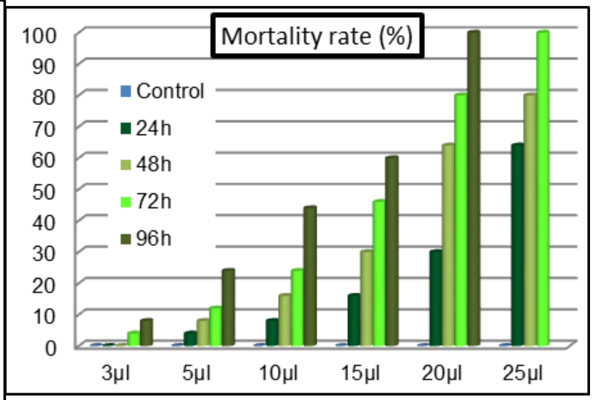

Figure 5. Evolution of the mortality rate of *R. dominica* under the effect of the essential oil of *S. molle*.

In the control no mortality is observed. the insecticidal effect of the two essential oils is highly significant compared to the controls. Figure 5 showed the evolution of the percentages of the cumulative mortalities of the adults as a function of the time and the dose of the essential oil of *S. molle* on the substrate. At low doses tested: 3, 5, 10 and 15 µL, the essential oil does not seem to have a significant effect on the longevity of the insects during the first 2 days of treatment.

The lethal effect is observed (100 % of mortality) the third day (72 h) under the effect of the treatment at the dose of 25 µL, on the other hand at 20 µL and after 96 h the same effect is recorded (100 % of mortality).

The mortality rate increases significantly according to the doses tested and the duration of exposure compared to the controls.Depending on the dose factor, the effect is highly significant when administering the dose of 25 µL [BUTNARIU and CORADINI, 2012, RODINO, *et al.*, 2014].

According to the duration of exposure factor, statistical analyzes showed no significant effect on adult mortality during the first day of treatment while a highly significant effect was recorded on 3rd day. The tests carried out showed that the three essential oils had an insecticidal effect on *R. dominica.*

According to the results, it appears that*:* The essential oil of *M. rotundifolia* is more effective with a mortality rate of 100 % at a dose of 3 µL (highly significant effect with F Cal = 3.49 and F Théo = 0.74). Followed by *S. Calamintha* with 100 % at a dose of 5 µL (F Cal = 3.49 et F Théo = 0.61) these results were also highly significant and in the last position *S. molle* (100 %) at a dose of 25 µL. (Fcal=3.29 and F Théo = 0.25) [CAN LI, 2012] noted that the percentage of mortality of adult reached 100 % at the concentration of 2000 ppm and exposure of 144 h by using essential oil from *Z. bungeanum* these oils also prevented adults from laying eggs normally. Many studies have demonstrated the action of essentials oils on the longevity of adults of different species of stored grain pests [AL JABR, 2006, NEGAHBAN *et al.*, 2007, AYVAZ *et al.*, 2009, CAMARA,2009, RIGHI et al., 2010, 2014, BUTNARIU, *et al.*, 2012].

Because of their high volatility, Essential Oils and their constituents exert insecticidal effects and reduce or disrupt the growth of the insect. The efficacy of these essential oils varies according to variations in the chemical composition of essential oils, qualitatively and quantitatively, these variations may be due to certain ecological factors, the part of the plant used, age of the plant and the period of the vegetative cycle, or even to genetic factors [ECH–CHAHAD *et al.*, 2013, BUTNARIU, *et al.*, 2005, CAUNII, *et al.*, 2015].

Repulsive effect of essential oils on filter paper. The results of the repulsion test are reported in table 2, which showed that the essential oils were shown to be repulsive to the adults of *R dominica* even at the lowest dose of 3 µL.

Table 2.

Percentage of repulsion of essential oils against adults of *R. Dominica* and their classification according to the method of MC Donald et *al*., (1970).

HE	3 µL	5 µL	10 µL	15 µL	20 µL	PR classement
S. molle	45%	56%	62%	82%	100%	50% III
S. calamintha	82%	100%	100%	100%	100%	100 V
M. rotundifolia	100%	100%	100%	100%	100%	100 V

It should be noted that after two h of treatment, we calculated a 100 % RP with *M.rotundifolia* and *S. calamintha*, and 50 % with S. molle. This percentage of repulsion is a function of time and dose.

Our results showed, that essential oils of medicinally plants are effective in protecting stored food against *R. dominica* attacks. They demonstrate significant variations in their repulsive activity which would depend on several factors, in particular the chemical composition of these oils [BUTNARIU *et al.*, 2006, GEORGIEVA, *et al.*, 2018, BUTU, *et al.*, 2014a].

In the light of these results, it can be noted that the three essential oils of *M. rotundifolia, S. calamintha and S. molle* not only have an insecticidal effect but also have an insect repellent activity against the adults of *R dominica.*

Many studies on other pests stored commodities demonstrated repellent properties of *C. schoenanthus essential oil on Sitophilus zeamais, Ocimum basilicum, O. Cannula, Callistemon viminalis* against adults of *Acanthoscelides obtectu* [NDOMO *et al.*, 2009] *Artemisia aucheri on Callosobruchus maculatus, Tribolium castaneum, Sitophilus oryzae and Sitophilus granaries* [SHAKARAMI *et al.*, 2004, BUTNARIU, 2014].

Toxicity by contact with test plants powders. The effect of powders on *R dominica* adults depends on the exposure time and the dose of the substances.

As shown in Figure 6, 7 and 8, It can be seen that the toxic effect of the powders was visible only after 48 h of exposure and that the mortality rates Which increase with time generally result in an increase in the intensity of the toxic effect this is called "dose–effect relationship".

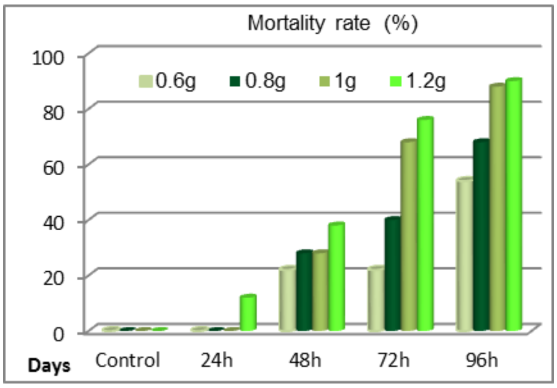

Figure 6. Evolution of the mortality rate of *R. dominica* under the effect of *S. Calamintha* powder

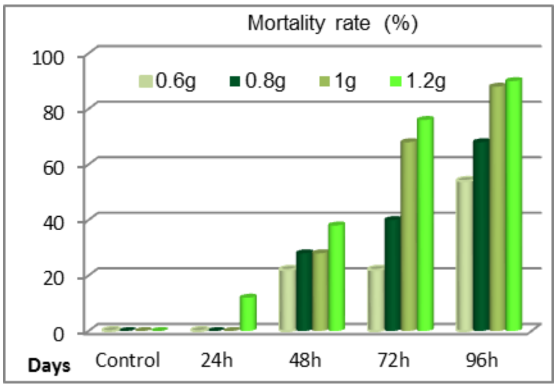

Figure 7. Evolution of the mortality rate *of R. dominica* under the effect *of M. rotundifolia* powder

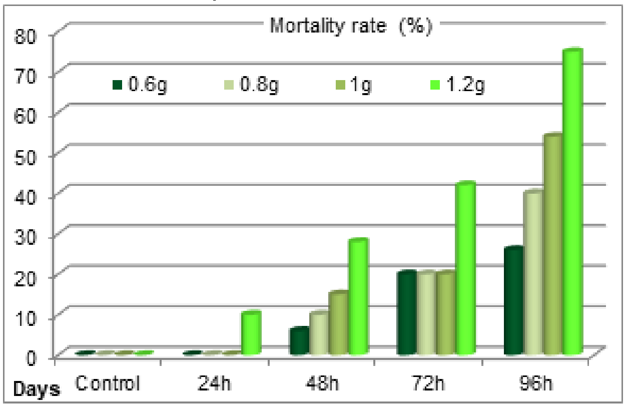

Figure 8. Evolution of the mortality rate of *R. dominica* under the effect of *S. molle* powder

For the different plants, the powders exerted their biocidal effect (1.2 g) with a mortality rate of 96, 90 and 75 % respectively for *S. calamintha, M. rotundifolia and S. molle* after 96 h of treatment. The study carried out by on more than 30 aromatic and medicinal plants showed that these plants were toxic to *Callosobruchus chinensis* and *Sitophilus oryzae* in several forms (powder and essential oil [KIM *et al.,* 2003, BUTNARIU and GIUCHICI, 2011, BARBAT, *et al.*, 2013].

Other authors conclude that the powder of *santolina chamaycipharisus and Thymus vulgaris* at doses of 0.5 and 1 g played a very important role of bio insecticide for testing their biocidal effect on different biological parameters studied on *C. chinensis*, they recorded a shorter life expectancy, low fertility, a low fertility rate and longer life cycle too short when compared to the control [RIGHI et al., 2010, NEOLIYA *et al.*, 2007IANCULOV, *et al.*, 2004].

Effect of plant powder against insects varies according to the mode of action, the tested part of the plant and the method of application.

Conclusions

The results of tests carried out for the first time in Algeria against *R. dominica* can confirm that the treatment of foodstuffs with essential oils and powders from aromatic and medicinal plants tested can be very effective in controlling this pest. These results encourage us to explore our spontaneous phytogenic resources by the discovery of new bioactive molecules, the purpose of which is to protect our stored products against this pest and many others and protect consumer health from insecticide residues.

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