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ORIGINAL ARTICLE

# Insecticide effect of pennyroyal and rosemary essential oils on the rice weevil

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The study aims at evaluating the potential insecticide essential oils from two plants of the family *Lamiaceae* pushing the spontaneous state in the Tiaret region (western Algeria). Insecticides tests were performed in the laboratory by the direct contact method. The results of these tests have shown that rosemary essential oil has remarkable insecticidal properties. They induced 100% mortality of adult rice weevils to 15µl dose after only 24 hours of exposition. The essential oil of pennyroyal induced 70 % mortality at the same dose and for the same exposure time. Lethal doses (LD50) are determined in the order of 5,58 for rosemary and 7,36µl for pennyroyal. The LD90 Are in order of 9.36 µl it mean between the second and third dose tested for the rosemary and of 12.52µl for the pennyroyal and in the same two essential oils experimental conditions prevent the development of rice weevil larvae *Sitophylus oryzae* to 5 µl dose.
**Key words**: essential oils; insecticides activities; *Mentha pulegium; Romarinus officinalis; Sitophylus oryzae*

## Introduction

Foodstuff are particularly attacked by insects when stocked (Sigaut 1978; El Camara, 2009) These insects are very feared, there presence alone depreciate the hole stock (Fleurrat-Lessard, 1982).

They got four stages life cycle from the egg to adult hood. Eggs, depending on the species are left inside or outside the cereal grain. To protect the stock of grains the rate of humidity must be maintained between 10 et 15%. This rate by hot air intake less than 65°C (Dupin, 1989). When stocks are infected a treatment is necessary to protect them from harmful insects. Chemical treatment of infected stocks is done by contact or fumigation (Cruz et al., 1988). The treatment by contact consist of recovering the grains with an insecticide, which act on depredators its effect is rather fast with longer persistence. The grains fumigation treatment is made by toxic gas which destroys eggs, larva and nymphs which have developed inside the grain. The chemical battle is harmful for human health and its wide use makes insects more resistant (Lee et al., 2001; Sanon & al, 2002). Nowadays researches are made to obtain a pesticide local aromatic plants (Vincent et *al*., 2007; Isman, 2006). They may be used alone and frequently without harm the rice weevil (*Sitophilus oryzae* L.) is a devastating insect of foodstuff. It reaches its adulthood in less than 28 days at 30 º C and at 15 % of humidity. The larva and the nymph are developed inside the grain (Balachowsky, 1962). The adult between 0.2 and 0.4 cm can fly and attack other cereals.

Our objective in this study is the development of some aromatic vegetal species and the evaluation of there on the rice weevil (*Sitophilus oryzae)*. The oil used is extracted from mint and rosemary on the other side rice weevil is raised in laboratory. This insect is responsible of heavy of cereals, of cryptogamic infections and toxin production (Kranz et al., 1977; De-Groot, 2004).

## Methods

### Insects breeding and essential oils

The insect S*ytophylus oryzae* was sampled from infected cereals from the Institute of Field Crops and Technology of Tiaret (ALGERIA). Its mass breeding in the laboratory of agrobiotechnology and of nutrition in semiarid zones, department of biology of Tiaret University in petri dishes on bread wheat grains (Fig. 1). The operation took place at constant temperature 27 °C and a humidity rate between 65% and 70%. The subjects used for testing are obtained by sieving wheat grains used for breeding.

Only larva L3 and adults are retained. The difference between males and female's adults was made by comparison of the rostrums and abdomen. The males got a thick and short rostrum and an abdomen concave at the tip (Delobel et Tran, 1993).

**Figure 1**. Laboratory culture of rice weevil

The vegetal equipment which served to the extraction of essential oils consist of pennyroyal and rosemary leaves (Fig. 2). The harvesting is carried out in blooming phase of the studied vegetal species. The drying is carried out in laboratory in ambient temperature and in shade for 10 days.

**Figure 2.** Plants view (from the left to the right: pennyroyal and rosemary).

**Extraction and analysis of essential oils**
Essential oils are obtained by leaves' (100 g) hydrodistillation during 4 hours in an appliance of Clevenger type. The essences less dense than water are collected by decantation in decanting ampoule and stored in opaque tubes at 4°C. The oils chemical composition is determined in gaseous phase by chromatography (Arpino et al., 1995) coupled to mass spectrometry (CG/MS) under the following

**Operating conditions:**
The analysis is carried out with a chromatograph in gaseous phase coupled to spectrometer of shimadza type equipped with flames ionization detector equipped with melted silica capillary column type QP2010C25 FS-OV1701 length 25m, internal diameter 0.25mm and 0.25μm film thickness. The column temperature is programmed from 60 to 220 °C at the rate of 3° C/min. The temperature of the injector is fixed at 240 °C that of the detector at 250 °C. The output of carrier gas (helium) is fixed at 1.5ml/min. The sample of pure essential oil injected is 0.1 μl. A sample of each essential oil is analyzed to determine its chemical composition

**Rice weevil was treated as follows**:
20 grams of healthy grains (variety hebda) were put in petri dishes of diameter 9 cm and height 2 cm. The healthy grains are imbibed separately by the two essential oils with different doses (5, 10, 15, and 20 μl). The second step consists to store 10 types of the insect *Sytophylus oryzae* in Petri dishes for 48 hours. The experimentation consists in five repetitions for each tested essential oil including the four doses, the sexes and the age of the insect.
Reference lots (untreated grains) are also considered for comparison. The dead insects in each Petri dish are counted regularly after 2h, 4h, 24h, and 48h.

**Figure 3**. Photos of the experimental protocol plan

**Statistical analysis**:

To estimate the impact of essential oils on insects was performed by Anova variance analysis with two classification criterions is carried out with the dead insect's number according to the concentration and the time with the Statistica 6.0. The toxic efficiency is measured by its DL50 and DL90 which represents the quantities of lethal toxic substances of respectively 50% and 90% of death in the same lot.

## Results

**Output and chemical composition**

The essential oils average outputs are calculated according to the dry leaves of each studied plant samples of *Mentha pulegium* give a rate of 1,38% higher than that obtained from *Rosmarinus officinalis* (1.13%).
The essential oils chromatographic analysis allowed the identification of nineteen components for the pennyroyal

**Table 1.** Major components of pennyroyal essential oil.

| Components | Pulegone | β-pinene | Linalool | Eucalyptol |
|---|---|---|---|---|
| rate% | 42.32 | 7.62 | 6.24 | 6.26 |

The major component is pulegone: 42.32%, followed by monoterpenes and sesquiterpenes. There is a presence of eucalyptol with nearly 6.26%, menthone with 1.32% and menthol with 2.35%, an interesting rate, very much in demand for food products aromatization. there are other components in low content as Verbenone 3.11%, carvone and caryophyllene with respectively 0.32 and 0.65%. The rosemary essential oil analysis allowed the identification of twenty-two (22) components representing 98.83% of the essence; the following table shows the major components of this essential oil

**Table 2**. Rosemary essential oil major components listing

| Components | **1.8 cinéole** | **Camphene** a | **Frechen** | le **géraniol** |
|---|---|---|---|---|
| Rates | 27.32% | 15.6**0%** | 6.1% | 8.32% |

The rosemary essential oil composition depends on its different chemotypes and on the degree of plant development. Many rosemary chemotypes have been listed in Algeria. Ours is 1.8 cineole as it is the major component.

**Insecticides tests results**

The death rate of adults *Sitophylus oryzae* specie is proportional to the dose of essential oils tested. The essence studied diminish highly (P<0.0001) the life of adults when the dose is augmented by 5µl to 20µl / 20g of grains. The results of essential oils insecticides tests of M*ental pulegium and Rosmarinus officinalis* showed an important insecticide activity.

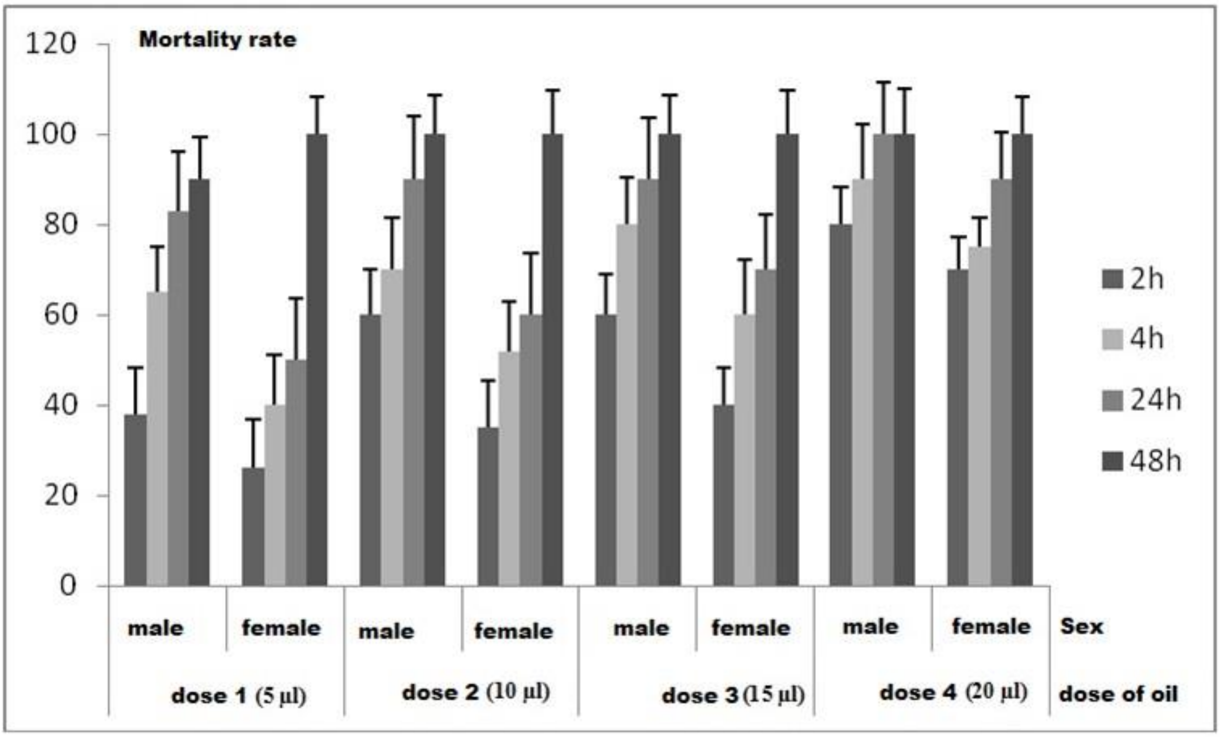


**Figure 4**. The degree of activity of these oils changes according to the sex, the dose used and treatment length of time

After forty-eight-hour contact, the adult Sitophilus oryzae mortality rate reached 100% for all the tested doses. Examination of Figs 4 and 5 also shows variability in the mortality rate between males and females. In fact, the essential oil of rosemary caused 83 ± 8.41% mortality for males and 50 ± 11.48% for females, at the lowest tested dose, after 24 hours of treatment. This rate is fluctuating for both sexes at a dose of 20 µl.
The essential oil of rosemary is more effective than that of pennyroyal mint; it recorded a mortality percentage of 38 ± 11.13% for males and 26% ± 18.24 for females, at the lowest dose (5µl) for only two hours of exposure. For the same dose of 5µl, 30% of male adults die after two hours of treatment with the pennyroyal essential oil and 40% with that of rosemary. The mortality rate increases gradually through exposure time and the dose of the essential oil to reach 100% after forty-eight hours.
The highly significant differences are confirmed through the test of by variance analysis (Table 3): plant effect and dose effect for the plant factor ($p < 0.001$) and the dose factor ($p < 0.001$).

**Table 3.** The effect of genotype and water treatment and their interaction on the viability of insects.

| | F | P |
|---|---|---|
| Oils effect | 8.673 | 0.0001 |
| Dose effect | 68.259 | 0.0000 |
| Interaction oil dose | 27.115 | 0.0000 |

The determined dose for lethal LD50 is 5.58 µl for rosemary and 7.36 µl for pennyroyal.
The DL90 lethal dose is ordered as follow 10.36 µl for rosemary; which comes between the second dose (10 µl) and the third one (15 µl) studied.
However, the pennyroyal mint order is of 12.52 µl, which is located between the third (15 µl) and the fourth (20 µl) tested dose.
The effect of Sitophylus oryzae essential oils on females is less remarkable compared to males, because at the dose of 5µl, the mortality rate rises from 15% to 40%, after 24 hours of exposure. At a dose of 15µl, the mortality in females increased from 45% for 2 hours of exposure to 80% for 24 hours of treatment.
Tests carried out with the two essential oils on L3 larvae aged 14-day-old reveal that they are toxic at the lowest dose, reaching 60% for rosemary oil and 36% for pennyroyal essence after only 2h of treatment (Fig. 5).
Compared to that of adults, the destruction of larvae over a shorter period, of twenty-four hours is noted for the two essential oils of two plant species. Under the same experimental conditions, the two essential oils prevent the development of the larvae by blocking their development cycle.

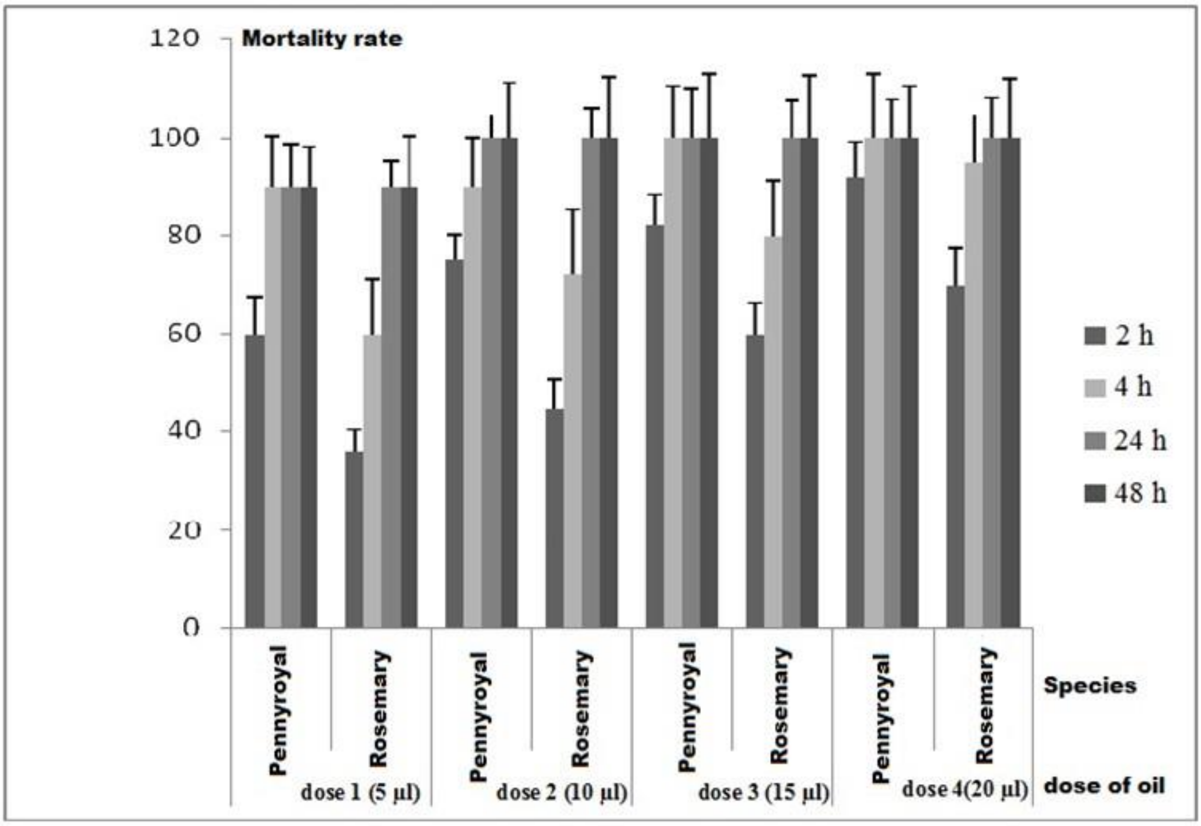


**Figure 5**. Mortality rate of Sitophylus oryzae larvae in relation to the dose and time of exposure to the essential oils of Rosmarinus officinalis and Mentha pulegium.

## Discussion

The composition of the essential oil of rosemary strongly depends on its different chemotypes and the degree of development of the plant (Afaltuni et al., 2005). Different rosemary chemotypes were recurrent in Algeria; the one identified is type 1.8 cineole. Atik Bekkara et al. (2007) reported that the spontaneous rosemary essential oil at the region of Tlemcen is characterized by the presence of α-pinene (23.1%) followed by camphor (15.3%), whereas that of cultivated rosemary is less rich in camphor (13.8%) and α-pinene (12.6%). On the other hand, the volatile species of the region of Algiers owns 1.8 cineole (52.4%) as the major constituent (Boutekedjiret et al., 1998),

The results of rosemary essential oil yields are slightly higher than those reported in other regions of Algeria. According to Djeddi et al. (2007), the productivity of rosemary of Algiers was estimated at 0.82%, and that of Tlemcen at 0.6%. Regarding the yield of pennyroyal of the volatile essence Brada et al. (2011) reported a return of 0.7 ± 0.1% in the Ain-Defla region.

The comparison of the results obtained with those found in the literature showed some differences at the level of the major compounds constituting the essential oil of rosemary as well as the percentage of the various components identified. This can be attributed to ecological factors, genetic differences, stage of plant development or the part of plants used (Guido et al., 2004; Zoran et al., 2009; Ozcan and Chalchat, 2008; Zekovic et al., 2009).

The different essential oils experienced of varying degrees brought insecticidal effects on the rice weevil. This property could be of interest in the production of crops and the preservation of stored food. This great insecticidal activity of these essential oils can only be explained by their chemical profiles rich in mono-terpenes (+ 75%). Indeed, Cseke and Kaufman (1999) define essential oils as secondary metabolites (biochemical complex) produced by plants as a defense against phytophagous pests.

The contact application of essential oils of rosemary and pennyroyal causes high mortality rates; both species show a great mortality reaching 50% on the insect "Sitophylus oryzae". These results are corroborated by the work of Kim et al (2003). Those of Benayad (2008) confirm that pennyroyal essential oil behaved like an insecticide after 24 hours of treatment.

The application of the essential oils of rosemary and thyme on *Rhyzopertha dominica*, by contact and by inhalation, made it possible to highlight their insecticidal effect. In fact, rosemary was effective in contact, at a dose of 1.384 mg/cm$^2$ causing 89.72% mortality, while thyme, under the same conditions, reached the rate of 100% (El Guedoui, 2003).

Essential oils have anti-appetizing effects, eventually affecting the growth, moulting, fertility, and the development of insects and mites. Thus Regnault-Roger and Hamraoui (1995) observed the effect of linalool, thymol and carvacrol on the fertility and the number of eggs laid from the bean weevil. As evidence, they showed complete inhibition of larval penetration in grains treated with linalool and thymol. In addition, thymol behaved as an inhibitor of adult emergence.

Tapondjou et al. (2005), have clearly demonstrated the insecticidal activity of essential oils rich of monoterpenes vis-à-vis *Sitophilus zeamais* but where they showed more than 50% mortality, after only 24 hours of exposure.

The toxic activity of essential oils vis-a-vis Sitophilus oryzae eggs has been proved; thus, the oils used prevented the emergence of adults where no survivors were counted, compared to the control.

## Conclusions

The local plants tested have appreciable yields of essential oils. They are ordered as follow: 1.28% for pennyroyal and 1.13% for rosemary. Chromatographic analysis of the essential oils of the plants leaves studied allowed to identify twenty-two terpene compounds in rosemary and nineteen others in pennyroyal mint. The major components are 1.8 cineole (27.32%) and pulegone (42.32%) respectively. The results of the insecticide tests prove their efficiency in the fight against the rice weevil (Sitophylus oryzae). Indeed, after 24 hours of contact with the lowest dose of the essential oil, the mortality rate has exceeded 50%. The lethal doses (LD50 and DL90) determined for both species confirm that rosemary is more toxic than pennyroyal when used against adult rice weevil. However, these two species are toxic, at the lowest dose in addition to the contact application of volatile species inhibits metamorphosis and prevents larvae of this insect to complete their life cycle and reach the adult stage. The results show that the essential oils of pennyroyal and rosemary could be the potential alternative towards the use of insecticides. Therefore, it is important to extract the active ingredients of these plants and test their insecticidal powers on a large panel of insects that resistant to insecticides at present.

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