Life cycle study of *Callosobruchus maculatus* (Fab.) (Coleoptera: Chrysomelidae) and insecticidal activity of essential oils of Origanum compactum

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Abstract

The development cycle of C. maculatus on chickpea seeds lasts from (29± 2 days) under laboratory conditions (28°C temperature and 75% relative humidity) with four developmental stages: egg, three larval stage, nymph and adult, as well as essential oils extracted by hydrodistillation from the aromatic plant Origanum compactum (Lamiaceae) were tested at different doses on the adults of Callosobruchus maculatus (chickpea bruchid) by contact and inhalation. Our results revealed that the insecticidal activity, EO caused total mortality of C. maculatus adults at a dose of 16 µL/L of air tested by contact during 24 h of exposure. A significant mortality of 80% was recorded with the high dose 20 µL/L of air tested by inhalation during 24 h of exposure.

Key words: Callosobruchus maculatus, insecticidal activity, essential oils, Origanum compactum, seeds, chickpea, life cycle.

Introduction

In Morocco, food legumes are located in regions with favorable rainfall. They cover 4.8% of the agricultural area, or an average of 445,000 hectares. Beans, chickpeas and dry peas are the main types of legumes that suffer considerable losses during storage. Callosobruchus maculatus (F.) and C.chinensis (F.) (Coleoptera:Bruchidae) are considered as the major pests of stored chickpea seeds. Each year, it requires protection measures by means of insecticides but the use of synthetic pesticides are the origin of several problems of sanitary, environmental and resistance phenomena [1]**.** Faced with the magnitude of the problems related to the use of insecticides and the risks of their application, the development of aromatic plants with insecticidal effect is becoming more and more important in research programs worldwide and particularly in Africa. These plants are exploited in several forms in order to limit post-harvest losses, either whole, or in the form of plant powders, essential oils, plant oils or plant extracts. Among these biological methods, the use of plants with insecticidal properties can be a safe, ecological and sustainable solution [2]**.** Oregano is one of the most important medicinal plants in Morocco and is widely used traditionally against several pathologies with a spectrum of use that varies according to the regions with regard to the pathologies, the parts used and the mode of preparation [3]**.** Several studies have reported that Origanum EOs contain many terpenoids and phenolic compounds such as carvacrol, thymol, γ-terminene and p- cymene [4]. These compounds have shown several biological activities such as significant antibacterial, anti-leishmania and antifungal activities [4]**.** Moreover, we found that the essential oil of Origanum compactum leaves present the strongest acaricidal activity against Tetranychus urticae individuals by causing a reduction of female oviposition exceeding 80% [5]**.** It is interesting to know if the essential oil of O. compactum leaves possess insecticidal action against other species. Thus, in this study, we evaluated the toxicity of essential oils and fruits of some plants on some biological parameters including the mortality of adults of the studied species.

Material and methods

- **Rearing of bruchids**

Mass rearing of *C.maculatus* bruchid was performed in glass jars on Cicer arietinum chickpea seeds. The jars were maintained at a temperature of 26 ± 1°C, a relative humidity of 70 ± 5% and a photoperiod of 14h (light) / 10h (dark) for several successive generations.

- **Plant material and method of extraction of the essential oil**

The plant *Origanum compactum* was harvested in the region of Rhafsaï (Taounate, Morocco)

After harvesting, the leaves of *Origanum compactum* were cleaned and dried at a room temperature of 21 to 24 ºC for ten days.

The extraction of the essential oils is carried out by hydro-distillation using a Clevenger for 4 hours at a rate of 90 g of dry plant for 1l of distilled water.

The essential oils obtained are dehydrated with anhydrous sodium sulphate and kept in the refrigerator at 4°C until use.

- **life cycle**

The 5 mated female bruchids (*C.maculatus*) as soon as they emerge are put 12 hours later in the presence of 5 adult males (*C.maculatus*) and 10 healthy seeds of chickpea which are placed in petri dishes, the samples are placed in a culture chamber at a temperature of 28 °C and a relative humidity of saturation. The determination of the different larval stages and pupation is done by regular observations (24 hours) with a binocular magnifying glass. In addition the opening of the seeds to be able to observe the hidden stages of *C. maculatus* inside them.

- **Inhalation test**

The test consists in studying the effect of essential oils of Oregano on the mortality of adults of *C.maculatus* by inhalation. In glass jars of one liter of volume, small masses of cotton are suspended with a thread attached to the inside of the lid.

To determine the effective concentrations of the essential oil of oregano, the following concentrations were tested: 4µl, 8µl, 16µl which were deposited in the cotton via a micropipette. Ten bruchids of *C. maculatus* (male and female) of an age between 0 and 24 hours were placed in each jar with a perfectly tight closure. An identical treatment without essential oil was used as a control. Each concentration was repeated three times. The biological activity of the tested essential oils was evaluated by the mortality rate of bruchids.

The observed mortality rate is calculated by the Abbott formula as follows:

$$\mathbf{Pc=100\times(P0-Pt)/(100-Pt)}$$

Where

Pc = corrected mortality in percent (%) ;

Po = observed mortality in the trial;

Pt = observed mortality in the control.

Contact test

For each oil, 100 g of chickpeas were used, infested with 5 pairs of the insects aged 0-24 h, packed in one-liter plastic containers duly closed with a perforated lid and covered with a thin transparent cloth. Oils were added to the grains using an automatic pipette, and subjected to manual shaking for two minutes. After 24 h of confinement, adult mortality was assessed.

Based on the results obtained in the preliminary tests, treatments at concentrations of (4, 8, and 16 µL/air) were performed. Controls for each test, containing 100 g of chickpeas without the oils, were also confined with 5 pairs of the parasite. At 48 h after the start of the experiments, mortality was assessed.

The observed mortality rate is calculated by the Abbott formula as follows:

$$\mathbf{Pc=100\times(P0-Pt)/(100-Pt)}$$

Where

Pc = percent corrected mortality (%);

Po = observed mortality in the trial;

Pt = observed mortality in the control.

Results and discussion

- **Life cycle:**

After using dissections of contaminated chickpea seeds we noticed that the life cycle of C. maculatus has four developmental stages: egg, four larval stages, pupa and adult.The life cycle of C. maculatus is on average (30 ± 2 days) in chickpea seeds.

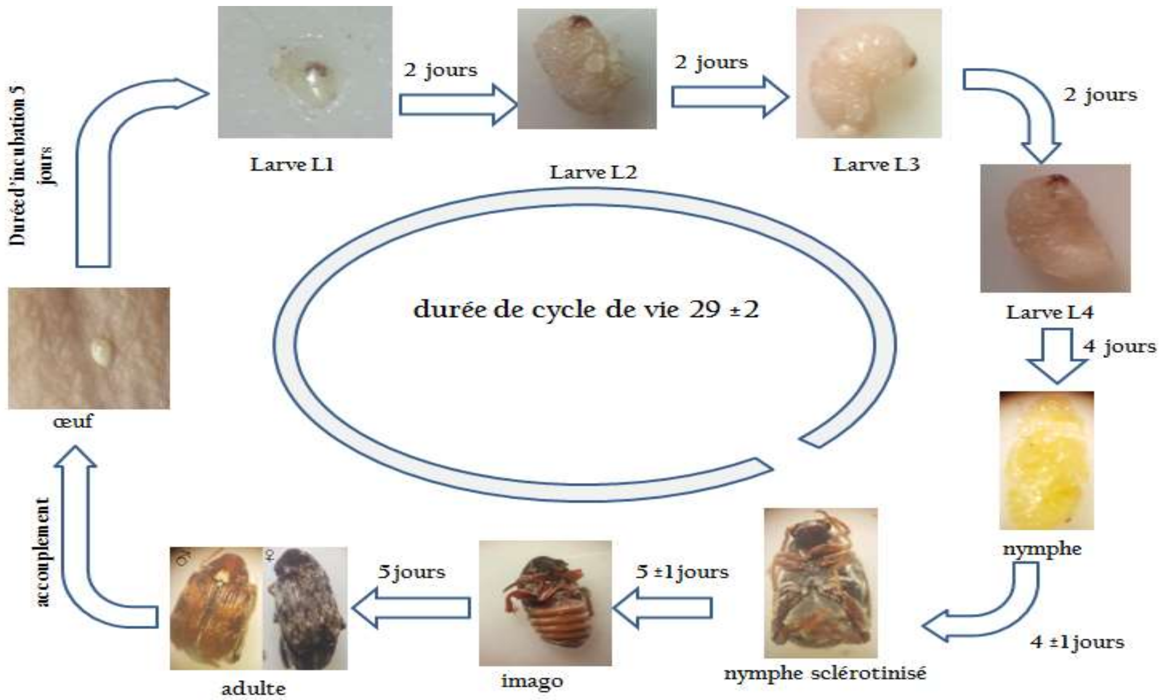

Figure 1 : Life cycle of *C. maculatus* on chickpea seeds under laboratory conditions

To effectively control insect pests of stock seeds, it is essential to control their developmental cycle. Our results show that the development cycle of *C. maculatus* on chickpea seeds lasts (30± 2 days) under laboratory conditions (28°C temperature and 75% relative humidity). According to [6]**,** the duration of the development cycle (from egg to adult) of the cowpea bruchid C.maculatus varies according to the variety of the seeds, it is shorter in the presence of the preferred host Vigna unguiculata (about 4 weeks).

The research of (Bouchikhi Tani et al., 2011) reported that the development cycle of A. Obtectus on the white variety is 30 days and that the duration between L1 and L2 is 2 days and between L2 and emergence is 23 days. The hidden stages (larvae and pupae) are predominant by their duration (23 days ±1.39 days). He added that the cycle length is 31 days on the black variety.

We observed that oviposition is important during the first days and that it is cancelled after the 7th day. The time course of oviposition in C. maculatus is reported by previous studies with different results.[7]

Parson and Credland (2003) recorded peak oviposition on day 2 after mating. These differences are probably due to inter-population variation in the species of A. Obtectus as in C. maculatus [8].

Kellouche (2005) found similar results on C. maculatus where the majority of eggs are laid during the first three days of the bruchid's life[6]. According to Righi-Assia (2010), this decrease is due to the aging effects of C[9]. chinensis males which have a reduced reproductive activity until they no longer mate with females after the 7th and 8th day.

Inhalation test

Inhalation treatments with essential oils of aromatic plants significantly affect the longevity of C. maculatus adults; mortality averages increase with increasing dose and duration of exposure. After 72h we observed a mean mortality of 100% of the bruchids for the 20 µl dose which allowed us to conclude that oregano essential oil has a greater inhalation effect on C. maculatus bruchid (Figure 2).

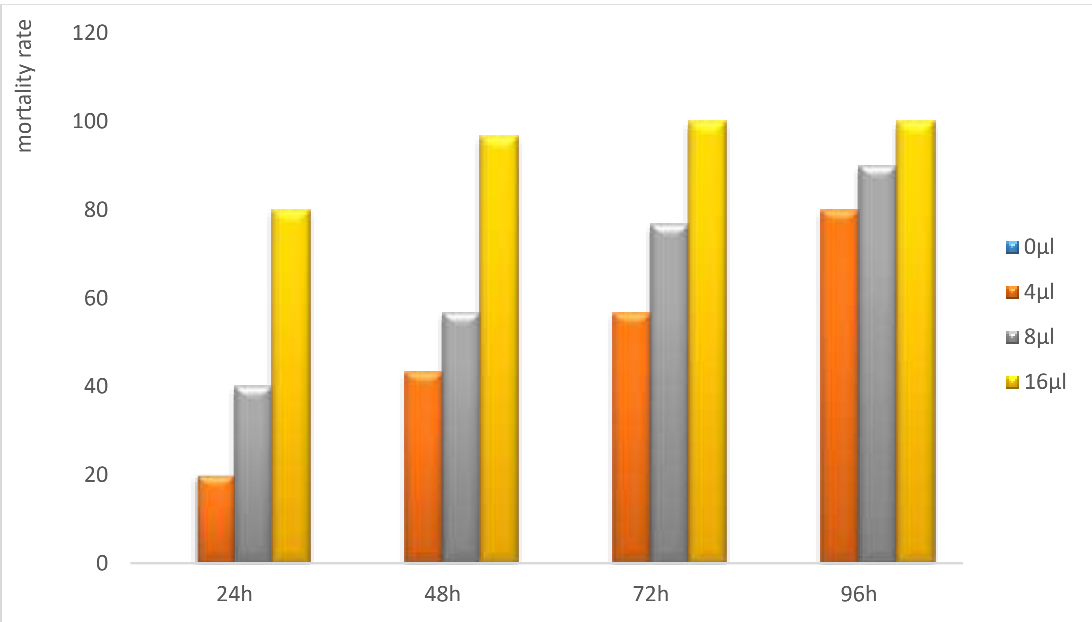

Figure 2 : **Inhalation test**

The analysis of variance with two classification criteria reveals that there is a significant difference for both the mortality factor (p = 0.021), and the date factor. Our results show that the essential oil of Oregano has an effect on the mortality of adults of C.maculatus. Other researcher showed a significant inhalant activity of some plants against callosobruchus maculatus, according to (Mansour, 2009) the mortality of C. maculatus adults is recorded after 24h of exposure to spearmint oil at 50µl dose. The same is true for Chami and Talbi (2007), who reported that laurel oil exerts strong inhalation toxicity on cowpea bruchid so that it induces 100% mortality after 24h of exposure at 14µl dose.

Contact test

The results obtained in this test show that the mortality rate of C. maculatus adults increases as the doses of essential oils increase (4µl, 8µl, 16µl/l of air) (Figure 3). After 13 days, we observe an average mortality rate of 100% for all the control batches (untreated batches). The EOs we tested all seem to have a toxic effect on the longevity of the adults. The most effective doses remain that of 16µl which presents a total mortality of all the individuals after 24h of exposure.

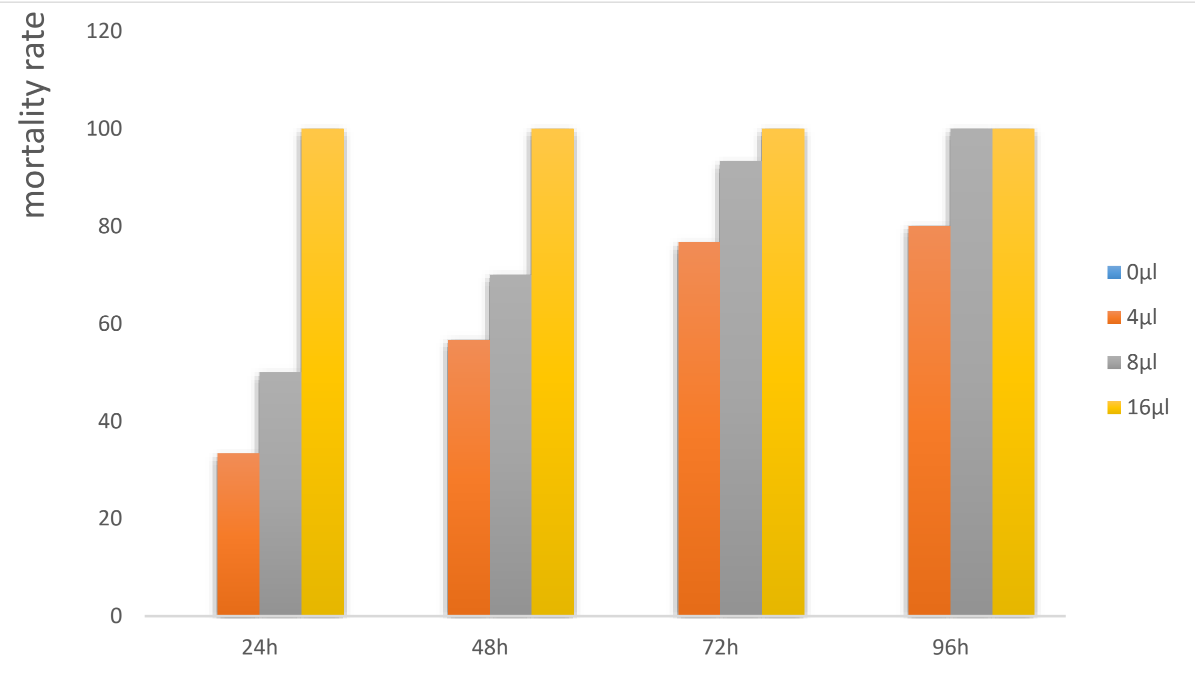

Figure 3 : **Inhalation test**

Our results show that the toxicity of oregano compactum essential oils becomes maximal when applied at high concentrations. Pavela et al. (2016) also demonstrated the insecticidal effect of essential oils of O[5]. compactum applied by fumigation on T. urticae adults. Several authors have observed the acaricidal/insecticidal effect of other oregano species, the essential oil of O. syriacum was shown to be effective by fumigation on T. cinnabarinus (Tunç & Sahinkaya, 1998)[10].

Aqueous extracts of O. majorana were also found to be effective against T. urticae (Pavela, 2016), According to Koschier (2008), the incidence of plant infestation is significant when treated with oregano. According to Koschier (2008), carvacrol-rich oregano oils exhibit significant activity against several insects, mites and plant pathogens [5], [11].

Compared to our results, other species of the genus Origanum showed significant efficacy against several pests of stored products, even when tested at higher concentrations. For example, Origanum acutidens oil rich in carvacrol (87.0%), showed a mortality of 68.3% and 36.7% against two adult insects Sitophilus granarius and Tribolium confusum, respectively (Kordali et al., 2008)[11]. Also the essential oil of Oregano showed strong insecticidal activity against Spodoptera littoralis larvae with an LD50 ≤ 0.05 ml/larva [5].

Similar results were obtained by Regnault-Roger and Hamraoui in 1994, who tested the efficacy of EOs extracted from 24 aromatic plants on A. obtectus[12]. They showed that it is especially the EO of Lamiaceae *(Thymus serpyllum, Origanum vulgare, Satureja hortensis, R. officinalis, Origanum majorana, Ocimum basilicum)* which are the most toxic inducing a 100% mortality after 1 to 4 days of exposure to the dose of 10-2 µl/ cm3. The same effect is observed only after 2 to 6 days at the dose of 5.10-2 µl/ cm3 for the EO of *T. vulgaris, Salvia officinalis, L. nobilis* and *Cinnamarrum verum* (Lauraceae). Similar to our results, the same study revealed that the EO of *C. limon* (Rutaceae) is the least toxic causing after 8 days of exposure, a mortality of 43% and 67% at doses of 10-2 µl/ cm3 and 5.10-2 µl/ cm3 respectively.

Conclusion

The use of essential oils to control insect pests in developing countries could be a complementary alternative approach to conventional insecticide treatments. Essential oils extracted from the aromatic plant Origanum compactum show a toxic effect on the longevity of C. maculatus adults. Despite the encouraging results obtained, the effectiveness of these essential oils has yet to be demonstrated in real-life situations. Further experiments are necessary to specify the nature of the compound(s) responsible for this activity, to optimize the effective doses, because it is well known that isolated and purified components act at low doses.

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