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Evaluation of the insecticidal potential of two medicinal plants and an entomopathogenic fungi against *Tribolium confusum* Jacquelin du Val. (Coleoptera: Tenebrionidae), a pest of stored foods

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Abstract

Stored products are among the most important foods in human nutrition. However; these products are under the pressure of many harmful organisms.
One of these pests is *Tribolium confusum* Jacquelin du Val. (Coleoptera: Tenebrionidae). In this study, effects of two essential oils (*Mentha rotundifolia* and *Satureja calamintha*) and an enthomopathogenenic fungi on *Tribolium confusum* has been tried under the laboratory conditions, contact and repellent effects of essential oils and contact effect of dry plant extract were determined.
As a result, essential oil, 3, 5, 10, 15 and 20 µl / ml doses of *Mentha rotundifolia* showed 100% effect after 24 hours, while *Satureja calamintha* reached 100% effect after 96 hours.
15 µl / ml dose of *M. rotundifolia* and 20 µl / ml dose of *S. calamintha* reached 100% detrimental effect after two hours. Powder doses of 0.6, 0.8 and 1.0 g of these plants, which were applied, caused the death of all the male and female individuals after 24 hours. In addition, the effects of $1x10^7$ conidia / mL dose of the entomopathogenic fungus *Beauveria bassiana* after 24, 48 and 72 hours were 87.5%, 97.5% and 100%, respectively. It was seen that both essential oils and *B. bassiana* were successfully in suppressing the depot pest *Tribolium confusum*.

Keywords

Beauveria bassiana, Cereals, Essential oils, *Mentha rotundifolia*, Repellent, *Satureja calamintha*

Introduction

Pests are a serious problem in grain during storage and its derived industry (Pérez et al., 2004).

Even if the problem is a global, it is more important in developing countries and in those of Africa in particular because of the climatic conditions favourable to their development (Stored food could be attacked by insects, fungi and rodents. Insect damage is the mostimportant. Even if the problem arises in such a wayoverall, it is more important in developing countries. Development and in those of Africa in particularbecause of the climatic conditions favorable to their development (Ndomo et al., 2009). Confused flour beetle "*Tribolium confusum*" is one of the most important species on grain stocks. It is found all over the world. The protection of cereals against these stock pests is of great importance for human survival. There are many obstacles to the timing of chemical use, including: increasing resistance in these pests and economic problems.

Following the increasing attention of the national and international scientific community on the risks related to the use of chemical pesticides, it is therefore important to seek new alternative control strategies to overcome this scourge. Hence the urgent need to search for natural insecticides which, while being equally active, volatile and available. These products have a bio-insecticidal activity and, above all, are not toxic for human. Which has been the subject of various studies (Righi et al., 2017; Kemassi et al., 2019; Benayad et al., 2012).

It is with the aim of making our contribution to this aspect of research concerning the protection of foodstuffs stored by natural means as an alternative to chemicals against this dreadful pest, that we have attempted to test new bioinsecticide molecules through the choice of two medicinal plants and the use of an entomopathogenic fungus *Beauveria bassiana*.

Materials and Methods

Insects and substrate

The strain of *Tribolium confusum* used originates from Mascara (Algeria) and its molecular identification was confirmed in a molecular entomology laboratory in Turkey. Several generations were obtained from mass rearing techniques which were carried out in glass jars filled with wheat flour. The whole is placed in an oven at a temperature of 28°c and a relative humidity of 70%.

Plant materials

The two plants (*Mentha rotundifolia* and *Satureja calamintha*) belonging to Lamiaceae family were harvested in March 2019 from Mascara region (West of Algeria). They are identified by Dr. Righi, a botanist in the Department of Agronomic Sciences of the University of Mascara. The aerial parts (stems, leaves and flowers) of each plant were dried and kept away from light and humidity. Part of the plant material of each of the two species is coarsely finely ground in an electric mill while the other part is used for the extraction of essential oils.

Extraction of essential oils

Essential oils were obtained by the hydro-distillation process using a Clevenger extraction device. The yield was 1.86% for *Mentha rotundifolia* and 1.77% for *Satureja calamintha.*

Bioassays

All bioassays were performed in the Entomology Laboratory of the University of Mascara under controlled conditions (28 °C, 70% RH).

Contact toxicity of essential oils

0.4g mass of flour substrate are put in petri dishes treated with *Mentha rotundifolia* and *Satureja calamintha* separately at different doses (5,10,15 and 20µL/ml) of acetone solution of essential oil and 3µL/ml only for *Mentha rotundifolia*. A batch of 10 adults male and female individuals is introduced into each petri dish. Five replicates were carried out for each dose and each plant. Mortalities in the treated plates (Mo) were expressed according to Abbott's (1925) formula of corrected mortality (Mc) taking into account the natural mortality observed in the control plates (Mt): Mo-Mt / 100-Mt x 1OO.

Repellent effect of essential oils

The repellent effect of the ssential oil towards adults of *Triboliumconfusum* was evaluated using the preferential area on filter paper method described by McDonald et al., (1975). Forexample, the 5cm diameter filter paper discs used for this purpose were cut into two equal parts, each with a surface area. Four doses of acetone solution of essential oil of *Satureja calamintha* and *Mentha rotundifolia* (5, 10 15and 20 µl/ml) and 3µL/ml for *Mentha rotundifolia* were used for this test. Each of these doses was spread evenly over one half of the disc; the other half received only 1ml of acetone. A batch of 10 adult insects, no more than 2 days old, was placed in the center of each disc. Four replicates were made for each dose and each plant. After two hours, the number of insects present on the part of the filter paper treated with essential oil (Nt) and the number present on the untreated part (Nc) were recorded. The percentage repellency (PR) was calculated using the following formula:

PR%= Nc - Nt X 100

The average percentage of repellency for the essential oil was calculated and assigned according to the classification of McDonald et al. to one of the different repellent classes ranging from 0 to V:

Class 0 (PR< 0.1%). Class III (PR= 40.1 - 60%).

Class I (PR= 0.1 - 20%). Class IV (PR= 60,1 -80%).

Class II (PR= 20.1 - 40%). Class V (PR= 80,1 - 100%).

Contact toxicity of test plant powders

10 adult male and 10 females *Tribolium confusum* individuals were introduced in petri dishes each containing 0.4 g of flour mixed with the powder of each plant studied. Three doses (0.6, 0.8 and 1 g) are tested, the experiments were repeated four times for each dose and each plant in the presence of a control.

Fungi and inoculum's preparation

Fungal strains of *Beauveria bassiana*isolated from soils in Isparta Province, Turkey in 2014 were obtained from Plant Protection Department, Agricultural Faculty, Isparta University of Applied Sciences, Turkey. The culture was maintained on a Sabouraud Dextrose Agar (SDA) slant at 4 °C. After were scraped with 10 ml of sterile water that contained 0.05% Tween 80. The spore suspension was adjusted to the desired concentration (10^7 conidia/ml) and counting with Malassez cell.

Application of entomopathogen fungi on insect

The 1x10^7 conidia/mL of the final concentration conidial suspension of isolate were sprayed two times from 30 cm distance with hand sprayers on the insects placed in the petri plates. Each assay consisted of 4 replicates with 10 insects. Control adult were treated with sterile distilled water with a 0.3% Tween-80. Petri dishes were kept at room temperature of 25 °C. The mortality rates of insects were evaluated on 1st, 2nd, and 4th day after inoculation. Tukey test was applied after one-way ANOVA.

Results

Contact toxicity of essential oils

The variation in the cumulative mortality rate of *T. Confusum* adults as a function of exposure time to essential oils extracted from two plants (*M. rotundifolia* and *Satureja calaminha*) was shown in Figure 1 and 2. Mortality rate in all doses of *M. rotundifolia* reached 100% less than 24 hours (Figure 1). According to figure 2, we noticed that the mortality rate of *S. calamintha* was 88% at the dose of 5 µl on the insects during the first 24 hours. After three days the mortality rate reaches 100%.

The analysis of variance reveals a significant effect with F Cal =3.49 and F Theo =3.15.

Contact toxicity of test plant powders

All tested doses (0.6g, 0.8g and 1g) powdered of *M. retundifolia* and *S. calamintha* killed all males and females of insects within the first 24 hours.

Repellent effect of essential oils

The results of the calculation of the percentage repellent of the two plants on the adults of *Tribolium confusum* by the method of Mc Donald et al., (1975) were shown in Table 1. After an exposure time of two hours, the results showed that the oil of *M. rotundifolia* is very repellent with a percentage of 96.3% compared to that of *S. calamintha* (90.37%) at the low dose of 3µl.

Effect of *Beauveria bassiana* on adults of *Tribolium confusum*

After the first few days of application, we observed the whitish mycelium of *Beauveria bassiana* curling over the entire surface of the adults of *Tribolium confusum*

According to time, an increase in mortality rates of adult *T. confusum* was observed (Table 2, Figure 3). The results reported in Figure 3 showed that *B. bassiana* caused with a mortality rate of 100% contrary to the control. No mortality was observed during the whole four-day exposure period.

Beauveria bassiana was very effective on the pest (Table 2). Numbers of dead insects in the 24th hour after treatment were 8.75 and it was different from control statistically. In the 48th hour, dead insect numbers were 9.75 and then all insects at 72nd hour were dead. Results of 98th and 72nd hours were different from other times statistically.

Discussion

The yields obtained are higher, whatever the species considered (*Mentha rotundifolia* 1.86% and *Satureja calaminha* 1.77%), than the values obtained in the literature (Lebyoud et al., 2015; Ech-Chahad et al., 2013; Bardeau, 2009; Righi et al., 2017; Bounihi, 2016).

These results can be explained by the fact that the values were calculated on the basis of the dry weight of the samples. Environmental, climatic and geographical conditions, the harvest period and the distillation technique influence the yield of essential oil (Lahlou, 2004).

According to Kemassi et al. (2019), mortality is the first criterion for judging the effectiveness of a chemical or biological treatment.

It was reported that the Lamiaceae family is the most effective family on the pests (Regnault-Roger & Hamraoui, 1993). In the present study, the two essential oils (*M. rotundifolia* and *S. calamintha*) demonstrated insecticidal activity on adults of *T. confusum* by contact with.

This is in line with the confirmations of several studies (Benayadet al., 2012; Righi et al., 2010; Butnariu et al., 2012; Righi et al., 2016).

According to Kim et al., (2003), the toxic effects of essential oils depend on the pest, plant material tested and the duration of exposure.

Tests carried out by Yahyaoui (2005), on the effectiveness by inhalation and contact of spearmint essential oils on *Rhyzopertha dominica* and *Tribolium confusum* showed that at a dose of 3.12% the essential oil acts practically in the same way on the two insects with 100% mortality. These results corroborate with those of (Benayad et al., 2012) in Morocco or *Mentha rotundifolia* was found to be too toxic with a mortality rate of 85% in the first day of exposure for 100% in the second day at the dose of 3µL.

At the low dose of 3µL/ml *Mentha rotundifolia* oil causes 100% mortality after 24 hours of exposure which corresponds to work of Righi et al., (2017), revealed that: the essential oil of *M. rotundifolia* is more effective on *R. dominica* 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).

The oils of seven plants belonging to the Lamiaceae family were more toxic causing a 100% mortality after 1-4 days of exposure, and at low dose 10^{-2} µL/cm^3 on the *A. obtectusbruche* (Regnault-Roger & Hamroui, 1994).

Similarly, the repellency increases with dose and exposure time.

Ndomo et al., (2009), reported that after 2 hours of exposure, *Callistemon viminalis* leaf oils caused a level of repulsion ranging from 36.6 to 80% against adult *A. obtectus* (Coleoptera: Brachidae), clearly showing that the percentage of repulsion increases with dose.

Other previous studies have also shown that repellency can be used as a control method. For instance, Righi et al., 2016 demonstrated repellent properties of *Mentha rotundifolia, Satureja calamintha* and *Schinus moll* essential oils against *R. dominica*. In addition 100% repellency of *M. longifolia* (L.) Huds essential oil was observed against *Sitophilus zeamais* (Odeyemi et al., 2008). While our own results show that *M. retundifolia* oil with (PR= 96,3% at 3µL/ml and *S. calamintha* with PR=90,37% at 5µL/ml) are very repellent.

Powders from the leaves of aromatic plants have a toxic effect on insects as they can act as a physical barrier Enobakhare (2007).

The Lamiceaes family plants have been considered among the most traditionally used species against bruchids and other stored food beetles.

Our results (consistent with the work of other researchers who have demonstrated the insecticidal effect of certain plant extracts in the form of powders against pests of stored seeds (Righi, 2016; Barbat, et al., 2013).

The results of present study indicate that *Beauveria bassiana* can be used successfuly against *Tribolium confusum*.

This is consistent with the work of other researchers who have found that Beauveria bassiana is a well-known entomopathogen fungi with a broad host range and is regarded as a safe biopesticide and has shown good results against many stored-grain insect species (Lord, 2005; Akbar et al., 2004; Athanassiou & Steenberg 2007, Ak et al., 2019; Sultan et al., 2019).

Akbar *et al.* (2004), in their research demonstrated that adults of *T. castaneum* exhibited very little susceptibility to *B. bassiana*.

Sheeba et al. (2001), applied *B. bassiana* against *S. oryzae* and recorded (86.2%) the mortality rate in adults after day. These results were also in line with Kassay et al. (2011) who tested 11 isolates of *B. bassiana* against adults of *S. zeamais* and *Prostephanus truncatus* (larger grain borer) (Coleoptera: Bostrychidae), and as a result, determined that *P. truncatus* was more susceptible to the *B. bassiana* than *S. zeamais*.

It was noted by Khashaveh et al. (2011), that the commercial product of *Beauveria bassiana* showed an interesting insecticidal effect against *S. granarius*, *Oryzaephilus surinamensis*, *Tribolium castaneum* with a mortality rate of 88.33, 78.31, and 64.99%.

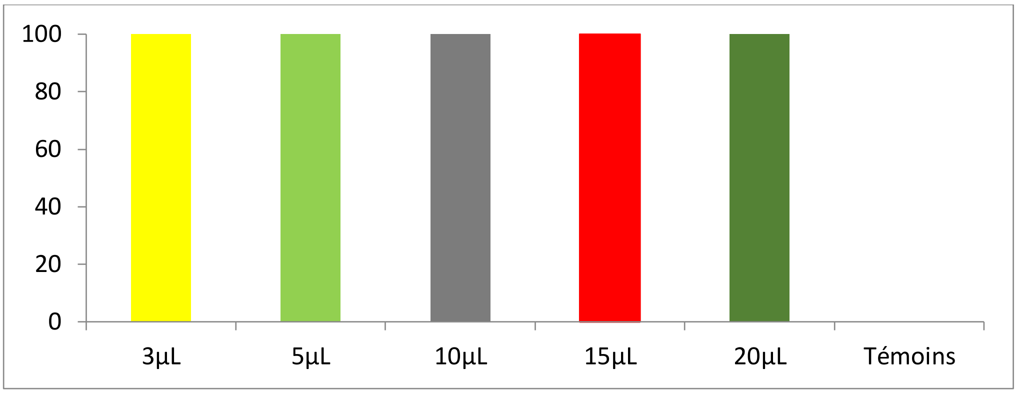

Figure 1. Bio-insecticidal contact effect of *Mentha rotundifolia* against *Tribolium confusum* after 24hours

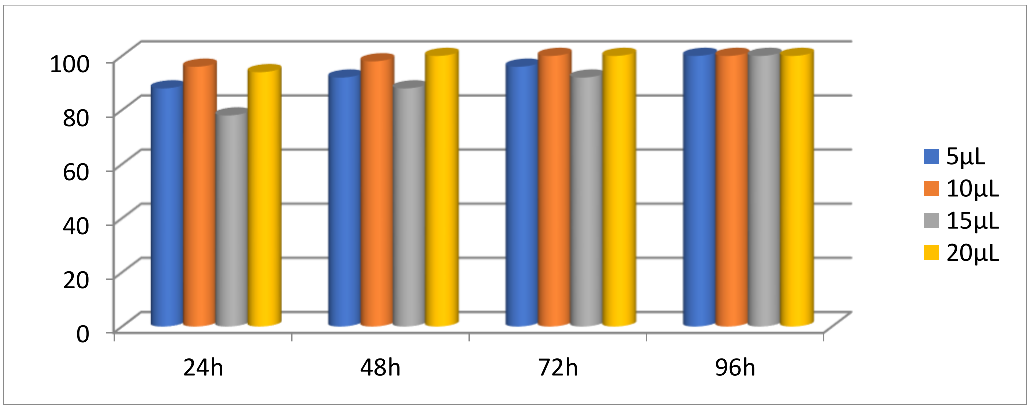

Figure 2. Bio-insecticidal contact effect of *Satureja calamintha* against *Tribolium confusum*

Table 1. Repellent effect of essential oils

Essential oils	3µL	5µL	10µL	15µL	20µL	PR %
S. calamintha	-	76,5%	89%	96%	100%	90 ,37%
M. rotundifolia	88,5%	95%	98%	100%	100%	96 ,3%

Table 2. Dead insect numbers according to time (Mean ±SE)

Time	Dead insect
98h	10.00±0.00* a
72h	10.00±0.00 a
48h	9.75±0.25 ab
24h	8.75±0.47 b
Control	0.00±0.00 c

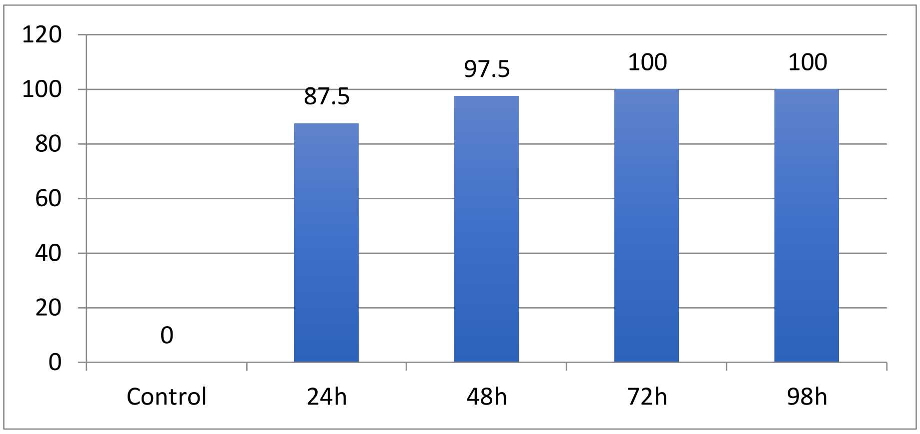

Figure 3. Mortality rates of *Tribolium confusum* treated by *Beauveria bassiana*

Conclusion

This study suggests that *Mentha rotundifolia* and *Satureja calamintha* medicinal plants as well as the entomopathogenic fungus *Beauveria bassiana* revealed that they contain active compounds with a remarkable insecticidal effect may therefore constitute an alternative to chemical control and may be potential protectors against *T. confusum.* and Thus, the study resuls have given the signals that they can be used against other storage pests too.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal.

All the authors read and approved the final manuscript.

All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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