Isparta Uygulamalı Bilimler Üniversitesi
Ziraat Fakültesi Dergisi
ISSN 1304-9984

Research Article/Araştırma Makalesi

Insecticidal Activity of Medicinal Plant, *Schinus molle* (Anacardiaceae) on *Tribolium confusum* (Coleoptera: Tenebrionidae)

Ziraat Fakültesi Dergisi,
Cilt 17, Sayı 2,
Sayfa 104-109, 2022

Journal of the Faculty of Agriculture
Volume 17, Issue 2,
Page 104-109, 2022

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Abstract: The insecticidal and repellent properties of essential oil and powder forms of *Schinus molle* were evaluated on *Tribolium confusum* in laboratory conditions. As a result of the studies, it was revealed that the average lifespan of *T. confusum* was 30±2 days. Egg incubation period was 10 days while the fecundity was 78 eggs/female. Sex ratio was observed as 1.06 female/male. In vitro insecticidal tests showed that the essential oil of *S. molle* was more effective with a mortality rate of 100% in 24 hours. The adults (males and females) of *T. confusum* survived for less than 24 hours in powder of *S. molle*. The repellence test of the essential oil provided variable levels of protection ranging from 78% to 100%.

Keywords: Essential oil, life cycle, mortality, powder, repellence, stored food

Tıbbi Aromatik Bitki, *Schinus molle* (Anacardiaceae)'nin *Tribolium confusum* (Coleoptera: Tenebrionidae) Üzerindeki Böcek Öldürücü Etkisi

Öz: *Schinus molle*'nin uçucu yağ ve toz formlarının böcek öldürücü ve kaçırıcı özellikleri *Tribolium confusum* üzerinde laboratuvar koşullarında denenmiştir. Çalışmalar sonucunda, *T. confusum*'un yaşam süresinin 30±2 gün sürdüğü saptanmıştır. Doğurganlık oranı dişi başına 78 yumurta olurken, yumurta açılma süresi 10 gün olarak belirlenmiştir. Eşey oranı 1.06 dişi/erkek olmuştur. Laboratuvar çalışmalarında *S. mole* uçucu yağının zararlı üzerindeki ölüm oranı 24 saat içinde %100'e ulaşmıştır. *S. mole*'nin toz olarak uygulandığı denemelerde *T. confusum*'un ergin erkek ve dişileri 24 saatten daha kısa süre yaşayabilmiştir. Uçucu yağın uzaklaştırıcı etkisi %78 ile %100 arasında değişmiştir.

Anahtar Kelimeler: Uçucu yağ, biyoloji, ölüm oranı, toz, uzaklaştırıcı etki, depolanmış ürünler

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Alınış (Received): 04/08/2022
Kabul (Accepted): 25/08/2022

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1. Introduction

Food security consist of an exact situation when all people at all time fairly access sufficient, safe and nutritious food that meets their dietary needs and preferences (FAO, 2006). In other words, food security is an expanded objective that refers to developing agriculture sector, as a food source, in such a way that people are satisfied in terms of quantity and quality food. So far, it became a global challenge to meet but it is much more special to African continent and there isn't a definite exception of Algeria. Its concerns are on both qualitative and quantitative importance of products in demand. A serious bottleneck arises at the level of food storage where storage pests damage give rise to significant quantitative and qualitative losses. Indeed, many pests (rodents, insects, viruses, bacteria, fungi) are the cause of losses.

In order to effectively control storage pests, it is essential to inhibit their development through well-defining bioecological conditions. The confused flour worm, *T. confusum*, is one of the most important storage pests on grains and is a worldwide threat. *T. confusum* is morphologically and biologically similar to the other species named *Tribolium castaneum* (Delobel and Tran, 1993). In addition, their behaviours are so close to the level that it is so probable that any common scientific work may report similar results (Mason, 2018; Goodnight and Craig, 1996). Confused flour beetle, *T. confusum* was named so due to the confusion on its specific identity; whereas for red flour beetle, *T. castaneum* is because of its red rust colour. However, they are distinguished with their antennae and thorax shapes (Mason, 2018).

Wherever *T. confusum* infests, its damage is not negligible. The use of chemical insecticides is currently the most practiced technique to fight against insect pests. The use of chemical products of plant origin appears as the best alternative to chemical pest control method as a safer control against these pests (Abbassi et al., 2005).

The object of our study is to seek the insecticidal potential of *S. molle* harvested in the region of Mascara in Algeria.

2. Materials and Methods

2.1. Insects rearing

2.1.1. *Tribolium confusum*

In order to obtain a homogeneous and sufficient population of insects, their mass rearing was carried out by collecting adults of *T. confusum* insects from a stock of soft wheat of a farmer in the Mascara region of Algeria. Its genetic identification at species level was confirmed in Molecular Entomology Laboratory (Ankara University, Agricultural Faculty, Plant Protection Department, Turkey). The rearing was carried out within glass jars (12 cm in length and 6 cm in diameter) each jar containing 250 g of flour and 5% of baker's yeast. The productions were carried out in the climate chambers of 25 °C temperature and 60% humidity.

2.1.2. Life cycle follow up

In order to observe the developmental stages of the confused flour worm, *T. confusum*, 1 g of flour was mixed with yeast as a food in Petri dishes of 9 cm diameter. A number of 60 pairs of insects were placed in each Petri dish and experiments were conducted in 10 replications. This development cycle took place in a climatic chamber set at the optimal conditions of 28 °C and 70% of Relative Humidity. Insects were controlled daily and observations were continued until the last adult died.

2.2. Harvesting and preparation of plant material

An aromatic plant *S. molle* belonging to the family Anacardiaceae, harvested in March 2020 in the region of Mascara was selected for this study. The plant was dried ten days. A part of collected plant materials was used for the extraction of essential oil, which was carried out on Clevenger (1928) device and yielded a finished essential oil. The other part was ground into powder using an electric grinder.

2.3. Harvesting and preparation of plant material

For powder application; 10 adult *T. confusum* individuals were introduced in petri dishes each containing 0.4 g of flour mixed with *S. molle* plant's powder.

For essential oil test; a batch of 10 *T. confusum* adult individuals was introduced into each petri dish. Four different doses, (5, 10, 15 and 20 µL/mL) along water as control treatment, were prepared to evaluate effectiveness against *T. confusum*. Each treatment was replicated four times in all bioassays. Mortality of *T. confusum* was considered only after they displayed immobility. Since the application day, data were recorded daily by counting dead adults under a stereo microscope. Mortalities were expressed according to Abbott's (1925) formula.

2.4. Repellency effect of essential oil

The insecticide test procedure used in this study was adopted from the method by McDonald et al. (1970). Petri dishes (9 cm diameter) lined with a filter paper divided into two parts were used. Four doses of *S. molle* solution (5, 10, 15 and 20 µL/mL) were applied on filter paper and then placed on one side and the opposite side stayed untreated.

After two hours, the number of insects that were present on one side with the filter paper treated with essential oil (Nt) and the number of insects present on the part not treated (Nc) were recorded. The percentage repellence (PR) of the essential oil was calculated using the following formula:

$$PR\ \% = Nc - Nt \times 100$$

The average percentage repellence for essential oil was calculated and assigned according to the classification of McDonald et al. (1970), with which different repellent classes range 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%)

3. Results

3.1. Life cycle of *Tribolium confusum*

Experimental observation showed that females of *T. confusum* laid their eggs directly on the flour substrates and sometimes on the walls of the Petri dishes 3 days after mating. Eggs incubation time was 10 days. As observed from the study, larval development of insect took place on substrate material. The larvae L1 that emerged from the egg were very mobile and could penetrate perfectly into both seeds and substrate. The 1st larval stage lasted 2 days after which the larvae reached the molting stage. The development of L2 takes 2 to 3 days in order to become the 3rd stage larva. Larvae stages: L4, L5, L6; L7; L8 are larger in size. The larval development generally last 18 days. However, it is so difficult to discretize larvae into stages as they differ in size without any differentiation in terms of external morphology.

This study resulted into an estimated fecundity of 78 eggs/female, a fertility of 69%, a sex ratio of 1.06 and 6 months of life span. The developmental cycle of *T. confusum* on flour powder lasted 30±1 days under laboratory conditions as 28 °C of temperature and 75% of Relative Humidity. The number of molted larvae varied from 4 to 8 larval stages according to Relative Humidity.

3.2. Contact effect of powder and essential oil of *Schinus molle* on *Tribolium confusum*

The adults (males and females) of *T. confusum* survived for less than 24 hours in the batches treated with *S. mole*'s powder and the mortality rate reached 100%. According to the results obtained, while the mortality rate for powder treatment reached 100% in early 24 hours, no death recorded in control treatments even during early 96 hours.

Figure 1 was indicated that the mortality rate of *T. confusum* adults evolved proportional to the rise in doses used (5, 10, 15 and 20 µL/mL) and to the duration of their exposure. Moreover, the mortality rate after 24 hours at 15 µL and 20 µL doses reached 100%. However, for essential oil treatments no mortality recorded in the control treatments during the whole exposure time. The lowest oil dose (5 µL/mL) showed a biocidal effect in early 24 hours of exposure on both females and males of *T. confusum*. The analysis of variance resulted into a significant effect with F Cal= 6.95 and F Theo= 2.94.

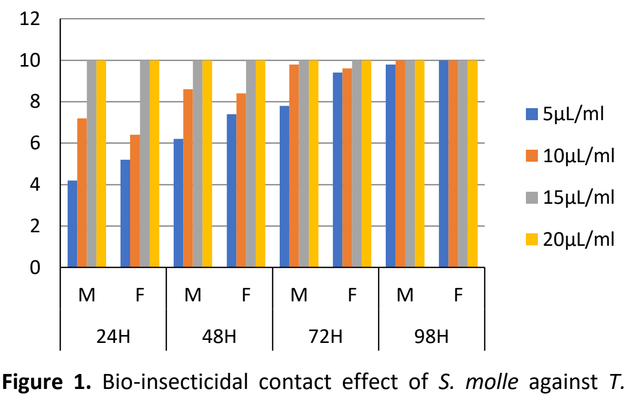

Figure 1. Bio-insecticidal contact effect of *S. molle* against *T. confusum* adults

3.3. Repellency effect of essential oil of *Schinus molle*

According to the results presented in Table 1, after two hours of exposure, the oil of *S. molle* into four doses (5, 10, 15 and 20 µL/mL) appeared significantly repellent to *T. confusum* adults at 78%, 86%, 95% and 100% respectively. It was determined that the repellent ratio increased as the dose of the applied oil increased.

Table 1. The repellent ratios of Schinus molle dosages

EO	5µL	10µL	15µL	20µL	PR %
S. molle	78%	86%	95%	100%	89.75%

4. Discussion

This study carried out on *T. confusum* especially on its biological parameters resulted into an average biological cycle period of 30±1 days under laboratory conditions (28 °C and 70% RH), by which larval and pupal stages are predominant in terms of lifespan. According to Mils (2012), adult stage of *T. confusum* was reached after 28 days of oviposition under optimal conditions of temperature (31 °C) and humidity.

This work's results come so related to those obtained by Gueye et al. (2001), who recorded a life cycle length of 30.42±2.02 days in *T. castaneum* when raised on broken millet under the temperature of 24.5-31 °C and Relative Humidity of 41-80%. According to Dawson (1964), the life cycle lasted 33 days at 28.5 °C of temperature and Relative Humidity varying between 65 and 75%. *T. castaneum* is easy to raise within a relatively short development cycle of 30 days. On the other hand, it possesses an exceptionally long life-span, varying from six months to four years and high fecundity (Bonneton, 2010). Young (1970) reported a life cycle of *T. castaneum*, divided into 4.7 days for eggs, 20 days for larvae and 6.7 days for pupae. The adult that emerged from the pupa lives for an average of 6 months but can survive up to a year and a half. Holdaway (1932) reported that the hatching rate increases with increasing temperature. The ideal temperature for hatching is 28.5 °C.

Eggs hatch after 5 to 12 days depending on temperature and Relative Humidity of *T. castaneum* (How, 1956). The temperature, Relative Humidity, type of availed food substrates are always the main factors affecting duration of the developmental cycle of *T. confusum*. For instance, the study of Diome (2012), one part of which highlights the effect of food on the bio ecology of this insect, has shown that among grains, those of millet are more favourable to the development of *T. castaneum*. Thus, the nature of the food provides a clear effect on the development of the subject insect. Gueye et al. (2015) noted that the larvae are so active and prompt feeding. They are white in hue with yellow and moult 5 to 11 times.

DOI: 10.54975/isubuzfd.1153369

Larvae go through 5 to 11 moults before reaching 5 mm long at their growth ending in time between 3 and 9 weeks. According to Diome (2012), rearing this insect on flour powder permitted a clear observation of the pupal development as it took place on the substrates. The adult emerged from pupa six days after pupation and its thermal optimum is between 32 and 33 °C. Camara, (2009) reported that its development has to cease below 22 °C and it resists very well to low levels of hygrometry.

Our results corroborate those of Gueye et al. (2015) concerning the fertility of *T. castaneum,* they noted a fertility of 84.31% on millet flour and a sex ratio 1.9±0.1 on the same substrate. According to Robinson (2005), egg viability ratio is 90%. At a temperature of 30 °C, eggs hatch after 5 days. However, incubation period requires at least 10 days under adverse conditions. The same author showed that under favourable conditions, there are 5 to 11 larval stages. Under 30 °C of temperature, adult emerges from the pupa six days after its formation, completing the cycle in 26 to 30 days.

In contact toxicity bioassays, Hassanali (2001) applied essential oils from the leaves of *S. molle* against *R. dominica* adults and recorded the highest effect, with LD_{50} values of 0.88 mg cm^2.

Descamps et al. (2008) recorded that the essential oil of false pepper was showing negative effect on adults and larvae of *Sitobion avenae* (Homoptera:Aphididae) after use of essential oil of false pepper on the adults of *Sitobion avenae* (Homoptera:Aphididae). In addition, Descamps et al. (2008) recorded a cl 50:30.71 mg ml^{-1} after 24h of treatment.

Lilian et al. (2011) demonstrated that the essential oils and extracts from *Schinu sareira* belonging to Anacardiaceae family displays an insecticidal activity. They found a contact and repellent toxicity to the larvae and adults of *T. castaneum.* In addition, Stefanazzi et al. (2006) found that an essential oil of *Tagetes ternifolia* (Asteraceae) produced a post ingestion toxicity on stored grain pest (*Tribolium castaneum*).

Righi et al. (2018) recorded a mortality rate of 100% after the third day of treatment with 25 µL/mL dose of *S. molle* oil. Recently, a number of studies of insecticidal and repellent properties of extracts from *S. molle* in different insects have been published (Asma et al., 2018; Righi et al., 2018; Abdel-Sattar et al., 2009; Benzi et al., 2009; Bayramoglu et al., 2008; Ferrero et al., 2007; Padin et al., 2007; Chopa et al., 2006; Gonzales., 2006; Ruffínengo et al., 2005).

The results obtained by Deveci et al. (2010) indicated that essential oil and hexanic extracts from *S. molle* showed a significantly potential effect in terms of repellency. Chopa et al. (2006) reported high repellence of an essential oils from ripe fruits of *S. mole* against German cockroach.

According to Abdel-Sattar et al. (2010), fruits and leaves of false pepper tree showed repellent and insecticidal effects against insects belonging to other taxonomical orders like *Trogoderma granarium* (Coleoptera:Dermestidae) and *Tribolium castaneum* (Coleoptera:Tenebrionidae). The recorded mortality rate was 53.3% after 2 days and 93.3% after 6 days respectively at the concentration of 1000 µL 10 mL^{-1} and with LC_{50}'s of 325.6 and 286.1 µL 10mL^{-1} respectively.

The insecticide efficacy of the extracts from leaves of *S. molle* has been observed also in their repellent and anti-feeding activities on the house fly, *Musca domestica L.* (Wimalaratne et al., 1996). Steinbauer and Martin (1995) in their investigations demonstrated that *S.molle* leaf powder had a repellent effect on *T. confusum*.

5. Conclusion

It is so noticeable that *T. confusum* is a threat to Algerian general economy and agriculture. This work is admitting the importance of knowledge on development and the behaviour of *T. confusum* toward its management. This study shows that the plant, *S. molle* is proven effective in both protection of storage products against the attacks of threatening insects by repellency and control in case of infestation through their lethality effect. There must be a hope for at least a reduction of the use of synthetic pesticides particularly for the storeroom grains. This must be so beneficial to health for there must be no contact of such pesticides with directly consumable produces.

Author Contributions

Sekrane FATİMA ZOHRA YOUSSRA; Righi Assia FATİHA; Righi KADA; Yahia BOUKHARI: Conceptualization, Validation, Resource/Material/Instrument Supply, Supervision/ Observation/Advice, Methodology, Investigation.
İsmail Karaca: Review and Editing; Validation, Visualization

Conflict of Interest

As the authors of this study, we declare that we do not have any conflict of interest statement.

Ethics Committee Approval

As the authors of this study, we declare that we do not have any ethics committee approval.

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