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INVESTIGATING THE IMPACT OF MYRTLE PLANT LEAF EXTRACT (MYRTUS COMMUNIS (L.)) ON THE ADULT RUSTY FLOUR BEETLE TRIBOLIUM CASTANEUM, (HER BEST) AN INTERMEDIATE HOST OF THE DWARF TAPEWORM HYMENOLEPIS NANA

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Abstract

This study was conducted for the prevention and reduction of the risk of infection with dwarf tapeworm disease caused by Hymenolepis nana

Using an extract from the leaves of Myrtus communis (L.) plant at concentrations of 10%, 15%, 20%, and 25%, the effect was tested for four treatments were used, with each treatment having five replicates for durations of 1 hour, 2 hours, 6 hours, 12 hours, and 24 hours، against the adults of Tribolium Castaneum (Herbest) from the family Tenebronidae and the order Coleoptera which is considered an intermediate host for this tapeworm

The results showed the highest mortality rate for red flour beetle adults was 72% and 84% for concentrations of 20% and 25%, respectively, after 24 hours of exposure. The lowest mortality rate was 8% for concentrations of 10% and 15% after only 1 hour of exposure

There were no significant differences between the time replicates when exposed to concentrations of 10% and 15%. However, there were significant differences between the replicates for the treatments with concentrations of 20% and 25%, at a significance level of $p \leq 0.05$.

Exposure to concentrations of Myrtus communis (L.) leaf extract leads to the death of the red flour beetle Tribolium castaneum (Herbst), which reduces the size of infection with the dwarf tapeworm Hymenolepis nana by decreasing the number of T. castaneum larvae that serve as intermediate hosts for the tapeworm.

Keywords: Dwarf Tapeworm Infection, Hymenolepis nana, Red flour beetle (Tribolium Castaneum), Myrtle (Myrtus Communis L.) leaf extract.

Introduction

Dwarf Tapeworm Infection is a disease caused by the Hymenolepis nana tapeworm, which belongs to the Hymenolepidae family, Order Cyclophyllidae, Class Cestoda, and Phylum Platyhelminthes (Al-Mekhlafi,2020).

Ong, and Qian, (2019) mentioned that the Dwarf Tapeworm, Hymenolepis nana, was discovered by Bilharz in Cairo in 1851, and its life cycle was described by Grassi in 1887. Infection with this parasite is common in hot regions such as India, parts of Russia, the Mediterranean region, Latin America, and Iraq.

The Dwarf Tapeworm, Hymenolepis nana, is the smallest tapeworm that parasitizes the small intestine of humans. Its head is small with four cup-shaped suckers and a crown made up of 20-30 hooks. Following the head, there is a long cylindrical neck, and it has around 200 body segments that are parallel-sided and more than four times as wide as they are long. Adult worms have three circular testes and a bipartite ovary, and the cystic uterus contains around 80-180 eggs. The single genital pore is located on the left side of the segment. The full segments dissolve before separation from the worm, so the eggs are mixed with fecal matter. More than one worm may be present in an infected person, with thousands of worms reported in some cases. The pathological effects and symptoms include diarrhea, loss of appetite, vomiting, insomnia, irritability, itching around the anus, skin rash, abdominal pain, and some neurological effects (Al-Haddawi & Awad, 2015).

As stated by Hotez, et al. (2020), the diagnosis of dwarf tapeworm infection relies on the presence of distinctive eggs in the stool. As for treatment, niclosamide can be used at a dose of one gram per day.

Wheat flour or grain from the plant Triticum aestivum is considered a major source of food as it is rich in proteins and carbohydrates and is a relatively cheap source of energy compared to other sources of calories. However, during storage, the grains are subject to significant losses due to infestation by various insect pests, which can cause significant damage, as well as contamination of the grains (Shahzad, et al. 2017)

El Khoury et al.. (2012) indicated that the importance of this crop's grains in human nutrition is attributed to its production of the best types of bread and its high content of carbohydrates, as well as quantities of fats, vitamins such as B1 and B2, and some mineral salts.

The myrtle plant, characterized as a compact evergreen shrub, predominantly thrives in damp and shaded environments. The plant has many branches that bear closely spaced, leathery, and fragrant leaves. The branches also bear flowers in white to pinkish colors, and the plant produces black fleshy fruits that are eaten when ripe and dried to be used as spices. The plant propagates through suckers and seeds and is known by other names such as hamblasis, mersin, and basil, but it is not the basil commonly known. In ancient Egyptian, it was called "khet-es", meaning "tomb's myrtle", while in Greek it was known as "amyrtos" and in Latin as "myrtus". In Arabic, it is known as "rihan", and in Egypt as "mersin" and in the Levant as "al-bustani" and "qaf wa nazur". Scientifically, it is known as Myrtus Communis (L.) and belongs to the Myrtaceae family and the Myrtales order (Ibrahim et al 2014).).

Ibrahim and Yassin,(2019) reported that the red rust flour beetle, Tribolium castaneum (Herbest), from the family Tenebronidae and order Coleoptera, is one of the major storage pests that is distributed in most parts of the world, especially in warm areas. It lives both as an adult and larvae on grains and their products, as well as on seeds, dried fruits, and tobacco. It does not infect healthy grains, but it lives on damaged grains and flour. The beetle infests flour, broken grains, biscuits, cakes, and chocolates.

During the storage phase of flour, grains, and other stored commodities, they frequently face infestation by various insects, leading to substantial damage. Approximately 20% of crops encounter destruction from pests during the post-harvest period, and this percentage escalates in developing nations, occasionally reaching up to 80% (Al-Iraqi et al., 2008). Marcio et al. (2007) elucidated that the damage rate can climb as high as 40% in countries that lack the implementation of modern storage technologies.

The challenge in dealing with insects lies in their association with flour and food products, where the use of pesticides can result in contamination. Hence, there is a growing demand for alternative approaches to chemical pesticides. One such alternative is the utilization of plant-based pesticides, which exhibit minimal inhibitory effects and low toxicity to mammals. These plant-based pesticides can be employed in various forms, such as powders and plant extracts, serving as feed inhibitors, toxic or growth inhibitors, as well as attractive or repellent substances (Narong, 2003; Rao et al., 2005; Timothy and Esther, 2009).

As a result of the foregoing, this research was conducted with the aim of :

1-- finding a safe and effective alternative method to pesticides, chemical powders, and freezing for use in the storage of flour and other food items where the use of pesticides is not feasible, such as grain products infested with the red flour beetle (Tribolium Castaneum). Different concentrations of plant extract from Asafoetida can be used in mills, government and local flour storage facilities, and homes to kill adult insects.

2 -Prevention and reduction of the incidence of Dwarf Tapeworm Infection caused by the Hymenolepis nana tapeworm, due to the presence of larvae of the red flour beetle Tribolium Castaneum (Herbest) that the tapeworm parasitizes or spends part of its life cycle in. Therefore, the presence of these insects in storage facilities increases the spread of this parasite

Materials and Methods :

Insect rearing: A colony of the red flour beetle Tribolium Castaneum was prepared from infested flour samples collected from local warehouses in Al-Amara city in August 2022. To prepare the insects, several beetles were placed in glass bottles. with a length of 13 cm and a diameter of 8 cm containing 200 g of flour. The bottles. were covered with mesh cloth and secured with rubber bands, then placed in an incubator at a temperature of (25 ± 2) °C with a relative humidity of (60-70%)%. A 30 g of KOH in 100 mL of water was added to the bottles. to adjust the humidity, which was monitored using a hygrometer. The bottles. were kept until used.

(Ahn, and Kim,2017) for preparing a leaf extract of Myrtle plant. The leaves were collected from gardens in Maysan province on the banks of the Tigris River in August 2022. After collection, the leaves were cleaned from any debris such as dust and damaged leaves. The leaves were then dried to remove moisture and placed in the shade at room temperature with continuous stirring to avoid damage and mold. The plant leaves were then ground using a mortar and electric grinder and the resulting powder was placed in medical plastic containers and stored until the extraction process was performed.

Table 1: Devices and materials used in the study.

The device name
Glass bottles
Acloth of malmal
Rubber band
Incubator
Oven
Sensitive Balance
Hygrometer
Filter Papers
Flaskes
Electric Mixer
Plastic bottles
Magnetic vibrator
Test tubes
Centrifuge
Refrigerator
Syringes

50 grams of the powder were added to 500 ml of distilled water in a 1000 ml glass flask and mixed using a magnetic stirrer for 15 minutes. Afterward, the mixture was left undisturbed for a period of 24 hours to facilitate settling. Subsequently, the settled mixture was filtered, and the resulting filtrate was divided into individual test tubes. These test tubes were then subjected to centrifugation at a speed of 3000 rpm for a duration of 15 minutes.

The sediment was discarded, and the supernatant was collected and dried in an electric oven. The resulting material was weighed and stored in a refrigerator until use. (Ahn, and Kim,2017)

The concentrations were prepared as follows :

The following dry weights (1, 1.5, 2, 2.5) grams were weighed and dissolved in 10 mL of distilled water, respectively, to obtain the concentrations of (10%, 15%, 20%, 25%) respectively.

The effect of the above concentrations was tested by using 500 red flour beetle insects for each treatment. For each concentration, 1.1, 1.5, 2, and 2.5 grams of dry material were dissolved in 10 mL of distilled water, respectively, to obtain concentrations of 10%, 15%, 20%, and 25%, respectively. Each treatment contained 125 insects, with five replicates for each replicate containing 25 insects in a medical plastic box measuring 6 cm in length and 5 cm in diameter with 50 grams of flour. The boxes were covered with mesh fabric and secured with a rubber band. The insects were sprayed with the recommended concentrations using a syringe and then placed in an incubator at a temperature of 25 ± 2 °C, with humidity maintained. Each treatment had 15 boxes with five replicates and one box per replicate, and the experiment lasted for different periods of time (1, 2, 6, 12, and 24 hours). The number of dead insects was recorded in Table (1).

The results were analyzed using a One-sample T-test to find significant differences between treatments and replicates at a significance level of $P \leq 0.05$, using the statistical software SPSS version 20). (BM Corp.,2011).

Results

The impact of various concentrations of leaf extract derived from the Myrtle plant on the mortality rate of red flour beetles.

1- Effect of 10% concentration:

The results of the study, as shown in Table (1), indicate no significant differences for the first treatment between the replicates or exposure periods at a probability level of $p \leq 0.05$ and t=2.029. The number of dead insects was (2, 3, 5, 7, 13) and according to the repetition periods (1, 2, 6, 12, 24) hours, the percentages of killing were (8%, 12%, 20%, 28%, 52%), respectively,

Table (1) Effect of 10% concentration of leaf extract of Myrtle plant on mortality of red flour beetle (Tribolium castaneum).

Percentage%	Number of Killed insects	Time
8	2	hour 1
12	3	2 hour
20	5	hour 6
28	7	hour12
52	13	hour 24

2-Effect of 15% concentration:

The results of the study, as shown in Table (2), revealed no significant differences between the replicates or exposure periods for the second treatment at 15% concentration at a probability level of $p \leq 0.05$ and t = 2.183. The number of killed insects was (2, 4, 6, 8, 15) according to the duration of the replicates (1, 2, 6, 12, 24) hours, and the percentages of killing were (8%, 16%, 24%, 32%, 60%) respectively.

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Table (2): The effect of 15% concentration of leaf extract of Myrtus Communis plant on the mortality of (Tribolium castaneum).

Percentage%	Number of Killed insects	Time
8	2	1 hour
16	4	2 hour
24	6	6 hour
32	8	12 hour
60	15	24 hour

3 - Effect of 20% concentration :

Table (3) shows the effect of 20% concentration of extract from the leaves of the Myrtus Communis plant on the mortality of (Tribolium castaneum).

Significant differences were observed between the replicates or time periods when exposed to the third treatment with a concentration of 20% at a significance level of $p \leq 0.05$ and $t=3.485$. The number of killed insects was (4, 8, 11, 14, 18) and according to the durations of the replicates (1, 2, 6, 12, 24) hours, the percentages of mortality were (16%, 32%, 44%, 56%, 72%), respectively.

Table (3) Effect of 20% concentration of leaf extract from the Myrtus Communis plant on the mortality of (Tribolium castaneum).

Percentage%	Number of Killed insects	time
16	4	1 hour
32	8	2 hour
44	11	6 hour
56	14	12 hour
72	18	24 hour

4- Effect of 25% concentration:

From Table (4), The findings of the study revealed notable variations between the replicates or time periods when exposed to a concentration of 25% with a probability level of $p \leq 0.05$ and $t=4.456$. The number of dead insects was (6, 10, 15, 17, 21) according to the durations of the replicates (1, 2, 6, 12, 24) hours, and the percentages of death were (24%, 40%, 60%, 68%, 84%) respectively, .

Table (4) Effect of 25% concentration of Myrtus Communis leaf extract on mortality of (Tribolium castaneum).

Percentage%	Number of Killed insects	time
24	6	1 hour
40	10	2 hour
60	15	6 hour
68	17	12 hour
84	21	24 hour

The effect of Myrtus Communis extract on reducing the size of infection with the dwarf tapeworm Hymenolepis nana :

The results of the study in the above tables showed that using different concentrations of the extract of Myrtus Communis leads to the death of (Tribolium castaneum) and reduces the size of the infection with the dwarf tapeworm (Hymenolepis nana). This is due to the reduction in the larvae of the red flour beetle, which the tapeworm infests or spends part of its life in. Therefore, the presence of these insects in warehouses increases the spread rate of this worm.

Discussion:

The results of the study in tables (1, 2) showed that the first and second treatments with concentrations of 10% and 15% of the extract of yarrow leaves, respectively, for repeated durations of (1, 2, 6, 12, 24) hours, at a probability level of $p \leq 0.05$ and $t=2.029$ and $t=2.183$, resulted in killing and destruction of the red rust flour beetle insects, where the percentage of mortality was (8%, 12%, 20%, 28%, 52%) and (8%, 16%, 24%, 32%, 60%) respectively. No significant distinctions were observed among the treatments regarding the repeated durations or exposure periods. It was observed that the killing or destruction increased with the increase of the extract concentrations for both treatments, reaching up to 84%, which was clearly noticed in the results of tables (3, 4) for the third and fourth treatments with concentrations of 20% and 25% of the extract of yarrow leaves, respectively, for repeated durations of (1, 2, 6, 12, 24) hours. The percentages of mortality were (16%, 32%, 44%, 56%, 72%) and (24%, 40%, 60%, 68%, 84%) respectively, at a probability level of $p \leq 0.05$ and $t=3.485$ and $t=4.456$. There were significant differences between the treatments regarding the repeated durations or exposure periods. The results showed that the percentages of mortality or destruction increased with the increase of the exposure time for all treatments with the studied concentrations. These results are consistent with the results of studies conducted by Al-Qazzaz (2010), which showed that the mortality increases with the increase of concentrations up to 100%, and Al-Jassani (2011), which showed that the percentage of mortality was 55% at a concentration of 4% of the cold water extract of eucalyptus leaves. There is a proportional relationship between the concentration of the extract and the percentage of mortality. Moreover, Al-Bayati et al. (2013) in Iraq showed that the treatment with ginger oil spray mixed with extracts of Henna and Myrtle plants resulted in the highest mortality rates, reaching 78.4%, while the lowest mortality rate was recorded in the control treatment, which was 0%.

The outcomes of this investigation deviate from the findings of a study conducted by Khalaf et al. (2006), which indicated a higher susceptibility of carpet beetle larvae and adults to chemical pesticides compared to the susceptibility observed in red flour beetle larvae and adults. Furthermore, these outcomes contrast with the results reported by Al-Hadidi et al. (2014), where the mortality rates were recorded as 50.050%, 4.350%, and 1.483% for clove, ginger, and coriander powders, respectively.

The study results showed that the four treatments with different concentrations of leaf extract of the Asiatic plant (Leycesteria formosa) contained some toxic substances that lead to the death of (Tribolium castaneum), which reduces the infestation rate of the dwarf tapeworm (Hymenolepis nana) by reducing the larvae of the red flour beetle, which the tapeworm parasitizes or spends part of its life cycle in. This is consistent with Qazzaz's (2010) findings, which clearly showed that the leaf extract of Asiatic plant containing some toxic substances caused the death of the red flour beetle. This is due to the toxic effects of these substances on the physiological functions inside the insect's body. These findings are also consistent with Rahman et al.'s (2007) study, which mentioned the use of ethyl alcohol in Melgota fruit to control rice weevil.

The conclusions are as follows:

1- The study showed that the leaf extract of Myrtus communis (L.) plant has an effect in killing or causing the death of Tribolium Castaneum (Herbest).

2- There is a direct relationship between increasing concentrations of the leaf extract of Myrtus Communis (L.) plant and the percentage of killing or causing the death of Tribolium Castaneum (Herbest).

3- There is an inverse relationship between the percentage of killing or causing the death of Tribolium Castaneum (Herbest) and the production of larvae that are infested with the tapeworm Hymenolepis nana, which in turn reduces the size of the infestation.

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