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Effectiveness of entomopathogenic fungi and plant extract against the rust-red flour beetle *Tribolium castaneum* (Coleoptera: Tenebrionidae) adults

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Abstract

The darkling beetle, also known as the rust-red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is a major pest that destroys grains and flour. It is also commonly used for research on ethology and food safety. The use of entomopathogenic fungi (*Beauveria bassiana*, *Metarhizium anisopliae*, and *Verticillium lecanii*) and chemical insecticides to control *T. castaneum* has been limited due to concerns about environmental sustainability. As a result, there is a pressing need to find environmentally friendly insecticides against this pest. This study evaluates the effects of entomopathogenic fungi and an extract from the green shoots of Arta, *Calligonum comosum* L'Hér., on adult *T. castaneum*. The fungi used in this study were isolated from soil in Egypt, while the plant extract samples were collected from natural vegetation in southern Sinai, Egypt. Different concentrations of the ethanolic plant extract (50, 75, and 100%) were applied to *T. castaneum*, and the effects were observed every 3 hours over a 24 hrs. period. The entomopathogenic fungi proved to be effective against adults of *T. castaneum*, *B. bassiana*, and *V. lecanii* causing 86.67% and 60.0% mortality, respectively, after seven days of infection at a concentration labeled as (C3). *M. anisopliae*, on the other hand, resulted in 100% mortality after seven days when infecting the (C3) concentration. The shoot extract of Arta, *C. comosum*, also exhibited effectiveness, with total mortality rates recorded after 48, 72, and 72 hrs. corresponding to concentrations of 100, 75, and 50, respectively. This study concludes that both the entomopathogenic fungi and *C. comosum* extract contain bioactive compounds that are toxic to *T. castaneum*.

Introduction

Around 10 to 40% of stored cereal grain worldwide is damaged by insect pests each year, particularly in tropical and subtropical regions of developing countries (Madrid *et al*., 1990; Shaaya *et al*., 1997 and Tripathi *et al*., 2009). Insects such as beetles, moths, and mites are responsible for destroying stored foods (Rajendran, 2002). Protecting stored grain and seeds from these pests is a significant challenge in post-harvest processes. As stored grain pests are spread

worldwide through human activity and seed transportation, they have evolved to adapt to different food sources. One of the most common and destructive stored-product insects is the confused flour beetle, *Tribolium castaneum* du Val., 1863 (Coleoptera: Tenebrionidae) (Aitken, 1975 and Hodges *et al*., 1996). These beetles have a large appetite and can consume various foods, including those stored in soils, warehouses, grocery stores, and houses (Via, 1999). They are especially abundant in cereal products such as wheat and flour (Aitken, 1975 and Hodges *et al*., 1996). When present in large numbers, confused flour beetles release a chemical mixture that can affect product quality as it contains carcinogenic quinones (Hodges *et al*., 1996). Fumigants and residual grain protectants are commonly used to control these pests, but fumigation has limitations (Mills, 1983; Taylor, 1989; Bell and Wilson, 1995; Bell, 2000 and Caddick, 2004).

The red flour beetle (*T. castaneum*) is another species of beetle in the Tenebrionidae family that is a worldwide pest of stored products, particularly food grains (Abdel-Raheem *et al*., 2016 a). It can cause damage to stored grain and other food products such as flour, cereals, pasta, biscuits, beans, and nuts (Abdel-Raheem *et al*., 2016 a). Some species of "*Tribolium*" may also spread the parasite "Hymenolepis nana" as an intermediate host (Jump, 2014). It can cause allergic responses but does not damage structures or furniture. The United Nations considers *T. castaneum* and *Tribolium confusum* (Jaquelin du Val) (Coleoptera:Tenebrionidae) to be the two most common secondary pests of plant commodities stored worldwide (Jump and Sallam, 2008; Sabbour and Abdel-Raheem, 2016; Sabbour *et al*., 2020 and Soliman *et al*., 2014).

Entomopathogenic fungi (EPF), which are used for biological control, may face challenges in the field due to environmental conditions that can affect their ability to infect and control pests. Factors like adverse temperatures and humidity can hinder the effectiveness of EPF treatments, making them less successful compared to conventional pesticides (Lord, 2001; Michalaki *et al*., 2007 and Jaronski, 2010). Other control methods, including synthetic chemicals and natural compounds, can interact with biocontrol agents and impact their efficacy (Samson *et al*., 2005; Friberg *et al*., 2005; Ismail *et al*., 2015 and Rabie *et al*., 2005). These interactions can be positive or negative for the growth and performance of EPFs in pest control. A study by Komaki *et al*. (2017) examined the effectiveness of seven entomopathogenic fungal treatments against *T. confusum* adults under laboratory conditions (Zaki and Abdel-Raheem, 2010). These treatments included *Beauveria bassiana*, *Paecilomyces farinosus*, *Isaria fumosorosea*, *Isaria farinosa*, *Lecanicillium muscarium* (2 isolates), and an extract of *L. muscarium* (Abdel-Raheem *et al*., 2009; Abdel-Raheem *et al*.,2016b; Abdel-Raheem *et al*., 2019; Abdel-Raheem, 2019 a, b, Abdel-Raheem 2020 a, b; Abdel-Raheem *et al*., 2015; Abdel-Raheem, 2005 and Zimmerman, 1986).

This work aims to study the effectiveness of entomopathogenic fungi and plant extract against the rust-red flour beetle *T. castaneum* adults.

Materials and methods

1. Entomopathogenic fungi:

Samples of soil were gathered from Kafr El-Sheikh and El-Behira Governorates to capture entomopathogenic fungi following the technique devised by using the larvae of the greater wax moth, *Galleria mellonella* L., as a vulnerable host of insects. Deceased larvae were obtained from the soil samples, cleaned with sterilized distilled water, and placed in Petri dishes on damp filter paper at a temperature of 24 ± 2 ºC and a relative humidity of 85 ± 5 %. *B. bassiana* and

Metarhizium anisopliae were derived from insects (*Cassida vittata* and *Scrobipalpa ocellatella*) as well as soil from Egypt. To generate a large number of conidia, isolates of *B. bassiana*, *Metarhizium anisopliae*, and *Verticillium lecanii* were cultivated on moistened rice. Two kilograms of wetted rice were rinsed in boiled water for a duration of 10 minutes and then placed in thermal bags. These bags underwent autoclaving at a temperature of 120ºC for 20 minutes, followed by infection with the isolates and incubated at a temperature of 26 ± 1 ºC for 15 days. The harvested conidia were obtained through the use of distilled water and filtered with cheesecloth to minimize the presence of mycelium clumps, with the addition of 80% Tween.

2. Preparing of the concentrations:

The fungal isolates' spores were collected by adding sterilizing water containing 0.5% Tween 80 to 14-day-old culture rice media. To prevent mycelium clumping, the suspensions were filtered through cheesecloth. The spores in the suspension were quantified using a haemocytometer with dimensions of 0.1 mm x 0.0025 mm 2. The suspension was then transferred into 2-liter plastic bottles. To restore their virulence, the isolates were passed through wax moth larvae *Galleria mellonella*, which served as their natural host. Three concentrations were prepared: (C_1) $1x10^6$ spores/ml, (C_2) $1x10^7$ spores/ml, and (C_3) $1x10^8$ spores/ml.

3. Bioassay test:

The insects were examined daily by spraying 15 adults with three different concentrations (C_1) $1x10^6$, (C_2) $1x10^7$, and (C_3) $1x10^8$ spores/ml from *B. bassiana*, *M. anisopliae*, and *V. lecanii*. Another group of 15 larvae were sprayed with water as a control.

4. Insect breeding:

Newly hatched adult beetles from flour infestations were collected and placed in 1-liter glass jars (Containing 10 pairs per jar). Each jar was filled with approximately 250 grams of flour and covered with a muslin cloth. The insects were reared in a controlled laboratory environment with temperature maintained at 26±2ºC and relative humidity at 70±5%, aiming to establish a consistent breeding population.

5. Plant materials:

The fresh leaves of *Calligonum comosum* were dried in the shade and crushed into a fine powder. Approximately 100 grams of the dried powder were subjected to extraction using 80% ethanol for 24 hrs. This process was repeated three times. The resulting extract was subsequently evaporated and stored for future use (Ismail *et al*., 2016 and Alkhalifa, 2013)

6. Biological study:

In the treatment, moist filter papers were positioned on four groups of three Petri dishes. A total of 240 insects were moved from the glass jar to each set of petri dishes, resulting in 20 insects per dish. To prepare the treatment, different concentrations (50, 75, and 100 %) of plant extracts were made from the stock solution, and 5 mL of each concentration was added to the corresponding set of dishes. Distilled water was utilized as a negative control. The number of deceased insects was tallied daily to monitor the impact of the diverse extract concentrations. The percentage of insect mortality was adjusted using the following equation:

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

$$\text{Percentage mortality} = \frac{\text{No. of dead insects}}{\text{No. of insects introduced.}} \times 100$$

Results and discussion

Table (1) demonstrates that the percentage of deaths with varying concentrations (C_1, C_2, and C_3) of *B. bassiana* isolated on the fifth day were 26.67%, 40.0%, and 53.33%, respectively. Similarly, the corresponding results with *V. lecanii* isolation were 20.0%, 40.0%, and 53.33%, respectively. On the fifth day, the percentages of deaths with all concentrations (C_1, C_2, and C_3) of *M. anisopliae* isolation were 40.0%, 46.67%, and 53.33%, respectively. In contrast, on the seventh day, the percentage of deaths with all concentrations (C_1, C_2, and C_3) of *B. bassiana* isolation reached 85%, whereas in *M. anisopliae* isolation, the mortality rate was 100% across all concentrations. These findings have been reported by Abdel-Raheem, 2015, 2019b and 2020a; Abd El-Salam *et al*., 2019; Abdel-Raheem *et al*., 2020; Acevedo *et al*., 2007 and Lawrence 1979.

Table (1): Percent Mortality of *Tribolium castaneum* adults treated with *Beauveria bassiana, Metarhizium anisopliae* and *Verticillium lecanii* isolates.

Days after infected	Percent of Moralities									
	Control	*Beauveria bassiana*			*Metarhizium anisopliae*			*Verticillium lecanii*		
		*C_1	C_2	C_3	C_1	C_2	C_3	C_1	C_2	C_3
2nd	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3rd	0.0	13.33	20.0	26.67	20.0	26.67	33.33	5.56	13.33	20.0
4th	0.0	20.0	26.67	40.0	26.67	40.0	46.67	13.33	26.67	33.33
5th	0.0	26.67	40.0	53.33	40.0	46.67	53.33	20.0	40.0	53.33
6th	0.0	33.33	46.67	66.67	46.67	66.67	80.0	26.67	46.67	53.33
7th	0.0	40.0	53.33	86.67	60.0	93.33	100	33.33	53.33	60.0
8th	0.0	66.67	99.33	100	86.67	100	100	60.0	80.0	86.67
9th	8.0	100	100	100	100	100	100	86.67	100	100

*** (C_1) 1x10^6, (C_2) 1x10^{7}and (C_3) 1x10 ^{8} Spores /ml.**

In this study, the lethal effect of Calligonum comosum's ethanolic extract as bioassay against the adults of *T. castaneum* was evaluated. The plant contains phytochemicals that are effective in controlling *T. castaneum.* Previous studies have proven the effectiveness of control programs (Kamil *et al*., 2000 and Abdel-Raheem *et al*., 2016c). The results of the current investigation indicate that the application of the plant extract to *T. castaneum* has a significant lethal effect. Table (2) observed the percent mortality differed according to the different extract concentrations. Based on our discovery, it has been revealed that the utilization of 5 ml of botanical extract containing 100, 75, and 50% concentration resulted in the elimination of the insects within 48 and 72 hrs., correspondingly.

Table (2): Percent mortality of *Tribolium castaneum* adults treated with plant extract.

Time (hrs.)	Plant extract concentrations			
	Control	**50 %**	**75 %**	**100 %**
3	0.0	5.56	13.33	20.0
6	0.0	5.56	13.33	20.0
12	0.0	13.33	20.0	20.67
18	0.0	13.33	20.0	26.67
24	0.0	20.67	26.67	26.67
48	1.0	26.67	26.67	53.33
72	3.0	26.67	30.33	53.33
Total death %	10.0	80.0	85.0	100

According to Table (3), it was found that *V. lecanii* had the smallest quantity of eggs deposited, with a value of 6.21, which showed a notable contrast compared to the maximum value of 49.46 observed in the control experiment. Other results regarding the number of eggs laid showed that when a high concentration of *M. anisopliae*, *B. bassiana*, and plant extract was applied, the mean number of eggs laid was 15.13, 14.20, and 12.47, respectively. Table (3), additionally, the mean number of dead *T. castaneum* was 1.01 in the control, which was significantly different from the mean values of 3.11 and 2.01 recorded at the time when *V. lecanii* and *B. bassiana* submitted our applications, individually.

Table (3): Effects of entomopathogenic fungi and plant extract on the development of *Tribolium castaneum*.

Treatments	Average No. of eggs laid ± SE	Average No. of eggs hatched ± SE	Average No. of dead *Tribolium castaneum* ± SE
Metarhizium anisopliae	15.13 ± 1.10b	3.10 ± 0.77c	2.0 ± 0.33c
Beauveria bassiana	14.20 ± 1.15b	3.28 ± 1.17c	2.01 ± 0.34c
Verticillium lecanii	6.21 ± 2.70c	3.29 ± 0.10d	3.11 ± 1.33b
Plant extract	12.47 ± 2.32b	5.36 ± 1.11b	3.9 ± 1.9b
Control	49.46 ± 4.11a	44.47 ± 2.11a	1.01 ± 1.18c

Sample means followed by the same alphabets are not significantly different from each other (DMRT 0.05). Table (4) revealed that the beetles exposed to flour treated with entomopathogenic fungi and plant extract exhibited varying levels of survival during the adult emergence phase. There was a substantial distinction in the average number of adults that emerged from flour treated with different entomopathogenic fungi and plant extracts.The smallest count of grownups (3.01) emerged when *V. lecanii* was applied, the highest number differed significantly from this (41.25) obtained from the control treatment where no entomopathogenic fungi and plant extract were used. The different treatments of entomopathogenic fungi and plant extract on *T. castaneum* resulted in varying total developmental periods (From egg to adult).

The period required for development with the lowest average duration of 15.5 days was observed when *B. bassiana* was applied, the longest period of development differed significantly from the one mentioned 23.2 days observed in the control experiment, but

not significantly different from the periods of 17.4 and 16.2 days observed when *M. anisopliae* and *V. lecanii* we have submitted our applications, individually.

The findings acquired regarding the gender distribution (males: females) indicate that the use of entomopathogenic fungi and plant extract altered the gender balance of *T. castaneum*. The 0:5 ratio demonstrates that *V. lecanii* influenced the number of males produced compared to the control treatment (6:4). The precise mechanism by which the entomopathogenic fungi and plant extract affect the sex ratio of *T. castaneum* offspring is not well understood and further scientific investigation may be necessary.

Table (4): Effects of entomopathogenic fungi and plant extract on the developmental period and sex ratio of *Tribolium castaneum.*

Treatments	Average no. of adult emergence ± SE	Total developmental period (egg – adult) (days)	Sex ratio (M:F)
Metarhizium anisopliae	3.10± 0.30c	17.4 ± 2.63c	8:7
Beauveria bassiana	6.20± 1.20c	15.5 ± 2.40c	4:5
Verticillium lecanii	3.01 ± 0.60b	16.2 ± 1.53c	0:5
Plant extract	4.10 ± 0.11b	19.02 ± 3.30b	7:2
Control	41.25 ± 5.33a	23.2 ± 4.34a	6:4

Groups of samples mean with identical letters are not statistically distinct from one another (DMRT 0.05). The mortality rate for all concentrations (C_1, C_2, and C_3) of *B.bassiana* on the 7th day was 40.0%, 53.33%, and 86.67%, respectively. The corresponding mortality rates for *B. bassiana* were 35%, 43%, and 51%, respectively (Abd El-Salam *et al.,* 2019). On the 7th day after infection, *M. anisopliae* resulted in 100% mortality at the highest concentration, while *V. lecanii* resulted in 60.0% mortality. The extract from *C. comosum* killed 100% of adults after 48 hrs. In studies conducted by Salem *et al.* (2016 and 2017) plant extracts were used against snails and *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), a pest that affects onion crops in Egypt. The mortality rate exceeded 50% after 72 hrs. and the 4th spray (Aldryhim, 1990; Samson *et al.* 2005; Jump and Grünwald, 2013; Abdel-Raheem 2015; Abdel-Raheem *et al.* 2020; Abdel-Raheem *et al.,* 2018; Abdel-Raheem and Youssif, 2020; Reyad *et al*., 2020 and Ismail *et al.,* 2014).

The evidence suggests that both entomopathogenic fungi and plant extract hold potential as effective alternatives to conventional pesticides for controlling stored product pests and can be incorporated into integrated pest management programs. This approach can help reduce environmental pollution, particularly when pests are below the economic threshold.

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