

Laboratory Evaluation of Two Species of Entomopathogenic Fungi as Biological Control Agents Against The Stored-Grain Insect Pest, *Sitophilus granarius* L. (Coleoptera: Curculionidae), and their Histological effect on Albino Rats

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Abstract

This study aimed evaluate to the efficacy of two entomopathogenic fungi, *Beauveria bassiana*, and *Metarhizium anisopliae*, against the adults of *Sitophilus granarius* (Coleoptera: Curculionidae), are stored grains primary insect pest at two temperatures 20 and 30 ±2 °C and relative humidity 65±5% under laboratory conditions. The residual impact of the two entomopathogenic fungus on the treated wheat was evaluated concerning histological changes in different organs of rats fed on the treated wheat. Five concentrations were tested from two entomopathogenic fungi, and dead individuals were counted daily, following treatments, for 21 days. Lethal time values (LT₅₀ and LT₉₀) for the two entomopathogenic fungi were calculated. The results demonstrated that the mortality varied according to the temperature and tested the two entomopathogenic fungi. The mortalities increased by increasing the temperature. The mortality percentages of the treated insect by the two entomopathogenic fungi at 10% concentration and 20 °C after 21 days were 87.77%, and 80% for *B. bassiana* and *M. anisopliae*, respectively. Whereas, when the temperature increased to 30 °C, the percentage of mortality increased to 90% for *B. bassiana* and 86.66% for *M. anisopliae*. LT₅₀ values for *B. bassiana* and *M. anisopliae* were 10.12 and 11.42 days, respectively, and LT₉₀ values were 24.33 and 29.96 days, respectively, at 20 °C for *S. granarius*. While, LT₅₀ values for *B. bassiana* and *M. anisopliae* were 9.51 and 10.09 days, respectively, and LT₉₀ values were 23.22 and 24.80 days, respectively, at 30 °C. Histological examination for the rats fed on treated wheat with entomopathogenic fungi was done. Overall, the liver; kidney, and lung were affected through the appearance of mild histological changes. This study indicated that *B. bassiana* and *M. anisopliae* had significant potential as biological control agents against *S. granarius*.

Keywords: Entomopathogenic fungi, *Sitophilus granarius*.

Introduction

Many insect pests are a major group cause of post-harvest losses in stored grains, which are between 10 and 25% per year. Among these insects, *Sitophilus* weevils, including *Sitophilus granarius* (L.) (granary weevil), *Sitophilus oryzae* (L.) (rice weevil), and *Sitophilus zeamais* (L.) (maize weevil) (Coleoptera: Curculionidae), are well-known stored-grain insect pests in Egypt and many other countries in the world. These weevils have a nearly cosmopolitan distribution, occurring throughout all warm and tropical parts of the world (Hong, et al., 2018). Damages caused by the insects not only contain the direct feeding harm resulting in loss of weight, but they also seriously decrease nutrients, lowering seeds germination rate, reducing quality, and lowering their marketing value due to the mass of waste, webbing, and insect cadavers (Abdel-Raheem, et al., 2015). Stored-grain protection against pests is currently based on the use of synthetic insecticides and fumigants (Arthur, 1996). As a result, these have caused problems including insecticide resistance and the contamination of food products with chemical residues and consumer demand for pesticide-free grain. Thus, there is a growing interest in using biological control agents against these pests as an alternative control method.

Entomopathogenic fungi are common natural enemies of arthropods worldwide, attracting attention as potential biological control agents. Fungal entomopathogens such as *Beauveria Bassiana* (Balsamo) and *Metarhizium anisopliae* (Metschnikoff) play an important role in the regulation of insect populations. Also, since they exist in nature, entomopathogenic fungi have a low environmental impact and are generally considered environmentally safe agents with low mammalian toxicity (Rumbos and Athanassiou, 2017). The use of entomopathogenic fungi for the control of insect pests in stored-grain products is one of the most promising alternative control methods (Moore, et al., 2000). Especially, the species *B. bassiana* and *M. anisopliae* have a wide host range and have been tested against most of the major stored-grain pests (Batta, 2018 and Rumbos, & Athanassiou, 2017).

Temperature plays a significant role in the effectiveness of entomopathogenic fungi, especially high temperatures affect negatively conidial viability and germination (Rumbos and Athanassiou, 2017). For example, *B. bassiana* was found to be more effective against *Rhyzopertha dominica*, (F.) *Sitophilus oryzae* at 26 °C than at 30 °C (Vassilakos, et al., 2006), and *S. granarius* (Athanassiou and Steenberg, 2007) in stored wheat. Similarly, found that *Isaria fumosorosea* (wize) was more effective at 20 °C than at 25 °C. In another study, *I. fumosorosea*

was effective against *Tribolium confusum* (Herbst) and *Ephestia kuehniella*, (zeller) but its effectiveness was highly dependent on the target species and life stage, exposure interval, and temperature (Michalaki, et al., 2007).

The objective of the present study was to test, in the laboratory, the insecticidal efficacy of the two entomopathogenic fungi *B. bassiana*, and *M. anisopliae*, against the storage-grain insect pest, *S. granarius* under two temperatures 20 and 30 ± °C by using different concentrations of conidial suspensions. Also, the residual impact of these entomopathogenic fungi on the treated wheat was evaluated concerning histological changes in different organs of rats fed on the treated wheat.

Materials and methods

Insect culture

The insects were reared in glass jars (approx. 500 ml) containing about 200g of sterilized and conditioned wheat kernels. The glass jars were covered with muslin fixed with a rubber band. Insect cultures were kept under controlled conditions of 28±2°C and 65±5% RH in the rearing room of the laboratory of stored product pests, Plant Protection Dept., Faculty of Agric., Moshtohor, Benha University. Wheat grains were treated by freezing at -18°C for 2 weeks before application to eliminate any possible infestation by any insect species. The moisture content of the grains was around 14%. About 1000 male and female adults of insects (1-2 weeks old) were introduced into the jars for laying eggs and then kept at 28±2°C and 65±5% RH, three days later, all insects were separated from the food, and the jars were kept again at the controlled conditions in the rearing room. This procedure was repeated several times to obtain a large number of the adults needed to carry out the experiments during this study. The foods in the jars were renewed when it was necessary. Adults of *S. granarius* (1-2 weeks – old) were taken for the experiment bioassay.

Entomopathogenic fungi

Two commercial entomopathogenic fungi were used in this study, namely, **1- Bio-Power** was obtained from Syngenta Agro Company and, contains the entomopathogenic fungi, *Beauveria bassiana* at a concentration of (1×10⁸ CFU's/gm). **2- Metmite** was produced by the Pesticide Production Unit of the Plant Protection Research Institute at Dokki and contains the entomopathogenic fungi, *Metarhizium anisopliae* at a concentration of (1×10⁹ CFU's/gm). The recommended dose of the two entomopathogenic fungi was 10g/L of water.

Bioassay tests:

Wheat grains (20g) wheat kernels were treated with the two commercial preparation of entomopathogenic fungi, whereas 1 ml of various bio insecticide concentrations: 10, 5, 2.5, 1.25, 0. 625 g/100ml distilled water. Thirty adults of insects (1-2

weeks old) were added to the treated grains. For the control, the grains were treated with distilled water only. Three replicates (Jars) were carried out of each concentration and incubated at 20 °C and 30 °C using an incubator. Dead adults were counted and recorded at 1, 3, 5, 7, 10, 14, and, 21 days after treatment. Accumulative mortality data were corrected by **Abbott's formula (1925)**. The adults were removed from the jars after 14 days of holdings periods and the reduction in the progeny was also, calculated. The bioassay was performed by using a completely randomized experimental design.

Histological study:

Twenty -seven male Albino Wistar rats, weighing around (100 - 120 g.) were obtained from Rodents laboratory at the Faculty of Agriculture, Moshtohor, Benha University. Experimental design and animal handling were approved by the Research Ethical Committee of the Faculty of Veterinary Medicine, Benha University, Egypt. All efforts were made to minimize animal suffering. After one week acclimation period, rats were randomly assigned to three groups (each group 9 rats); the first group fed on treated grains with *B.bassiana* and the second group fed on treated grains with *M. anisopliae*, while the third group fed on untreated grains as control. All rats of the three groups were subjected to feed for 5 days. Three replicates (each replicate 3 rats) were carried out for each group. Specimens from vital organs (liver, kidney, and lung) of treated male rats were collected on the 7th; 14th, and 21st days after feeding on treated grains. Specimens from these organs were taken at the same interval times from control rats for comparison. All specimens were fixed in 10% neutral buffered formalin. The fixed specimens were dehydrated in ascending grades of ethyl alcohol, cleared in xylene, and blocked in paraffin. Paraffin blocks were cut in sections of -5 micron thickness. Sections were stained with hematoxylin and eosin for general structure. All histological changes were examined and photographed by a Leica DM3000 microscope.

Statistical analysis:

The obtained mortality data were subjected to Probit analysis (Finney, 1971), A computer program (Noack and Richmuth, 1978), which was used for determining the dosage mortality response for the bio-insecticides.

Results and discussion

The efficacy of the two commercial preparations of entomopathogenic fungi, *B. bassiana* and *M. anisopliae* against adults of stored grain pest, *S. granarius* at two temperatures (20- 30°C) was evaluated.

1. Efficacy of *B. bassiana* and *M. anisopliae* against adults of *S. granarius* at two different temperatures (20- 30°C).

The results show that the mortality percentages were increased by increasing temperature degree, entomopathogenic fungi concentration, and period of exposure. whereas at the highest concentration of 10 g/100 ml, the adult mortality was 7.77 % after 3-days and increased gradually to reach 90% at 21 days post-treatment at 30°C. While at the same concentration at low-temperature 20°C, the mortality was 4.44% after 3-days and increased after 21 days post-treatment to 87.77%. On the other hand, at the lowest concentration, 0.625 g/ml, the mortality was 2.22% after 3-days and increased to 48.88% after 21 days post-treatment at 30°C. While at low-temperature 20°C, no mortality was observed after 3-days and recorded 8.88% after 5 days, then increased to reach 46.66% after 21 days post-treatment. (Figs. 1&2 and Table 1)

The same trend of results was obtained with the commercial preparation of *M. anisopliae* against the adults of *S. granarius*, where the highest concentration effect (86.66%), was recorded at 30°C at the end of 21 days with the highest concentration of 10g/ml, while at the same concentration of *M. anisopliae* at 20°C the mortality recorded 80% after 21 days post-treatment. At the lowest concentration, 0.625 g/ml, the mortality was 1.11% after 3-days and increased gradually to reach 42.22% after 21 days

post-treatment at 30°C. While at 20°C, the mortality began to appear after 5-days (4.44%), then increased to reach 40% after 21 days post-treatment. (Figs. 3&4).

The lethal times (LT₅₀, LT₉₀ and LT₉₅) of *B. bassiana* used against the adult of *S. granarius* at two temperatures of 30 and 20 ° C are shown in Table (1). The results reveal that the time required to obtain 50% mortality (LT₅₀) at the highest concentration 10 mg/100ml. were 9.51, and 10.12 days for *S. granarius* at 30 and 20° C, respectively. While, the time needed to achieve 90% mortality (LT₉₀) was 23.22, and 24.33 days for the same insect pest and two temperatures, respectively. On the other hand, the time required to obtain 95% mortality (LT₉₅) was 29.91 and 31.198 days for the same insects and two temperatures, respectively.

the LT₅₀ values of the *M. anisopliae* (10.09 and 11.42 days, respectively) used against was lesser effective than *B. bassiana*. considering the LT₉₀ values of *M. anisopliae* were 24.80, 29.96 days, respectively at the same concentration and the two temperatures 30-20° C.

The results indicated that *S. granarius* was more sensitive to the *B. bassiana* at the higher temperature than *M. anisopliae*.

Table 1. The efficiency of *B. bassiana* and *M. anisopliae* biopesticide against the adults of *S. granarius* after exposed period .

Concentration (W/W) %	Temp.	(%) Adult Accumulative mortality after an indicated period (days)					
		3	5	7	10	14	21
<i>Beauveria bassiana</i>							
10	30°C	7.77	15.55	31.11	50	71.11	90
	20°C	4.44	14.44	30	48.88	65.55	87.77
5	30°C	5.55	14.44	28.88	44.44	63.33	82.22
	20°C	3.33	13.33	27.77	42.22	64.44	82.33
2.5	30°C	4.44	12.22	23.33	35.55	53.33	71.11
	20°C	2.22	11.11	21.11	38.88	52.22	70
1.25	30°C	3.33	11.11	22.22	34.44	47.77	66.66
	20°C	1.11	10	17.77	37.77	51.11	65.55
0.626	30°C	2.22	6.66	14.44	23.33	34.44	48.88
	20°C	0	8.88	15.55	22.22	33.33	46.66
	20°C	0.	0	0	0	0	0
<i>Metarhizium anisopliae</i>							
10	30°C	5.55	14.44	30	47.77	67.77	86.66
	20°C	4.44	10	26.66	43.33	60	80
5	30°C	4.44	11.11	26.66	43.33	62.22	80.80
	20°C	3.33	8.88	23.33	40	55.55	73.33
2.5	30°C	3.33	10	25.55	37.77	50	67.77
	20°C	2.22	7.77	21.11	35.55	48.88	66.66
1.25	30°C	2.22	7.77	20	32.22	45.55	64.44
	20°C	1.11	6.66	18.88	26.66	40	62.22
0.625	30°C	1.11	5.55	13.33	20	28.88	42.22
	20°C	0	4.44	11.11	22.22	32.22	40

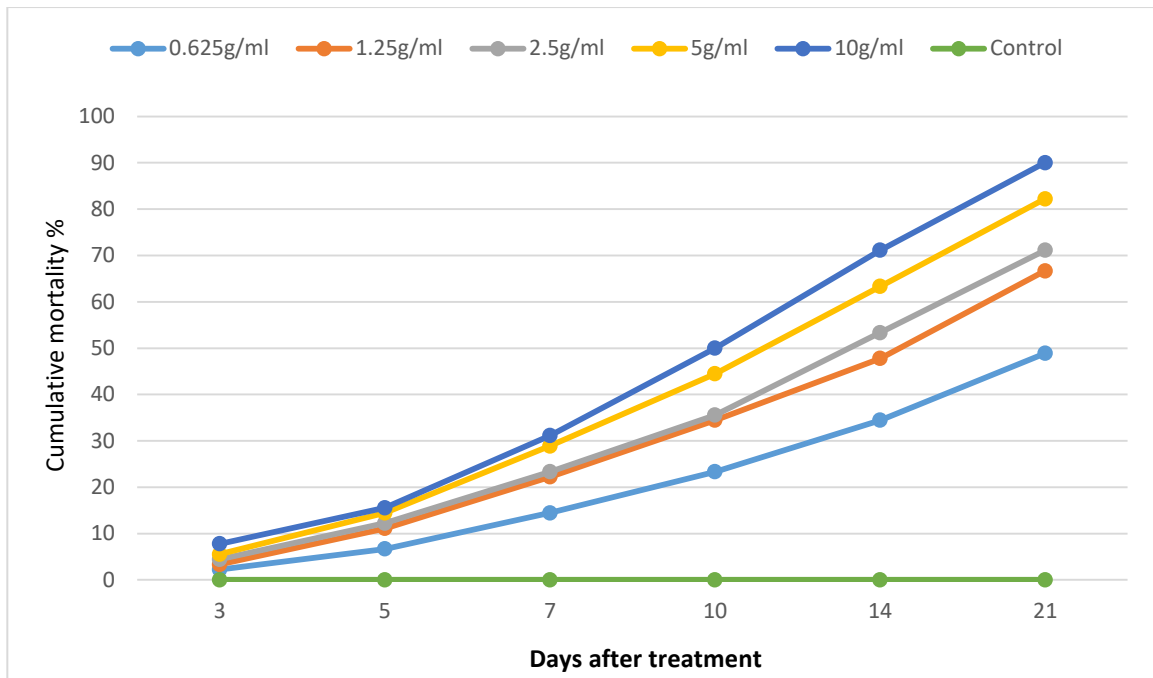
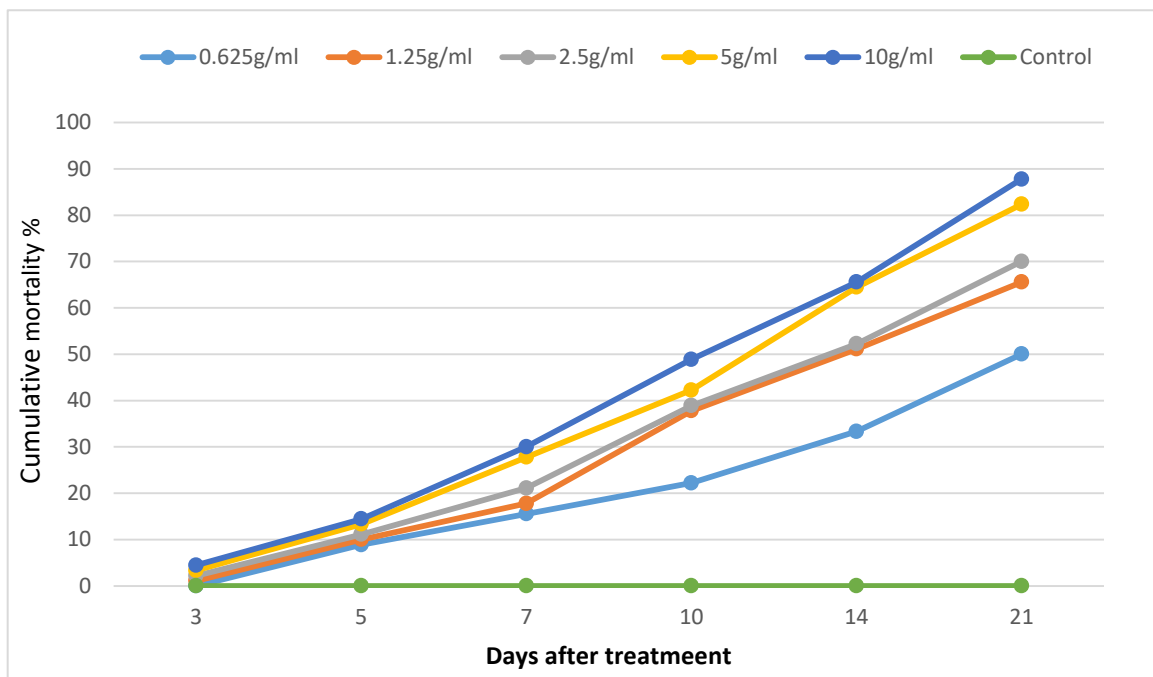


Fig. 1: Mortality % of *S. granarius* treated with *B. bassiana* at 30°C.

Fig. 2: Mortality % of *S. granarius* treated with *B. bassiana* at 20°C



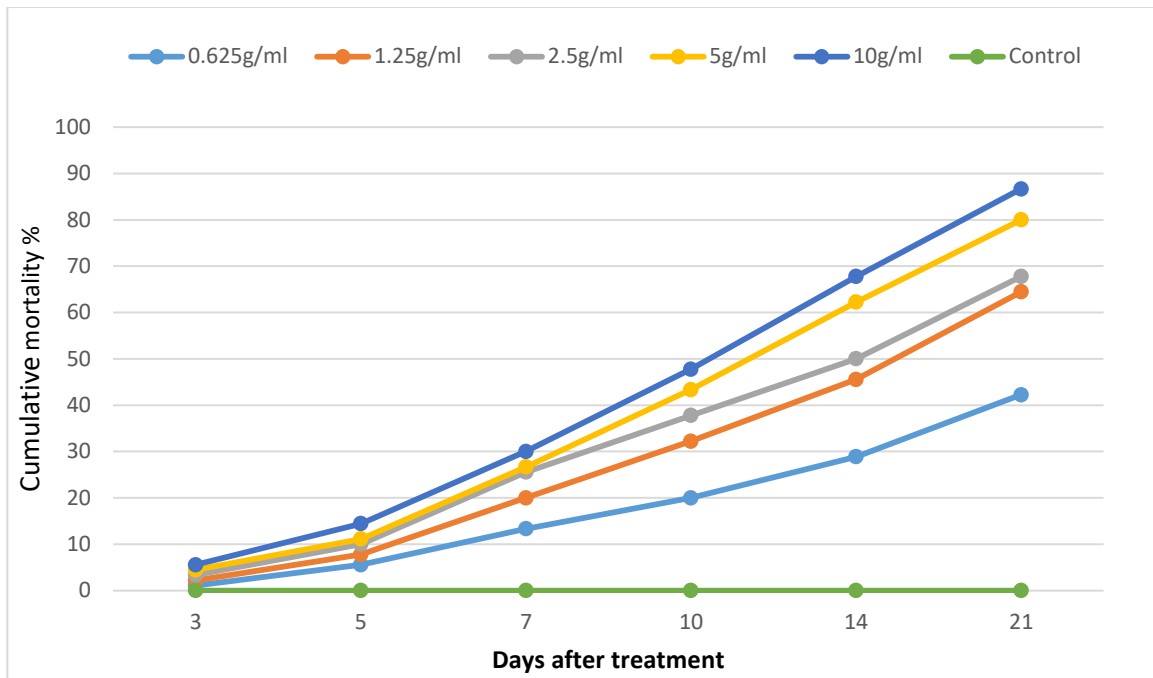


Fig. 3: Mortality % of *S. granarius* treated with *M. anisopliae* at 30°C

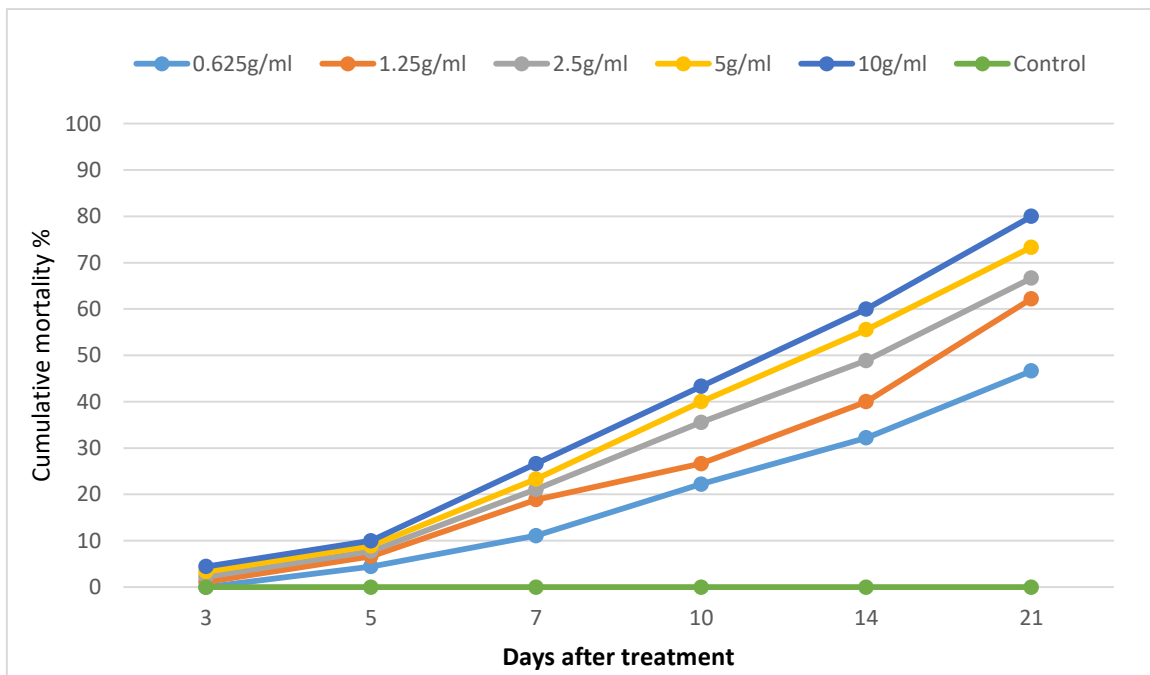


Fig. 4: Mortality % of *S. granarius* treated with *M. anisopliae* at 20°C

Table 1. Lethal times (LT₅₀, LT₉₀ and LT₉₅) for adults of *S. granarius* treated with the tested entomopathogenic fungi, *B. bassiana* and *M. anisopliae* at two different temperatures.

Conc.	Temp.	Lethal times and their 95% confidence limits (days)			Slope ± SD	R
		LT ₅₀	LT ₉₀	LT ₉₅		
<i>Beauveria bassiana</i>						
10gm/100ml	30°C	9.511 (8.736-10.385)	23.226 (19.979-28.292)	29.913 (24.982-38.006)	3.305±0.261	0.991
	20°C	10.123 (9.306-11.061)	24.331 (20.899-29.716)	31.198 (26.022-39.72)	3.365±0.267	0.998
<i>Metarhizium anisopliae</i>						
10g/100ml	30°C	10.091 (9.262-11.046)	24.809 (21.212-30.506)	32.015 (26.552-41.111)	3.28±0.263	0.997
	20°C	11.422 (10.413-12.655)	29.962 (24.942-38.4)	39.382 (31.65-53.1)	3.06±0.26	0.992

R= Correlation Coefficient of the regression line

SD= Standard deviation of the mortality regression line

2. Histological studies of the effect of the two commercial entomopathogenic fungi, *B. bassiana* and *M. anisopliae* on rat organs.

The lung, liver, and kidney sections of control rats throughout the experiment showed normal histological structures (Plates. 1A, 2A, and, 3A). The histopathological effects of both fungi on these organs were similar as shown in plates (1-3). Also, no fungal germination was identified on any of the examined organs throughout the experiment. On the 7th day, lung sections showed congestion of blood vessels and perialveolar and peribronchial inflammatory cells infiltration (Plates. 1B and 1C) which decreases in its severity on the 14th day (Plates. 1D and 1E) however nearly normal lung

structure was seen on day 21 with slight congestion of blood vessels (Plates. 1F and 1G).

Liver sections on 7th day showed dilated and congested central veins in addition to hydropic degeneration in hepatocytes (Plates. 2B and, 2C). These features decreased on days 14 and 21 where only congested central veins were seen on the 14th day (Plates. 2D and 2E) but T day 21, congestion of central vein was not seen (Plates. 2F and 2G).

Atrophied renal glomeruli, as well as peritubular congestion, were identified in kidney on 7th day (Plates. 3B and 3C) but on kidneys of day 14 (Plates. 3D and 3E) and day 21 (Plates. 3F and 3G) showed normal sized renal corpuscles. However, peritubular congestion still persisted in kidneys at 14th days (Plates. 3D and 3E).

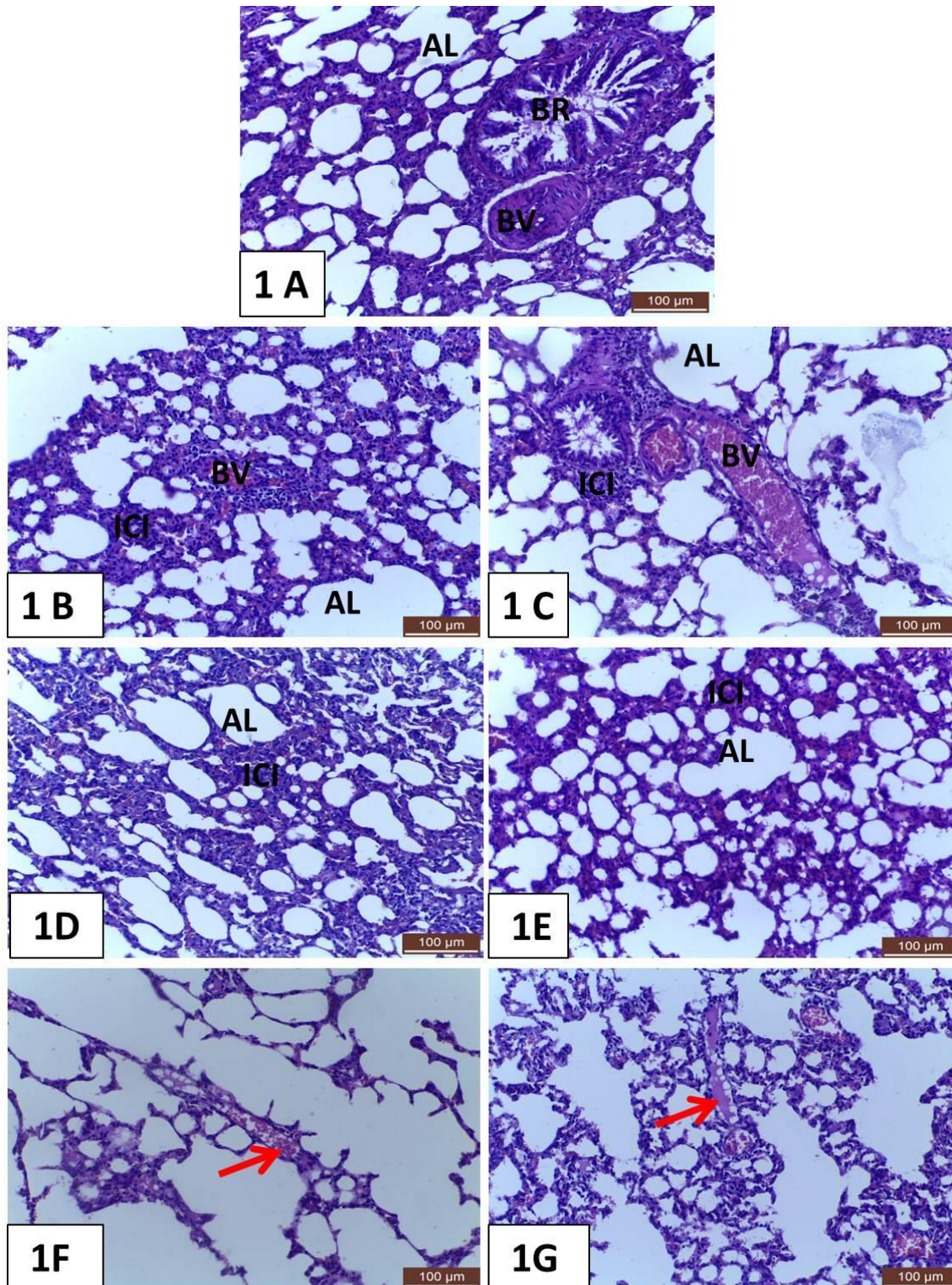


Plate 1. Photomicrograph of lung of all groups. (A): control rats, (B, D, F): treated rats with *B. bassiana* and (C, E, G): treated rats with *M. anisopliae*. (B, C) at 7th day, (D, E) at 14th, and (F, G) on day 21 of the experiment. AL, alveoli; BR, bronchiole; BV, blood vessels; ICI, inflammatory cells infiltration; red arrow, slight congestion. H&E stain. Scale bar = 100 µm.

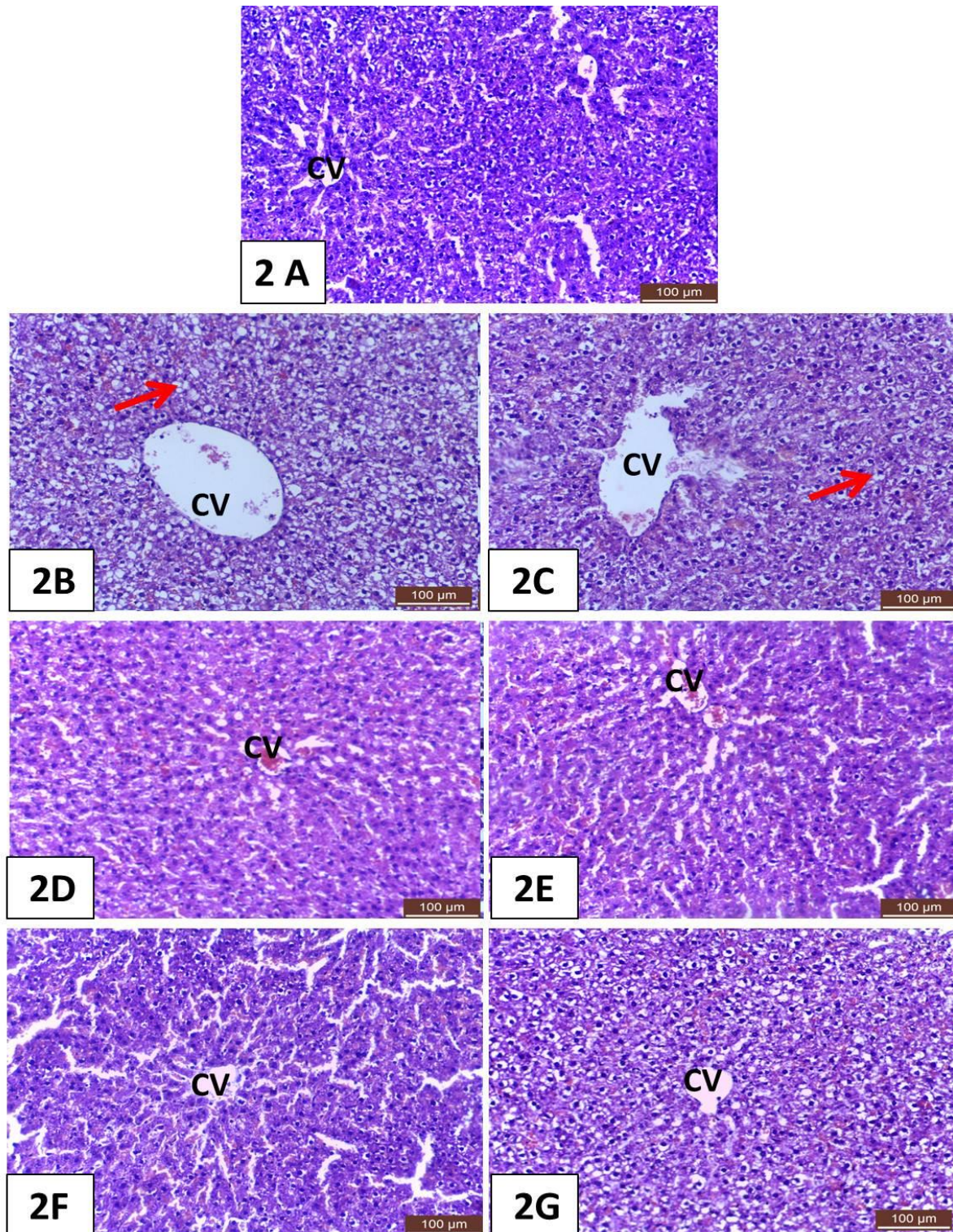


Plate. 2. Photomicrograph of liver of all groups. (A): control rats, (B, D, F): treated rats with *B. bassiana* and (C, E, G): treated rats with *M. anisopliae*. (B, C) at 7th day, (D, E) at 14th, and (F, G) on day 21 of the experiment. CV, central vein; red arrow, hydropic degeneration. H&E stain. Scale bar = 100 µm.

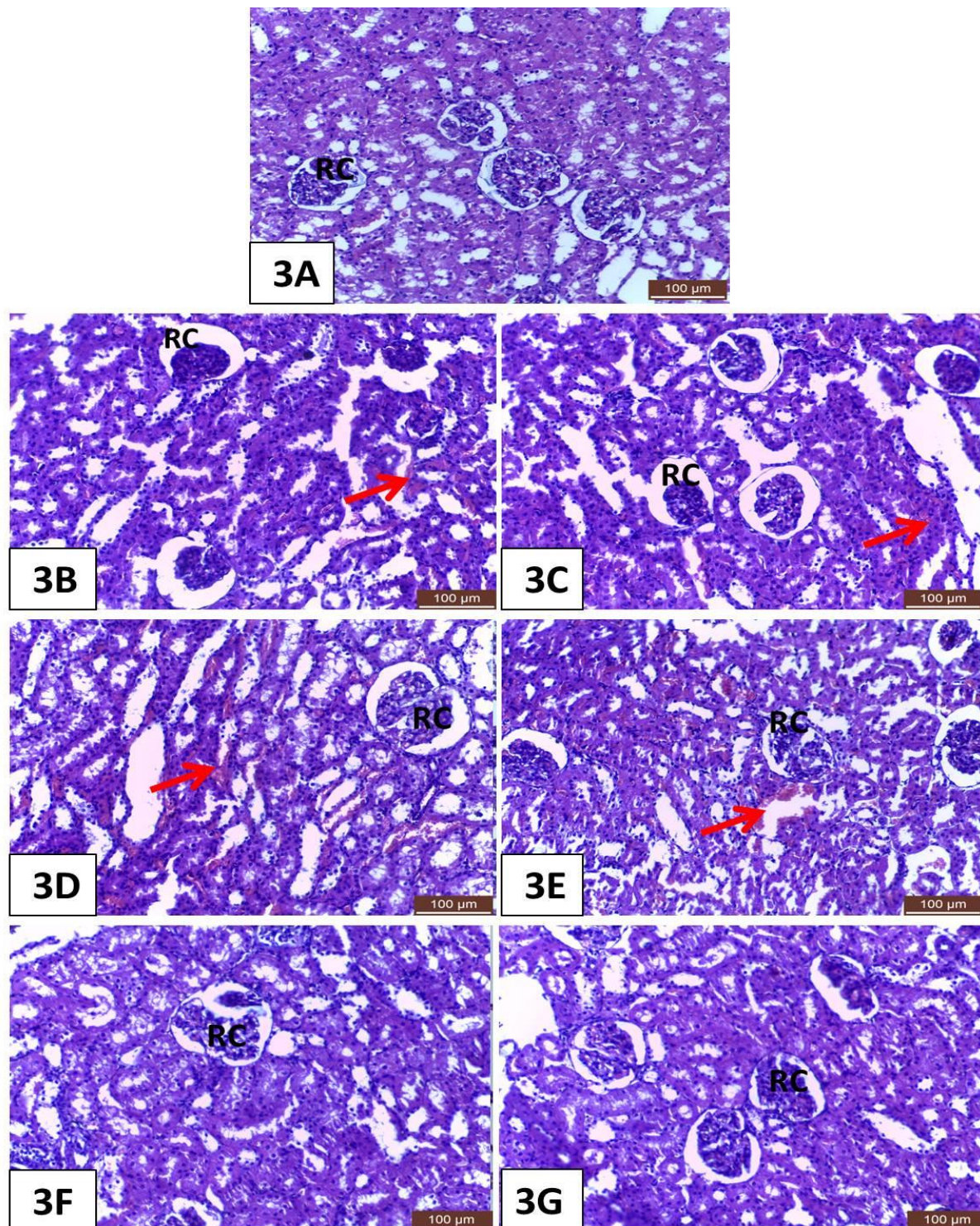


Plate. 3. Photomicrograph of kidney of all groups. (A): control rats, (B, D, F): treated rats with *B. bassiana* and (C, E, G): treated rats with *M. anisopliae*. (B, C) at 7th day, (D, E) at 14th, and (F, G) on day 21 of the experiment. RC, renal corpuscle; re arrow, peritubular congestion. H&E stain. Scale bar = 100 µm.

Discussion

Entomopathogenic fungi are being developed worldwide for the control of insect pests and some products are already available commercially. (Miller, et al., 1995). There is increasing evidence that habitat selection drives the pathogenicity of Entomopathogenic Fungi species. Thus, results from our study indicate that screening of potential isolates should not be limited to those isolated from the original host.

Sitophilus granarius is one of the less-studied storage pests as far as myco-biological control is concerned. (Shams, et al., 2011). recorded 60 % mortality of *S. granarius* after 13 days, when weevils were immersed in a conidial solution of *B. bassiana*. They also estimated LT_{50} at 10.45 days via probit analysis. When *S. granarius* adults were exposed to grain sprayed with a *B. bassiana* conidial suspension, the results demonstrated very low mortality (3%) (Hluchý and samsin, 1989)

Moreover, the insecticidal efficacy of Entomopathogenic fungi is highly influenced by several other factors such as the insect's behavior, population density, age, nutrition and genetic information, environmental conditions, as well as the effect of host physiology and morphology on its sensitivity to biological control agents such as Entomopathogenic fungi. Therefore, the differences in insect susceptibility to Entomopathogenic fungi could not be explained solely as a function of the applied conidial concentration.

In general, the results indicated that the percentage of mortalities increased by increasing the temperature degree. Among the two species of entomopathogenic fungi applied on *S. granarius* at 30 °C, *B. bassiana* showed the highest effect (90%) at 10g/ml concentration after 21 days. Whereas, when the temperature increased to 30 °C, the percentages of mortality increased, where *B. bassiana* showed the highest effect followed by *M. anisopliae* with all concentrations used. It means that temperature plays a significant role in the effectiveness of entomopathogenic fungi. (AK, 2019).

Also, among the two species of entomopathogenic fungi, *S. granarius* adults were more sensitive to *B. bassiana* than *M. anisopliae* fungi. In this respect, (Cherry, et al., 2005). demonstrated that different isolates of *M. anisopliae* and *B. bassiana* can provide good control of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) by immersion bioassay at 12 days, whereby *B. bassiana* was reported to be more virulent than *M. anisopliae*, so *B. bassiana* can be successfully used against stored wheat pests. Similarly, reported that some *B. bassiana* isolates can achieve 100% mortality of *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) (organophosphate resistant strain) after 10 days of treatment with a dose of 1×10^8 conidia/mL. In

previous studies, mortality of stored grain pests reached 80–100% after 10–20 days. (Rice and Cogburn, 1999) and (Padin, et al., 1997).

Kassa, et al., (2003) also reported that *B. bassiana* isolates were virulent against *Sitophilus zeamais* L. (Coleoptera: Curculionidae), but only at doses higher than 107 conidia/mL, and as such, variability among the different *B. bassiana* isolates were apparent. (Hidalgo, et al., 1998) also pointed out that it is possible to achieve a useful level of control of *S. zeamais* by using formulated *B. bassiana* conidia.

(Batta, 2005) recorded high mortality of *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae) after 7 days of treatment with *M. anisopliae*. Greater mortality of stored grain pests was achieved when these were inoculated with *B. bassiana* rather than *M. anisopliae* isolates (Moino, et al. 1998) and (Dal Bello, et al. 2006). In contrast, (Dal Bello, et al., 2000) reported that treatment of *S. oryzae* with *M. anisopliae* was not effective.

The histological evaluation was carried out on days 7th, 14th, and 21th days after treatment. The control rats, throughout the experiment, showed normal histological structures of lung, hepatic and renal tissues. Our histopathological findings revealed that the effects of both fungi on lung, liver, and kidney were similarly that was supported by (Kamarudin, et al., 2019) who reported that both *B. bassiana* and *M. anisopliae* perform similarly as an bioinsecticide. Prialveolar and peribronchial inflammatory cell, infiltrating hydropic degeneration of hepatocytes, and atrophied renal glomeruli along with preitubuar congestion were noted severly at 7th days of treatment. This histopathological effect decreased gradually from the 7th day to the 14th day till be of nearly no effect on day 21th of the experiment. No fungal germination was found on any of the examined organs throughout the experiment. Our histopathological findings regarding the lung were similar to those of (Tsai, et al. 1994), Tsai, et al., 2003), and (Escobar, et al., 2015). For us and after extensive literature, this study is considered the first to explore the effect of *B. bassiana* and *M. anisopliae* on the liver and kidney of rats.

Conclusion:

In Conclusion, the two species of Entomopathogenic fungi evaluated in this study showed that they were effective against *S. granarius* and may be considered as alternative to chemical control. In addition, *M. anisopliae* and *B. bassiana* showed about 86.66 and 90% efficacy against *S. granarius* at the end of 21 days. thus, the two species of Entomopathogenic fungi are promising against stored product pests and should therefore be further investigated as potential biological control agents and as a valuable component of stored-product IPM. Considering the propensity of *Sitophilus* species to develop resistance

to synthetic chemicals, the exploration of alternative biological control methods appears even more necessary. Although this is only a preliminary investigation into the use of entomopathogenic fungi, the fungal isolates we tested showed encouraging insecticidal effects which, however, need to be extensively followed up. Future research steps include establishing the biosafety of these fungi for non-target organisms.

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تقييم معلمي لنوعين من الفطريات الممرضة للحشرات كأحد عوامل المكافحة البيولوجية ضد حشرة الحبوب المخزونه

Sitophilus granarius وتأثيرها الهستولوجي على فئران الألبينو

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تعتبر سوسة القمح من اهم الافات الحشرية الأولية التي تصيب الحبوب المخزونة، تهدف هذه الدراسة الي تقييم فاعلية نوعان من الفطريات الممرضة للحشرات وهما *B. bassiana* and *M. anisopliae* ضد الطور الكامل لحشرة سوسة القمح علي درجتين حرارة مختلفتين وهما 20 ± 2 - 30 درجة مئوية ورطوبة نسبية 65 ± 5% تحت الظروف المعملية، كما أجري تقييم الأثر المتبقي لتلك الفطريات علي حبوب القمح علي التغيرات الهيستولوجية لبعض أعضاء الفئران التي غذيت علي القمح المعامل. تم اختبار خمس تركيزات لتلك الفطريات الممرضة وتم حساب نسبة الموت يوميا بعد المعاملة ولمدة 21 يوم، وتم حساب قيم الوقت اللازم لقتل 50-90-95% من الحشرات (LT₅₀ - LT₉₅) للفئران. وأظهرت النتائج أن نسبة الموت زادت بزيادة درجة الحرارة والتركيز المستخدم، وكانت نسبة موت الحشرات الكاملة لسوسة القمح عند تركيز 10% عند درجة حرارة 20م بعد 21 يوم من المعاملة بفطر *B. bassiana* (87.77%) بينما كانت لفطر *M. anisopliae* (80%)، وعندما زادت درجة الحرارة الي 30م ارتفعت نسبة الموت فكانت لفطر *B. bassiana* (90%) وأظهرت أعلي تأثر بينما كانت لفطر *M. anisopliae* (86.66%). وكانت قيم LT₅₀ لكل من *B. bassiana* و *M. anisopliae* (10.12) و (11.42) يوم، وقيم LT₉₀ كانت (24.33) و (29.96) يوم علي التوالي عند درجة حرارة 20م. بينما قيم LT₅₀ لفطري ال *B. bassiana* و *M. anisopliae* (9.51) و (10.09) يوم علي التوالي وقيم LT₉₀ (23.22) و (24.80) يوم علي التوالي عند درجة حرارة 30م. كذلك تم فحص التغيرات الهيستولوجية للفئران التي تغذت علي القمح المعامل بالفطريات، وكانت النتائج المتحصل عليها حدوث تأثير بشكل عام لكل من الكبد، الكلية والرئتان وذلك من خلال ظهور تغييرات نسيجية خفيفة. تشير هذه الدراسة بأن كل من فطري *B. bassiana* و *M. anisopliae* لديهم كفاءة عالية في مكافحة حشرة سوسة القمح ومن الضروري إجراء المزيد من الدراسات لتقييم فاعلية الفطريات الممرضة للحشرات علي الأوقات تحت ظروف التخزين.