Bionatura
Latin American Journal of Biotechnology and Life Sciences

Article

Efficacy of the entomopathogenic fungus, *Metarhizium anisoplae*, against Khapra beetle *Trogoderma granarium* (Coleoptera: Dermestidae) under laboratory conditions

Battol Qasem Kteo[1], Akram Ali Mohammed[1], Hanaa H. Al-Saffar[2]*

[1]Faculty of Agriculture/ University of Kufa/ Najaf/ Iraq; akrama.abodarb@uokufa.edu.iq
[2]Iraq Natural History Research Centre and Museum/ University of Baghdad/ Baghdad/ Iraq; hanaahani2014@gmail.com
**Correspondence: Battol Kteo; batt2018alhussein@gmail.com*
Available from. http://dx.doi.org/10.21931/RB/2023.08.04.59

ABSTRACT

The khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), is an important pest of stored wheat worldwide. This study assessed the efficacy of two isolates of *M. anisoplae* (commercial and local isolates). Different conidial concentrations (1×10^8, 1×10^6, 1×10^4) conidia/ml^{-1} and fungal filtrate (100, 75, 50) % of *M. anisoplae* were evaluated. In both fungal isolates, corrected mortality of *T. granarium* after exposure varied according to fungal concentrations conidial concentration of conidia/ml$^{-1,}$ and the fungal filtrate at a concentration of 75% caused the highest mortality rates. In addition, mortality rates were significantly varied according to the beetle's developmental stage. Both isolates of *M. anisoplae* caused between 41 and 67.6 % reduction in total fecundity of the female adults. The results demonstrate no significant differences between the local isolate of *M. anisoplae* and commercial formulation (Met 52 EC). Further studies under commercial storage conditions are required.

Keywords: Insects storage pests, biological control, Entomopathogenic fungi

INTRODUCTION

The khapra beetle, *T. ganarium*, is one of cereals' most common and damaging insect pests [1]. It is considered one of the most dangerous pests of stored crops due to its multiplicity of food hosts, and it infects wheat, barley, and industrial powdered milk [2]. The larvae of this insect can enter into a long hibernation and endure unsuitable conditions. It can also remain without food for several years and, most of the time, hide in cracks, making it difficult to control with chemical insecticides. However, extensive use of synthetic insecticides has led to insecticide resistance in stored insect pests and environmental pollution with harmful side effects on human health[3]. Recently, there has been increasing awareness of environmental concerns related to the use of insecticides, and these issues have encouraged the development of alternative control methods [4]. The efficacy of entomopathogenic fungi against insect pests of stored grain products, including *T. granarium*, has been evaluated in many regions worldwide [5, 6]. However, there is little information on the potential of Iraqi isolates of entomopathogenic fungi for *T. granarium* control. Studies on the virulence of these fungal isolates and the factors influencing their efficacy are still required to evaluate the potential of these fungal isolates against *T. granarium*. The susceptibility of different developmental stages of coleopteran insect pests such as *Otiorhynchus sulcatus* F. and *Sitona lineatus* L. to fungal infection has been studied [7]. In addition, the susceptibility of adults and larvae of *T. granarium* to infection by some Iranian isolates of *M. anisopliae* has been investigated [8]. The objectives of this study were to evaluate different conidial concentrations and fungal filtrates of two isolates of entomopathogenic fungus, *M. anisopliae* (commercial isolate

based on Met 52 EC and Iraqi isolate) against different *T. granarium* life stages, and to investigate sublethal effects of fungal infection on the fecundity of individual *T. granarium* adults

MATERIALS AND METHODS

The media used

Medium Potato Dextrose Agar (PDA)

To prepare potato infusion, 200 g of boiled potato were sliced and placed in 1 liter distilled water for 30 min using a glass beaker. Then, it was filtered through cheesecloth to obtain the potato infusion. The potato infusion was mixed with 20 g of Dextrose and 20 g of Agar. The mixture was autoclaved at a temperature of 121°C and a pressure of 15 pounds/ing2 for 20 minutes. The medium was poured into the Petri dishes according to the required experiments, and some medium was kept in the refrigerator until use. This medium was used to grow the fungi under study.

Potato Dextrose Broth (PDB)

This media was prepared in the same way mentioned in the previous paragraph. However, without adding Agar, this medium was used to grow fungi to obtain fungal secretions.

***Metarhizium anisoplae* isolate sources.**

The local isolate of the fungus was obtained from the Faculty of Agriculture, University of Babylon, and Prof. Jamal Hussein diagnosed the fungus. A formulation of *M. anisopliae* (Met 52 EC by Planet Natural, Canada) was obtained from the Faculty of Agriculture, the University of Kufa, which was kept at 4 _°C until use.

Collection and diagnosis of *Trogoderma granarium* Everts

A pure colony of Khabra *Trogoderma granarium* was obtained from the entomology laboratory of the Faculty of Agriculture / University of Kufa. The insect was diagnosed as a Khapra beetle by Dr. Rasha Abdul Razzaq Jawad / Plant Protection Department / College of Agriculture / University of Kufa, and the insect was raised on sterilized wheat grains. The temperature was incubated at 30±1, and the culture was constantly monitored and renewed after each generation.

Preparation of conidial concentrations of the fungus *Metarhizium anisoplae* (local and commercial isolates)

The conidial concentrations of *M. anisoplae* were prepared by activating and multiplying it through transferring several times using the media PDA, and a Petri dish containing the developing fungus was taken for 7 days and washed with 5 ml of sterile distilled water containing 0.02% Tween 20, and stirred well then 1 ml of it was taken and diluted in a ratio of (10:1) with sterile distilled water containing 0.02% of tween to the first dilution, and then the second, third and fourth dilutions etc.

As for the commercial, it was prepared by taking 10 gm of loaded fungi and adding it to a liter of sterile distilled water, which contains 0.02% of tween compound, and thus the first dilution was obtained 1×10^{10} and 1 ml was taken from it and added to 9 ml of sterile distilled water. Thus, the required dilutions were obtained in the experiment: (1×10^{8}, 1×10^{6}, 1×10^{4})conidia /ml^{-1}.

Preparation of the filter for *Metarhizium anisoplae*

This method used glass flasks with a volume of 250 ml and a broad base. 150 ml of the PDB culture medium, which had been prepared previously, was placed in these flasks. After cooling the medium, the flasks were inoculated with discs, each with a diameter of 0.5 cm of fungi colonies of *Metarhizium anisoplae* a week old, which were grown on Petri dishes containing PDA with (1-3) fungi discs/beaker for all fungi

isolates used in the experiment [9]. The flasks were incubated in the incubator at a temperature of 25±2 °C for 28 days, with the flasks agitated every three days to split the mycelium and separate the spores. After the end of the period, the fungi farms were filtered using a glass funnel and filter paper of the type What man No.1, and the filtrate was kept until it was used in glass containers [10].

Preparation of different concentrations of filtrate of the *Metarhizium anisoplae* (local – commercial isolates)

Different concentrations of the produced fungal filtrate were prepared, which are (100, 75, and 50) % for each fungal isolate, by taking a quantity of the fungal filtrate according to the required concentration and dissolving it in distilled and sterile water. Dishes were sprayed with a fungicide according to the concentration of each treatment using a small hand sprayer with a capacity of 100 ml. In contrast, the control dishes were sprayed with distilled water only, and all treatments were incubated at approximately 30°C for 10 days.

Obtain different larval stages of the Khapra beetle.

The method described by [4] was used to obtain a similar age of different developmental stages of *T. granarium* (third and fifth instars, as well as adults). Adults were transferred from a stock culture into small plastic jars (15 adults per jar) and allowed to produce eggs for 1 day at 42, 32 or 21 days before bioassay to allow adults, fifth and third instars, respectively, to be available for experimental use on the same treatment date. The adults were removed, and the eggs were allowed to develop for 41, 31 or 20 additional days before beginning the experiment in an incubator at 30 ± 1°C, 60 ± 5% RH and continuous darkness.

Effect of conidial concentration and fungal filtrate of *M. anisoplae* on the productivity of adults of Khapra beetle *T. granirum*

To determine the sublethal effect of fungal infection on the fecundity of *T. granarium* adult females, male-female (1–2 day old) pairs (each pair is a replicate) were introduced into each 9-cm Petri dishes containing Whatman No. 1 filter papers that pairs were exposed to 1 ml of either 10^8 conidia ml^{-1} or fungal filtrate at a concentration of 75% for each isolate. Each treatment was replicated 15 times. After 24 h, each male-female pair was transferred to an individual sterile Petri dish with 5 g of wheat grains covered with muslin cloth, secured using a rubber band and kept at 30 ± 2°C, 65 ± 5% RH and 12:12 (L:D) h photoperiod. The number of eggs produced by each female was recorded daily until the female's death using a magnifying lens. In the control treatment, adults were sprayed with sterilized water only. Experiment design and statistical analysis: The laboratory experiments were carried out according to a Complete-Randomized-Design (CRD) with one factor, and the averages were compared using the Least-Significant-Differences (LSD) and under the probability level of 0.05. The percentages of fatalities were corrected according to Abbott's equation [11]. The fixed loss percentage was calculated as follows:

$$\textbf{Corrected Death percentage} = \frac{\textbf{\% Death in Treatment} - \textbf{\% Death in Control}}{\textbf{100} - \textbf{\% Death in Control}} \times \textbf{100}$$

The loss percentages were converted into loss percentages, and the corrected percentage of death was transformed into angular values to be included in the statistical analysis[12].

RESULTS

Effect of different concentrations of the spore suspension of *M. anisoplae* on the mortality rate of the different instars of Khapra beetle

Table (1) indicates the effect of the interaction of different concentrations of the conidial suspension of the commercial *M. anisoplae*, as well as the time duration on the corrected mortality of the third, fifth instar larvae and adult of *T. granirum*, as the results showed that the highest effect of the different concentrations of

the spore suspension factor was at a concentration of 1×10^8 conidia/ml after 7 days of treatment for adults, the corrected mortality was (58.8), while for the third and fifth larval stages, the concentration of 1×10^8 conidia/ml gave the highest corrected mortality after 10 days of treatment with the spore suspension, which was (80 and 62.5) respectively. The lowest death rate was at the concentration of 104 x 1 conidia/ml in all insect stages, reaching (32.5) in adults after 7 days of treatment, while the third and fifth larval instars reached (52.9 and 43.4), respectively, after 10 days of treatment. As for the effect of time, it was great after treatment with sporozoites, as the largest corrected death rate was on the seventh day of the adult stage in all concentrations and the tenth day in the third and fifth stages also in all concentrations, and the lowest death rate was on the third day, as the excretion of the spore suspension started on the third day of treatment for both adults, the third larval instar and the fifth larval instar. The corrected mortality ratio for the concentration was 108 x 1 conidia/ml (0.5, 10, 7.5), respectively.

Developmental stage	Conidial concentration conidia ml^{-1}	Corrected mortality (%)				
		1 day	3 days	5 days	7 days	10 days
Adult	1×10^4	0.0	0.0	5.0	32.5	
	1×10^6	0.0	0.0	17.4	40.1	
	1×10^8	0.0	0.5	30	58.8	
Fifth instar larvae	1×10^4	0.0	0.0	2.5	17.5	43.4
	1×10^6	0.0	0.0	7.5	30.0	55.0
	1×10^8	0.0	7.5	17.5	35.0	62.5
Third instar larvae	1×10^4	0.0	5.0	12.6	37.8	52.9
	1×10^6	0.0	7.5	25.4	47.3	72.2
	1×10^8	0.0	10.0	37.5	52.5	80

Table 1. The corrected mortalities of different developmental stages of *T. granarium* treated with different conidial concentrations of commercial formulation (Met 52 EC) based on *M. anisopliae* strain F52. Mortality at the adult stage was recorded for 7 days only because it was the maximum lifetime of the adult stage to survive.
LSD for developmental stage = 3.45; LSD for Conidial concentration = 2.56; LSD for days = 4.32

Table (2) indicates the effect of the interaction of different concentrations of the spore suspension of the local fungus *M. anisoplae*, as well as the time duration on the corrected percentages of the mortality of the third, fifth and adult larval age members of *T. granirum*, as the results showed that the highest effect of the different concentrations of the spore suspension factor was At a concentration of 108 x 1 conidium/ml after 7 days of treatment for adults, the corrected mortality rate was (42), while the third and fifth larval stages gave the concentration 1×10^8 conidia/ml the highest corrected death rate after 10 days of treatment. The ratio was (77.4 and 60.5) Consecutively, the lowest death rate was at a concentration of 104 x 1 conidia/ml in all insect stages, reaching (32.5) in adults after 7 days of treatment at the third and fifth larval stages. It reached (60.9 and 45.5) respectively, after 10 days of treatment. Its effect was significant after treatment with sporozoites, as the largest corrected death rate was on the seventh day of the adult stage in all concentrations and the tenth day in the third and fifth stages also in all concentrations. The lowest death rate was on the third day due to the effect of the sporozoites. It started on each adult's third day of treatment, the third and fifth larval instar. The corrected mortality ratio for the concentration was 108 x 1 conidia/ml (2.5, 5, 5.5), respectively.
The results showed that the third larval stage gave the most mortality in all concentrations, followed by the fifth larval stage and then the adult stage, and this is due to being more sensitive due to its thin chitinous wall, which is easily penetrated by the enzymes of pathogenic fungi, then depletion of its internal contents and then death. What [13] found that the modern stages of the rusty red flour beetle *Tribolium castaneum* gave the highest mortality rate of 97% for concentration 108 x 5 conidia/ml. The results also agree with [14] when studying the

effect of the fungus *Lecanicillium lecanii* on the larvae of the saw-breasted beetle *Oryzaephilus surinamensis* L. The results showed that the mortality rates for the larvae in the early stages were high, reaching 60% at the concentration of 1×10^3 conidia/ml. The chitinous wall has fewer tanning materials than the advanced stages, making it easier for the fungus to penetrate the larva's body and consume its internal contents, leading to the larva's death. The killing rates increased with the increase in the concentration of the fungal suspension, and these results agreed with [15], where the killing rates of the hairy beetle increased with the increase in the concentration of the fungal suspension of *Beauveria bassiana*.

Developmental stage	**Conidial concentration conidia ml^{-1}**	**Corrected mortality (%)**				
		1 day	3 days	5 days	7 days	10 days
Adult						
	1×10^4	0.0	0.0	2.5	32.5	
	1×10^6	0.0	2.5	10.4	37.5	
	1×10^8	0.0	2.5	12.5	42	
Fifth instar larvae	1×10^4	0.0	0.0	5.0	17.5	45.5
	1×10^6	0.0	0.0	5.5	27.5	52.2
	1×10^8	0.0	5.5	15.0	35.0	60.5
Third instar larvae	1×10^4	0.0	0.0	15.6	33.5	60.9
	1×10^6	0.0	2.5	20.4	47.5	70.0
	1×10^8	0.0	5.0	27.5	50.5	77.4

Table 2. The corrected mortalities of different developmental stages of *T. granarium* treated with different conidial concentrations of a local isolate of *M. anisopliae*. Mortality at the adult stage was recorded for 7 days only because it was the maximum lifetime of the adult stage to survive.
LSD for developmental stage = 2.91; LSD for Conidial concentration = 3.72; LSD for days = 5.43

Effect of different concentrations of the filtrate of *M. anisoplae* on the mortality rate of the different instars of the grain beetle

The results presented in Table (3) showed the effect of the commercial fungi filtrate by direct spraying and the time duration on the corrected mortality percentage of adults and the third and fifth larval instars of *T. granirum*. The highest cumulative death rate at the concentration was 100%, and it was (39) after 7 days for the adults. As for the third and fifth larval stages, it reached (50 47.5), respectively, after 10 days of treatment, while the lowest cumulative death rate was at the concentration of 50% after the passage of 7 Days of adult treatment, the corrected percentage of mortality was (18), while the third and fifth larval instars were after 10 days of treatment, where it gave mortality percentage (27.5 and 25), respectively. As for the effect of the time, the 7 days for adults significantly outperformed the rest of the periods at 5,3.1 in all concentrations. In the third and fifth larval instars, the period was 10 days more than the rest of the periods 7,5,3.1 also in all concentrations, while the lowest death rate was on the third day of treatment for both adults and instars. The third larvae and the fifth larval instar had the corrected mortality rate of the concentration 100% (12.5, 10, 5), respectively, and we conclude from that that the period has an essential effect on biological control, as there is a direct relationship with the mortality rates.

Developmental stage	EPE filtrate concentration	Corrected mortality (%)				
		1 day	3 days	5 days	7 days	10 days
Adult	50%	0	2.5	5	18	
	75%	0	10	20	27	
	100%	0	12.5	22.5	39	
Fifth instar larvae	50%	0	2.5	7.5	20	27.5
	75%	0	10	12.5	20	40
	100%	0	5	12.5	25	47.5
Third instar larvae	50%	0	2.5	10	15	25
	75%	0	10	12.5	12.5	40
	100%	0	10	15	27.5	50

Table 3. The corrected mortalities of different developmental stages of *T. granarium* exposed to different concentrations of filtrates of commercial formulation (Met 52 EC) based on *M. anisopliae* strain F52. Mortality at the adult stage was recorded for 7 days only because it was the maximum lifetime of the adult stage to survive.
LSD for developmental stage = 3.08; LSD for Filtrate concentration = 1.75; LSD for days = 3.77

Table (4) shows the effect of local fungus filtrate and the time duration on the corrected mortality percentage of adults and the third and fifth larval instars of *T. granirum* by direct spraying. The concentration of 100% on the two concentrates exceeded 75.50% in the death rate of insect instars, as the highest cumulative death rate in adults after 7 days of treatment was at (42.5), while the third and fifth larval instars reached (47, 44) respectively after 10 days. From the treatment, while the lowest cumulative death rate at the concentration was 50% after 7 days of adult treatment, the corrected percentage of mortality was (20). The factor of the effect of the time showed that the time of 7 days for adults significantly exceeded the rest of the periods 5, 3, and 1 day in all concentrations, while in the third and fifth larval instars, the time of 10 days exceeded the rest of the periods 7, 5, 3, 1day. The lowest mortality rate was on the third day of treatment for adults, third larval instars, and fifth larval instars, and the corrected mortality rate for the concentration was 100% (10, 7, 7.5), respectively.

Developmental stage	EPE filtrate concentration	Corrected mortality (%)				
		1 day	3 days	5 days	7 days	10 days
Adult	50%	0	2.5	7.5	20	
	75%	0	5	20	32	
	100%	0	10	25	42.5	
Fifth instar larvae	50%	0	2.5	7.5	20	27.5
	75%	0	12.5	16	20	31
	100%	0	7.5	17.5	25	44
	50%	0	5	11.4	17.5	22

Third instar larvae	75%	0	10	17.5	24.5	38
	100%	0	7	15	23.5	47

Table 4. The corrected mortalities of different developmental stages of *T. granarium* exposed to varying concentrations of filtrates of a local isolate of *M. anisopliae*. Mortality at the adult stage was recorded for 7 days only because it was the maximum lifetime of the adult stage to survive.
LSD for developmental stage = 1.53; LSD for Filtrate concentration = 2.16; LSD for days = 3.66

Effect of conidial concentration and fungal filtrate of *M. anisoplae* on the total fecundity of adults of *T. granirum*

Treatment	Total fecundity mean No. of eggs per female (±SE)
***M. anisopliae* (Met 52 EC)**	11.9 ± 3.2 a
***M. anisopliae* (Local isolate)**	13.7 ± 2.1 a
Fungal filtrate (Met 52 EC)	19.9 ± 4.3 b
Fungal filtrate (Local isolate)	22.6 ± 5.6 b
Control	37.7 ± 4.7 c

Table 5. filtrate at 75% on the mean numbers of eggs produced per adult female of *T. granarium* compared to the control. Means within a column followed by different lowercase letters indicate significant differences among treatments at each insect species at *P* = 0 .05 using the LSD test.

Table (5) shows the significant effects of the conidial concentration and the fungal filtrate on the mean number of eggs produced by adult females of *T. granarium*. The conidial concentrations were outperformed in reducing the number of eggs compared to the fungal filtrate. The number of eggs decreased significantly to its lowest levels, reaching (11.9 ± 3.2 a 13.7 ± 2.1 a) eggs per female treated with a concentration of 1×10^6 conidia/ml for the two commercial and local isolates, respectively, While the number of eggs for females treated with the fungal filtrate was (19.9 ± 4.3 b, 22.6 ± 5.6 b) eggs for each female treated at 75% concentration for the two commercial and local isolates, respectively, compared with (37.7 ± 4.7 c) eggs/female in the control treatment. The issue by adults is due to the fungi's consumption of nutrients inside the insect's body. This result was observed when the fungus *M. anisopliae* infected the pupae of *R. ferrugineus* [16]. These results also agree with the findings of [17]. that the use of the suspension the spore suspension of the entomopathogenic fungus *Clonostachys rosea* against three types of insects, *Trogoderma granarium*, *Tribolium castaneum* and *Callosobruchus maculatus*, showed effectiveness in decreasing the total fertility of the three treated insect pests.

CONCLUSIONS

The fungus *M. anisoplae*, in its commercial and domestic isolation, showed activity against *T. granirum* under laboratory conditions. A decrease in the fertility of the treated insect was also recorded. However, more studies are needed to confirm the efficacy of *M. anisoplae* in commercial storage conditions. In addition, the potential effects of the fungus should also be evaluated in conjunction with some other control methods.

REFERENCES

1. Al-Azzawi, A.F. and Mahdi, M.T. (1983). Warehouse insects. Ministry of Higher Education and Scientific Research, University of Mosul, 464.
2. Omar Khaled Attallah, Thafer Thabit Mohammed and Nasr Nuri Al-Anbari. Effect of Adding Grape Pomace and Resveratrol on Some Physiological Traits and Gene Expression to Prevent Hemorrhagic Fatty Liver Syndrome in Laying Hens. IOP Conference Series: Earth and Environmental Science.2022, 1060 (1), 012076. doi:10.1088/1755-1315/1060/1/0120.
3. Al-Iraqi, R.A., Hamid, A.A. and Al-Hadidi, I.K. (2008). Effect of some plant powders on the life of *Trogoderma granarium* Everts (Coleoptera: Dermestidae). ***Tikrit J. Pure Sci.***, 13 (1): 60-64.
4. Mohammed, A. A, Jamal, H.K and Ahmad, M.H (2019). Laboratory evaluation of entomopathogenic fungi for the control of Khapra beetle (Coleoptera: Dermestidae) and their effects on the beetles ' beetles'fecundity and longevity. J. Agric Urban Entomol., 35(1) :1-11
5. Alkhateeb, A. .; Ibrahim, W. I.; Taha, A. E. . Body Conformation with Daily Milk Yield Relationship On Buffaloes. JLSAR 2022, 3, 1-5.
6. Bilal, M., Ranjha, M. H. and Bashir ,N. H.(2017). Comparison of *Beauveria bassiana* with IGRs against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae). Journal of Entomol. Zool. Stud. 5:113–117.
7. Poprawski, T. J., Marchal, M. and Robert ,P. H.(1985). Comparative susceptibility of *Otiorhynchus sulcatus* and *Sitona lineatus* (Coleoptera: Curculionidae) early stages to five entomopathogenic hyphomycetes. Environ. Entomol. 14: 247–253.
8. Khashaveh, A., Safaralizadeh, M. H. and Ghosta, Y.(2011). Pathogenicity of Iranian isolates of *Metarhizium anisopliae* (Metschinkoff) (Ascomycota: Hypocreales) against *Trogoderma granarium* Everts (Coleoptera: Dermestidae). Biharean Biol. 5: 51–55.
9. Thabit S S, Awad M M, Abdulateef S M. Effects of In-Ovo Injection of sialic acid on Chick's embryonic development and physiological traits. Revis Bionatura 2023;8 (2) 49. http://dx.doi.org/10.21931/RB/2023.08.02.49
10. Al-Jubouri, A.N.H. (2006). Integration of aphid resistance using some local fungicide isolates and the insecticides Chess and Polo. Master's Thesis - Technical College / Al-Musayyib.
11. Alaaraji, S.F.T., Mohisen, M.A., Awad, M.M. Assessment serum levels of neopterin, IL-6, IL-1β, hs-CRP, TNF-α and MMP 9 in iraqi rheumatoid arthritis patients (2020) Systematic Reviews in Pharmacy, 11 (12), pp. 88-93.
12. Al-Rawi, K.M. and Khalaf Allah, A.M.(2000). Design and analysis of agricultural experiments. faculty of Agriculture. Second Edition. University of Al Mosul. Ministry of Higher Education and Scientific Research. The Republic of Iraq.
13. Al-Anbaki, H.A.M.; Al-Amiri, N.A.K.; Sultan, A.A.; Saadi; H. and Alwan M. (2013). The use of some pathogenic fungi in controlling the red rust beetle *Tribolium castaneum*. Diyala Journal of Agricultural Sciences, 5 (2): 282.
14. Al-Douri, M.R.T. (2017). Biological control of the saw-breasted beetle *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae) using the fungus (Zimm.) *Lecanicillium lecanii*. Master Thesis, College of Science for Women, University of Baghdad.
15. Al-Hadithi, OTHA (2016). The use of microwave radiation and the biological fungus *Beauveria bassiana* in controlling the *Trogoderma granrium* (Everts) (Coleoptera: Dermestidae) and the red rusty flour beetle *Tribolium castaneum* (Herbest) (Coleoptera: Tenebriondae). Tikrit University.
16. H. Kh. Al-Khater, S., I. Neamah, S. Effect Of Growth Regulators 2,4-D And Kintein In The Inducation Of Callus From Helianthus Annuus L. Cotyledon. Anbar Journal Of Agricultural Sciences, 2023; 21(1): 174-187. doi: 10.32649/ajas.2023.179728.

17. Akram, A.M; Amal , S.Y. and Abdulla, N.A .(2021). Efficacy of *Clonostachys rosea*, as a promising entomopathogenic fungus, against coleopteran stored product in set pests under laboratory conditions. ***Egypt J Biol Pest Control***., 31: 55.

Received: 26 September 2023 / Accepted: 15 April 2023 / Published:15 December 2023

Citation: Kteo, B.; Mohammed, A.; AL-Saffar, H. Efficacy of the entomopathogenic fungus, *Metarhizium anisoplae* against Khapra beetle *Trogoderma granarium* (Coleoptera: Dermestidae) under laboratory conditions. Revis Bionatura 2023;8 (4) 59. http://dx.doi.org/10.21931/RB/2023.08.04.59