Laboratory evaluation of two isolates of \textit{Meterhizium anisopliae} and \textit{Beauvaria bassiana} to control infesting by \textit{Trogoderma granarium} (Coleoptera: Dermestidae) larvae

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**Abstract.** \textit{Trogoderma granarium} Everts (Khapra beetle) is a serious pest of stored barley and wheat worldwide. The current research evaluates the efficacy of local isolates of \textit{Meterhizium anisopliae} and \textit{Beauvaria bassiana} compared to an imported isolates when khapra larvae sprayed with concentration of later isolates under laboratory condition, mortalities were recorded for more than two weeks after treatment. Results demonstrated that the larvae were more susceptible to the local isolates of the two fungi than the imported isolates. Cumulative mortalities were 86.67 and 50 when larvae sprayed with $10^9$ conidia/ml of local and imported isolates of \textit{M. anisopliae} respectively, and 86.67 and 66.67 when larvae sprayed with $10^9$ conidia/ml of laocal and imported isolates of \textit{B. bassiana} respectively after two weeks. The findings of the current study clearly showed that the local isolates caused higher mortalities than the imported one so they may be used as a good candidates for pragmas of integrated pest management of stored grain pests.

**Keywords.** \textit{Meterhizium anisopliae}, \textit{Beauvaria bassiana}, \textit{Trogoderma granarium}, Larvae, Barley.

1. Introduction

Khapra beetle, \textit{Trogoderma granarium} Evert (Coleoptera, Dermestidae) is a serious pest of stored grain product especially under warm dry condition, it is considered to be one of the worst invasive pests worldwide [1]. Weight losses and damage by sever infesting can be up to 30% and in extreme cases 73% [2]. Grains that heavily infested become unpalatable and unmarketable by consumers, high infestation by khapra beetles reduces content of fats, sugar protein and carbohydrates, of the grains along with increase of level of uric acid [3]. Moreover this pest was responsible for health problem for man like asthma and dermatitis caused by its skin [4]. Khapra beetle undergoes five larval instars and the development period for these instars may range between 30-50 days depending on relative humidity and temperature of storage conditions, but under un adversed conditions these larvae may enter into diapause where it may molt but does not feed while keep inactive, and larval period prolonged up to four years, therefore the infested food products are vulnerable to their attack, the
diapaused larvae are tolerant to common insecticides and other control methods that are very effective against other pests of stored grains [5, 6]. Fumigations the stored products by methyl bromide was the most promising way against khapra beetle since it acts as suffocating against, killing the insects by blocking its breathing. Recent studies revealed that methyl bromide was a major ozone depletion agent there for many countries minimize its usage under strong controlled conditions [7]. Previous concerns led to look out for other alternate ways to control khapra beetles such as biological control methods [8]. Controlling khapra beetles by using predators such as *Xylocoris flavipes* (Hemiptera: Anthocoridae) had been studied [9]. Potential of the larval parasitoid *Anisopteromalus calandra* (Hymenoptera: Pteromalidae) and *Laelius pecdatus* (Hymenoptera: Bethylidae) to control larvae of khapra beetles in cereal had been studied [10, 11]. Entomopathogenic agents such as bacteria and fungi investigated to control this pest, [12] reported that the *Bacillus thuringiensis* was very effective against larvae of khpra beetles. Entomopathogenic fungi were applied and used to control many pests, one of them khapra, since these fungi infects the insect topically (by contact) then it penetrates its cuticle and kills it by the toxins and enzymes that they secret inside the insects body, so these fungi considered to be a good alternatives to control these pests [13]. Moreover entomopathogenic fungi are specific to the hosts that they infected, therefore they have no effect on mammalian animals and fishes since it is a natural enemies of insects [14]. Many species of entomopathogenic fungi such as *Meterhizium anisopliae*, *Beauvaria bassania* and *Isaria farinosa* were known to be good natural enemies of many species of stored grain pests, there for these fungi seem to be the most promising and native strategy to control these pests, especially they are available and registered for using and commercially produced. It is safe to treat the grains directly without any decreasing of its quality and marketability [15]. The aim of this study is to examine the efficacy of *M. anisopliae* and *B. bassiana* (Two isolates of each one introduced while the other local) against the larvae of *T. granarium* under lab conditions.

2. Materials and Methods

2.1. Rearing of rest insects

Infested barely with *T. granarium* (one Kg) was brought from the local market to the laboratory of entomology in Department of Biology, College of Education for Pure Science (Ibn-Al-Haitham) at September 2018, fifty adult beetles were collected than added to sterilized plastic jar (1L) containing 500 gm of sterilized barely, two replicates were done, Jars kept in an incubator at 30±5°C° and 50±5% relative humidity. The emerged second-third instar larvae of *T. granarium* was used for the later experiments, adults beetles were identified by Iraqi natural history museum, University of Baghdad [16].

2.2. Entomopathogenic isolates and preparation of fungal suspensions

Two isolates of each *M. anisopliae* and *B. bassiana* were examined against *T. granarium* larvae each of fungus were isolated from soil of Baghdad area and marked as a local isolate, so we have two local isolates one is the a local isolate of *B. bassiana* and the other is a local isolate of *M. anisopliae*. The other fungus isolate was marked as imported by Agricultural research center-Ministry of Science and Technology so we have two imported isolates, one of them *B. bassiana* imported isolates and the other *M. anisopliae* imported isolate. All isolates (The four isolates) were cultivated and suspension concentrations (10^7, 10^8, 10^9 conidia/ml) were prepared as in [16].

2.3. Bioassays

Ten larvae of *T. granarium* were placed in a petri dish, then sprayed topically by hand sprayer with two ml of each fungal suspension sprayed, larvae transferred to another petri dish containing 10 gm of
sterile barely grins. Three replicates of each of the three concentration, for the four fungal isolates were done for control treatment larvae were spread with sterile distilled water with tween-20 (0.01%). Experimental petri dishes kept in an incubator at 28±2°C and 55% relative humidity [17]. Larvae were monitored every 24 hr to record number of dead insects, and photographed them by camera fitted with a simple microscope (Dissecting). Cumulative mortality were calculated.

2.4. Statistical analysis

Percentages of mortalities were recorded and corrected according to Abott’s equation [18]. Subsequently SAS was used to analyze the data which show the sensitivity of the concentrations of the fungal isolates in larvae mortalities [19]. The significant differences were compared between mean and least significant differences.

3. Results and Discussion

Insects infected by many kinds of pathogens, which cause a deadly disease fungi are one of these pathogens, infections may be by a direct contact, after the conidia/spores land on the body wall of the insects they will start to germinate and penetrate forcibly through the cuticle into the insects body which kill the insects, in case of the conditions are suitable the fungus start to sporulate and make more spores which continue the infection, finally caused a slowly death to the insects. These fungi can be used as a biological control agents by causing natural epizooties diseases and reduced insects populations [20]. Laboratory assessments of isolates of M. anisopliae and B. bassiana (Imported and local) against T. granarium larvae were done under laboratory conditions to decide whether these isolates are good candidates for biological control of T. granaria larvae. Many researches had been done by the local isolates of M. anisopliae and B. bassiana and proved to be effective against many species of insects [16, 21, 22, 23]. Our study proved that the local isolate of M. anisopliae was very effective against larvae of khapra beetles. Table (1) shows that 10⁹ and 10⁸ conidia/ml led to cumulative mortality by 86.67%, and this isolate was more effective than the imported ones which have the concentrations 10⁷ and 10⁶ conidia/ml led to cumulative mortality by 50 and 26.67% respectively. Figure (1) shows that mortalities depend on time, insects death started after about four days of treatment since the fungus takes time to penetrate through the body wall of the larvae and invade the cavities of its body, producing hyphal mass and many kinds of toxins and secondary metabolites such as Destruxin, conidial growth demonstrated that the larvae died by the fungus which appeared greenish (Figure 2).

Table 1. Cumulative mortalities of Trogoderma granarium larvae sprayed by concentrations of Meterhizium anisopliae (Imported and local isolates).

| Concentration Conidia/ml | Cumulative mortalities % | Imported isolate Mean%±S.E | Local isolate Mean%±S.E |
|--------------------------|--------------------------|----------------------------|-------------------------|
| Control                  | 3.33±3.33                | 3.33±3.33                  |
| 10⁷                      | 10.00±0.00               | 56.67±8.81                |
| 10⁸                      | 26.67±12.02              | 86.67±6.67                |
| 10⁹                      | 50.00±5.77               | 86.67±3.33                |

*S.E.: Standard error.
Since *M. anisopliae* had known to cause the green muscardin disease. The current finding of our study confirm with [23] who proved that the some local isolate of *M. anisopliae* was effective against *Chrysomya megacaphalo* larvae. Similar result was reported by [16] when they revealed that the same local isolate of *M. abisopliae* was very effective against Tribolium castanum adult and 10^9 conidia/ml caused 80% cumulative mortality after about two weeks and they revealed that the local isolate was more effective than the imported isolate. Another research had proved that the local isolates very effective against pest of storage grain, [24] proved that Iranian isolates of *M. anisopliae* were effective against *T. castanum* and *Oryzaephilus surinamensis* when they evaluated these isolates through bioassays and found that 8.5×10^7 conidia/ml of one of these isolates led to 77.8% mortality percent of *O. surnamensis* and 89.9% mortality percent of *T. castanum* after ten days. The final finding of our study about *M. anisopliae* is that the local isolate was more effective against larvae of *T. granarium* than the imported isolate so it may use as a promising microbial against to control this pest. *B. bassiana* was known to be one of the most effective entomopathogenic fungi since it has high ability to infect and kill as host of many different insect species, our finding listed in Table (2) shows that 10^9 conidia/ml of the imported and local isolates led to 66.67 and 96.67 respectively as accumulative mortality percent when *T. granarium* larvae were sprayed with, after about two weeks (Figure 3), these results refer that the local isolate was more effective than the imported isolate against the larvae.
Dead larvae appeared distorted and growth of fungal hyphae spinning above then and their bodies were covered with white fungal growth (Figure 4).

**Table 2.** Cumulative mortalities of *Trogoderma granarium* larvae sprayed by concentrations of *Beauvaria bassiana* (Imported and local isolates).

| Concentration | Imported isolate | Local isolate |
|---------------|------------------|---------------|
| Control       | Mean%±S.E        | Mean%±S.E     |
|               | 10.00±0.00       | 10.00±0.00    |
| $10^7$        | 46.67±12.02      | 46.67±6.67    |
| $10^8$        | 60.00±0.00       | 50.00±15.28   |
| $10^9$        | 66.67±15.28      | 86.67±6.67    |

These results were in a good agreement with those of [21, 22]. Also these results agreed with [25] who revealed that the treatment of larvae of *Agrotis ipsilon* with a concentration of *B. bassiana* ($2 \times 10^8$ conidia/ml) led to mortalities after one week and hyphal fungi emergence from dead larval bodies, after 10-13 days. Mortality rates of the treated larvae were attributed to the growth of the fungus inside...
the insect body which was accompanied by secretion of toxin and enzymes that finally led to destruction and decaying the host body, the most effective enzyme of the fungus were protease and chitinase which analyzed the insects body and allowed the fungus to enter inside the larval body, then the fungus started to grow inside the insects hemocole. Some toxins such as Beauverien was secreted by the fungus, followed by a growth of hyphae outside the dead insect body that carry spores/conidia of the fungus, which considered to be source of infection [26]. The present findings are in line with [27] who revealed that local isolates of entomopathogenic fungi \( B. \) bassiana and \( M. \) anisopliae which recovered from samples of soil fields (Crops, Orchards and Vegetables) were very effective against \( R. \) ferrugineus, therefore these isolates would be the most promising biocontrol agent against \( R. \) ferrugineus which is a serious date palm orchard pest. Similar results recorded by [28] who assessed five Iraqi isolates of entomopathogenic fungi including \( B. \) bassiana against \( T. \) granarium larvae, found that the younger larval instars were more susceptible to the fungus than the last larval instar and adult, and mortalities percentages were recorded after 10 days post treatment, these results demonstrate that these isolates (\( B. \) bassiana) have a good potentials biological control agent against larvae of \( T. \) granarium. Further investigations are required to determine the virulence of the local isolates which may be play an important role for the safer management of pests that attack stored grains since it is environment friendly agents instead of chemical insecticides.

4. Conclusion

This study shows higher susceptibility of \( T. \) granarium larvae to the tested concentration of the local isolates of \( M. \) anisopliae and \( B. \) bassiana than the imported isolates. Thus they can be used as promoting biocontrol insecticides agents in terms of practical application that verified from similar studies

5. References

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