Laboratory Evaluation of *Beauvaria bassiana* and *Meterhizium anisopliae* isolates against Rust-Red Flour Beetle *Tribolium castanum* (Herbst) (Coleoptera:Tenebrionidae)

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**Abstract**

Two strains of each *Beauvaria bassiana* and *Meterhizium anisopliae* were tested against adult of *Tribolium castanum* at 3 conidial concentrations (10⁶, 10⁷, 10⁸ conidia/ml) by direct spraying, under Laboratory conditions, one of each fungus was isolated from soil of Baghdad city while the other was imported. The findings demonstrated that the bring out isolate of *B. bassiana* was more effective against the insects than the local one, since the cumulative mortalities at 10⁷ conidia/ml have 53.33 and 43.33 respectively after more than 14 days. Whereas the local isolates of *M. anisopliae* was more effective and pathogenic to the adults of *T. castanum* than the imported isolate.

Insects treated by spraying with 10⁸ conidia/ml led to cumulative mortalities percentages as 76.67 and 33.33 respectively after 16 days. The results also appeared that native isolate of *M. abisopliae* more pathogenic and effective for adults of *T. castanum* than *B. bassiana* at the same circumstances and concentration. The current result indicate that this isolate could be apomising bio control agent against adults of *T. castanum*.

**Keywords:** *Tribolium castanum*, Biological control, Stored Product, Entomopathogenic fungi

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**Introduction**

*Tribolium* beetles are inclusion *Tribolium castanum* Rust-red flour beetle and *T. confusum* (Confused flour beetle) (Coleoptera:Tenebrionidae) as well as know stored-grain pests in allower the world.

These beetles have a cosmopolitan allocation during all tropical parts and all warm countries of the world. It is considered to be the MostCommon destructive test of various nutrition stuffs (Karunakaran et al., 2004). *T. castanum* have a large appetite for a variety of food such as grocery stores, warehouses including biscuit, beans, pasta spices, crackers, seeds and nuts (Beeman et al., 2012). Its known to be the most common pest attaching stored wheat and barley (Devi and Devi, 2015). Both adult and larvae attack and feed on grains and cereals and spend its life cycle outside the kernels of the grains. In addition, infesting food (Such as flour) it has adisagreeble odour making it not fit for intake by human, and may result an allergic recation (Prakash et al., 1987). Therefore storage grain beetle are the main cause of quantitative and qualitative destruction to the stored food such as quality, discreasing in weight seed viability commercial value (Hill, 1990).

There are many ways have been followed to control at thisPest are: First of all, Sanitation carefully to avoid infestation. Second way to kill this insect is chemical agent (Insecticides). This factor is considered the most have been use but its has toxicity to birds, fish and mammals and its effects in non-target living organism as well as has some risks of whole environmental such as chemicalpollutions so may be to replaced this chemical agent by other methods which are safe and known to be friendly to the environment (Rechcigl and Rechcigl, 2000). Biological control regarded as one of aviable strategies for pest management.

Entomopathogenic fungi were utilized to control many pests, more than 700 species of fungi belong to about 90 genera were to be known pathogenic to insects (Lacey and Goettel, 1995). Entomopathogenic fungi infects the insect by penetrata the cuticule and kill the insect by sécrétion the enzymes and toxines that they secret inside the pest body. Therefore these type of microorganism could be an alternative to insecticides (Monlar et al., 2010). Moreover this mold have no sids-effects, and lowhuman toxicity since it is natural enemies of the pests (Jaronski, 2010).

*Meterhizium anisopliae*, *Beauvaria bassiana* as well as *Isaria farinosa* are known as anamorphic entomopathogenic fungi are natural enemies of alarge number of insect pests (Roberts and St Leger, 2004). As a result, utilizing these species of Mold for the control of stored-grain pests seems to be the most actiev way to kill them, especially they are commercially imported, available and recorded for employing in storage facilities. Thesosrt of microbes also can be treat the employ stores to die all pests before bringing the new
harvest in it, and its safe to applied then directly to the grain and commodity whitout any reduction of its marketability and quality (Khshaveh et al., 2011).

The goal of our study was to assess the efficacy of *B. bassiana* and *M. anisopliae* (both study isolates of each) against the adult of *T. castanum* under laboratory circumstances.

**Methodology.**

1- **Insect culture:** Firstly, It have taken 1kg of infested barely with *T. castanum*. Then, it is brought to the laboratory from a local market. Next, collect about fifty adult beetle then added to sterilized plastic jar involving 500 g of sterilized barely. After that, three replicates have made then the jar was kept in an incubator at 30±2 C° and 50±5 % relative humidity. Finally, adults have emerged of *T. castanum* were used for experiments as well as Adults were diagnosed by Iraqi natural history Museum, University of Baghdad.

2- **Fungal isolates and suspension concentrations préparations:** Two strains of each *B. bassiana* and *M. anisoplaiae* were tested against *T. castanum* adult for each fungus. These two isolates have imported by Agricultural Research Center-Ministry of Science and Technology mated with *B. bassiana* (Imported) *M. anisoplaiae* (Imported), on other hand, two strains have isolated from Baghdad area and marked with *B. bisiana* (Local) and *M. anisoplaiae* (Local). Four isolates were seeding on PDA with supplemented with 100 ppm chloramphenicol to prevent bacterial growth Petridishes and incubated at a température of 27±2 C° for 7-11 days. Study Fungus has isolated from the soil of Baghdad area. This soil identified by chief Scientific Research Dr-Hadi M. Aboud/ Head of Biotechnology center directorat of Agricultural Research, Ministry of science and technology (Aboud et al., 2017). Three suspensions concentrations of each isolates of both fungi *B. bassiana*and *M. anisoplaiae* (*B. bassiana* imported and local and *M. anisoplaiae* imported and local) 10^7, 10^8 and 10^9 conidial/ml were prepared utilizing stérile distilled water added tween-20 (0.01%) to study its effect on the adults of *T. castanum*.

3- **Bioasys:** 10 adults of *T. castanum* (male and female) have put in aPetridishes, then they were spraying directly with about two ml of each fungal suspension concentrations, from adistance of about 10 cm utilizing hand sprayer, next the insects transfend to another steril plates including about 10 gm of barely, three replicates of each concentration, of each isolatehave made, control treatmentin volves spraying the adults with stérileDW with 0.01% of Tween-20. Experiplates were left in an incubator of 28±2 C° and 65% relative humidity during all expérimental time (Kavallieratos et al., 2014). The insects were monitored every day to record the number of death insects, cumulative mortality was calculated the killed insects were photographed by camera fitted with adsection microscope.

4- **Statistical analysis:** Mortalities pourcentagehavedone according to Abottéquation (Abott, 1925). Subsequently, SAS (SAS, 2012) was did to analysed the data which was the sensitivity of the concentrations of the Mold isolates in insects mortalities, the significant différencesfor compared between mean and least significant variation.

The efficacy of four different strains of *B. bassiana* and *M. anisoplaiae* against adults of *T. castanum* was determined at laboratory conditions. Among the isolates, imported and local isolates of *B. bassiana* led to cumulative mortalities by 53.33 and 43.33 respectively when adult were treated with 10^7 conidia/ml (Table 1) after 16 days at treatment (Fig 1) showed that mortalities percentages recorded after about 7 days of treatment since the pathogenic fungus need time to pénétrâd during thecuticule an d reachd to insects hemolymph (Cavity), next hyphal bodies developed by the fungus. There are many secondary métabolites have produced bythis sort of mold which they acting as immunosuppression, facilitation infection to the insect such as Bassiantin and Beauvericin which are causing the insect Dead (Fig 2) (Mannino et al., 2013 ; Pedrini, 2018).

Findings in Fig (1) shows mortalities percentages based on time and concentrations, our results is consistent with Mehdi (2015) results who showed that the some imported and local isolates of *B. bassaiana* were pathogenic and active against *Chrysomya megacephala* larvae and cumulative mortalities depend on concentration and period. Similarly, our findings were similar to Bilal et al. (2017) who evaluated the concentrations of *B. bassiana* suspensions against *T. castanum* and *Trogoderma gronarium* adults and found that a higher concentration 9x10^7 conidia/ml provided 66.80% mortality after 21 days. Study of Komaki et al. (2017) recorded 100% mortality in *T. castanum* adults when sprayed with 10^7 conidia/ml of *B. bassiana* strain (ARSEF-4984) after 10 days. *T. castanum* as apest of grain-stored products appeared particulary good candidate for biocontrol by *B. bassian* as was done by Wakil et al. (2014).

Results in Table 2 revealed that the two isolats of *Manisoplaiae* (Imported local) were pathologic to the adults of *T. castanum*, and the cumulative percentages mortalities when the insect sprayed by 10^7 conidia/ml were 36.67 and 80 respectively, with significant variations between the percentages after 16 days of tratement. It seems clearly that the local isolate was more effective and pathogenic to the insects then the imported isolats.

Fig (3) shows mortalities recorded after 9 days of administration since the pathogenic fungus was generally slow acting and it needs many days to penetrate and causes the infection, and the mortalities became higher gradually day after day and the main sight of infection was during the abdomen (Fig 4) since the cuticle...
of the wings (Elytra) was so rigid and hard and mycosis in insects cadaver recorded after more than two weeks. Our findings agreed with Al-Samarae (2017) who tested the same isolates (Imported and local) against the larvae of Chrysomya megacephala and mortality percentages depend on concentrations and time. Ak (2019) study showed that anative isolate of M. anisopliae was very effective against adults of Sitophilus granarius and S. oryzae when they sprayed by $1 \times 10^8$ conidia/ml and cumulative mortality percentages at 25°C recorded as 90.48 and 93.66 respectively both at the end of 7th day, thus this isolate was promising biocontrol agent for these pests.

The scientific work of Mrchala et al. (2006) have shown that using M. anisopliae against T. castanum killed the larvae after 1 week so survival rate of the insects was ratively high. Similarly in Kavaliertosote et al. (2006) study who found that spraying adults of Rhizophythera dominica and S. oryzae and I. confusus recorded after more than one week and this fungus was very effective against these insect findings of our study is in line with Yassin et al. (2017) who confirmed the lethal action of M. anisopliae isolates from soil specimens against adults of red palm weevil, Rynchophorus ferrugineus, when they tested two various concentrations ($10^7$ and $10^8$ conidia/ml) of these isolates by direct contact causing about 75% mortality.

Based on the our findings, all isolates had moderate to high mortality percentages impact, therefore the local isolates considered as a good candidates for biological control against T. castanum adults.

Conclusions.
The current study has concluded the indication that B. bassiana isolate could be apomising bio control agent against adults of T. castanum as well as it was more effective against the insects than the local one. In addition, it was appeared that native isolate of M. abisopliae more pathogenic and effective for adults of T. castanum than B. bassiana.

References


Table 1: Mortality of Tribolium castanum adults sprayed by concentrations of Beauvaria bassian (Imported and local isolates).

<table>
<thead>
<tr>
<th>Concentration conidia/ml</th>
<th>Cumulative mortality %</th>
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<tr>
<td></td>
<td>Imported isolat Mean±S.E.*</td>
</tr>
<tr>
<td></td>
<td>23.33±3.33</td>
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<tr>
<td></td>
<td>40.00±5.77</td>
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<td>53.33±8.82</td>
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*S. E.: Standard Error.

Table 2: Mortality of Tribolium castanum adults sprayed by concentrations of Meterhizium anisopliae (Imported and local isolates).

<table>
<thead>
<tr>
<th>Concentration conidia/ml</th>
<th>Cumulative mortality %</th>
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<tbody>
<tr>
<td></td>
<td>Imported isolat Mean±S.E.*</td>
</tr>
<tr>
<td></td>
<td>13.33±3.33</td>
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<tr>
<td></td>
<td>33.33±3.33</td>
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<tr>
<td></td>
<td>36.67±3.33</td>
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*S. E.: Standard Error.
Fig (1): Mortality mean of *Tribolium catanum* sprayed by *Beauvaria bassiana* (A: Imported isolate, B: Local isolate).

Fig (2): Post-mortal mycelia and conidial growth in *Tribolium catanum* infected by *Beauvaria bassiana*, A: Imported isolate, B: Local isolate.

Fig (3): Mortality mean of *Tribolium catanum* sprayed by *Meterhizium anisopliae* (A: Imported isolate, B: Local isolate).
Fig (4): Post-mortem mycelia and conidial growth in *Tribolium catanum* infected by *Meterhizium anisopliae*, A: Imported isolate, B: Local isolate.