# Biological control of mealybug ( *Brevennia rehi* ) on rice by *Metarhizium anisopliae* fungus in Iraq.

**Ameer S. A. Al-Hedad; Majeed M. Dewan and Hadi mezel AL-rubaei**

[1]*PhD Candidate, University of Kufa, Najaf, Iraq. E-mail:* ameer.sahib1983@gmail.com

[2]*Associate Professor, University of Kufa, Najaf, Iraq, (corresponding author).*

[3]*Associate Professor, University of Babylon, Al Hillah, Iraq, (corresponding author*

## Abstract

The laboratory study on different concentration of *Metarhizium anisopliae* fungus suspension and dry biomass were carried out to control mealybug on rice. The Nymphs stage and Adult of treated mealybug (*B. rehi* ) showed that the percentage of mortality with highest concentrations (50%) reached to 82.97 %. While in Nymphs the percentage was 84.97%. However, the results also showed a significant differences between the high and low concentrations of fungal suspension. The percentage of mortality of the Adult treatments with highest conc. 25 mg / ml were reached to 75.73%. while it was 79.43% in nymphs. For all three cultivars Yasamen , Anbber33, and Furat1 of the rice, there was not a difference in concentration between them at the level of 0.05. The efficacy of *M. anisopliae* in the field rice ( enhanced with 1% ash rice ) concentration $6.1\times10^6$ was assessed against *B.rehi*. the result revealed 37.98% mortality in first spare, the second spare was 48.68% mortality on 14 days. The mean of mortality on rice cultivars were 34.86 and 51.66% on Anber and Furat respectively after 14 days when the *B. rehi* treated with suspension of *M. anisopliae* enhanced with 1% rice ash.

## 1. Introduction

Mealybug are plant-sucking, relatively immobile insect and belong to the family of Pseudococcidae. They secrete white filaments of wax to cover themselves. They are widely distributed throughout the world and are considered economically important pests of a variety of host plants that include horticultural crops. Among them are coffee, cacao, and citrus;, potato as sorghum and rice. The rice mealybug *Brevennia rehi* (Lindinger) is newly recorded in Iraq, Azerbijan and Brazil. (Ben-Dov, 2008).

Isolated 40 species of entomopathogenic fungi from the genus, which are: *Verticillium*, *Hirsutella*, *Beauveria*, *Paecilomyces*, *Metarhizium* and *Tolpocladium*. Similarly, the laboratory bioassays showed that entomopathogenic fungi could be used in the biological control of *V. destructor* (Kanga *et al.* 2002; Kanga,2010 ).

The Deuteromycetes pathogenic fungus, *Metahizium anisopliae* promise to be a biocontrol agent against locust. *M. anisopliae* caused reduction in the feeding activity and induced a significant mortality percentage in the nymphal instars of locust (Arthurs, and Thomas, 2000).

Application of chemical is practically uneconomical, difficult and associated with high cost, environmental pollution and other problems. Hence, there is a strong need for the development of alternative strategies for the control of white grubs, which are ecofriendly and economically feasible. The use of bio-control agents in general and fungal based as myco-insecticides in particular are lacking in the country. About 90 genera and 700 species of fungi representing a large group of entomophthroals (*Metarhizium* spp., *Beauveria* spp., and *Verticillium* spp.) which are entomopathogenic have been reported. Among these, *Metarhizium* is of greater importance in the management of white grubs. *Metarhizium anisopliae* can be effectively utilized as one of the components in the management of white grubs (Mohi-ud-din *et al*., 2006; Chroton, 2007). The fungus is eco-friendly, cost effective, highly persistent and also self-perpetuating in nature. Moreover the microclimate of sugarcane eco-system is ideal for the fungus to multiply. Further, rainfall, high humidity and soil with high organic content also help the fungus to perpetuate itself in nature

## 3- Materials and study methods

### 2.1 insect rearing

Insect were cultured on host plants (rice) .The Mealybug insects (*B. rehi*) were collected from the different parts of the infected rice plants. After identified the mealybug insects, they puck up to the laboratory of the department of plants protection in the College of Agriculture, University of Kufa, Iraq. Three different cultivars were tested, which are: Yassamen, Anbber 33, and Al-Forat rice. All these cultivars were increased to utilize for experimental tests.

All seeds of the rice were brought from the Rice Research Station located in the Al-Mechkaab city, Najaf-Iraq. The seeds were officially validated by the Ministry of Agriculture, Iraq. The seeds were completely washed with the clean water. Then, they were placed in the plastic sieves having a capacity of 250 mg including the solution of sodium hypochlorite sterilization with 10% of pure concentration. This solution was used to sterile the seed from bacterial and fungal infestation for 15 minutes.

The seeds, after that, were carefully washed with the clean water and placed again in the clean sieves to remove the Sodium hypochloride. They were placed on the new sheet inside plastic cans having dimensions of 6 × 12 cm (D×H) with 30 tables of the seeds for each can. The cans, then, were placed inside the incubator with the temperature of 27 ± 1 ˚C and relative humidity of 65 ± 5% for 12 hours. This is to get the seeding at the age of 4 to 5 cm, for 3 to 5 days for each piece.

### 2.2 Preparation of spore suspension from *M. anisopliae* fungus

The *M. anisoliae* was grown on the solid medium which was Potato Sucrose Agar (PSA). One ml of prepared Stock solution from three dishes that were grown completely. For each dish, 10 ml of distilled sterile water were added. The spores were harvested from each dish using a soft brush.

The contents of the dishes were filtrated using a sterile gauze cloth which was fixed on the sterile glass funnel with sterile conical flask had capacity of 250 ml. To ensure falling of the spores, 30 ml of the distilled sterile water was added around the gauze cloth inside the funnel to wash all the spores . The filtrated solution which represents the stock suspension of

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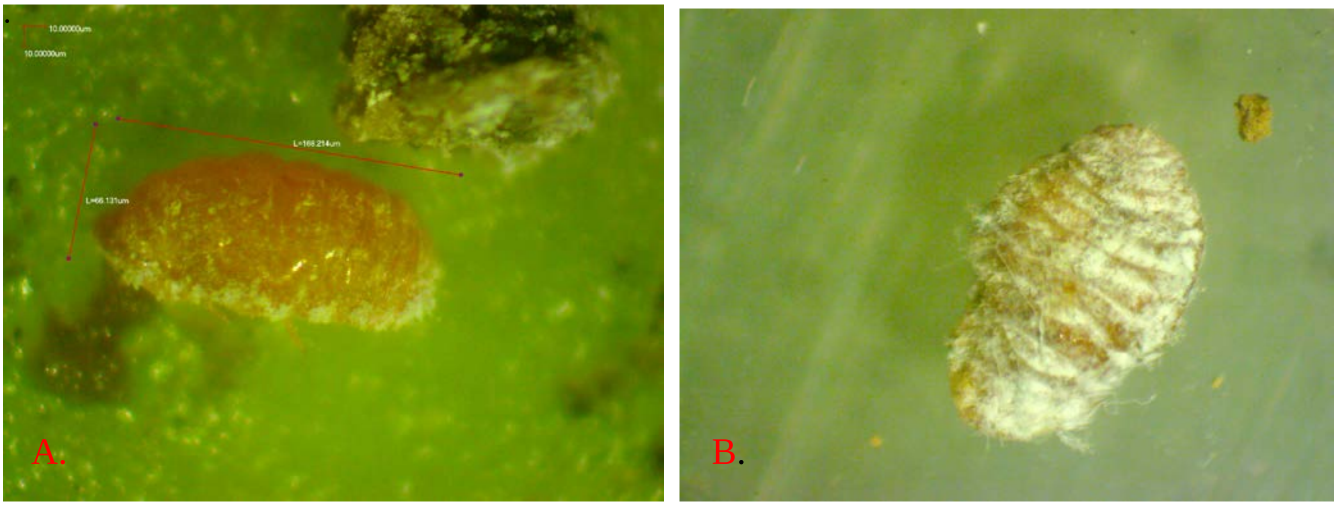
fungal spores was taken and added to 9 ml of distilled sterile water. Series of dilutions was worked to reaching $10^{-6}$.




**Figer 1. A.  normul insect B. inffected insect by *M. anisopliae***

One ml from the diluted suspension was taken and added in the petri dish  containing PSA medium.  . The Petri dish were  incubated was nestled within the temperature of 25 ± 2 ˚ C for 24 hours. The number of fungal colonies  were calculated accordingly, the number of germinated spores the concentration of spores was determined and aseal as stock suspension. The concentrations of 10, 20, 30, 40, and 50% were made from that suspension by adding the distilled sterile water to the suspension according to the above percentages (Dewan, 1989).

### 2.3 Exudates Preparation of Exudates  from *M. anisopliae*

The Fungal Exudates was prepared by using the potato sucrose broth (PSB)medium in flasks.  The flasks were incubated within   25 ± 2 ˚ C for 28 days.   The Fungal Exudates of *M. anisopliae* in liquid medium was filtrated by using the Whatman No. 1 filter paper.

The filtrated exudates, after was put in the tubes and placed in the Centrifuge with the speed of 3000 rpm. The filtrate was taken again from the tubes and filtrated by  using a Millipore filter (45um). The exudates in liquid medium were dried and the concentrations of 5, 10, 15, 20, and 25 mg/ml were taken. This is to conduct the experimental tests.

### 2.4 effect of fungal suspension and dried filtrate of *M. anisopliae* on the female nymph and adult of *B. rehi* on rice.

Fungal suspension and dried fungus filtrate of *M. anisopliae* were used on the female nymphs and adult. Ten individuals of them were taken from each dish by about three repetitions for each treatment for each piece. The nymphs were treated with concentrations of 5, 10, 15, 20, and 25 mg/ml by a manual filter had a capacity of 500 ml with the amount of 2 ml for each dish. All dishes were covered and incubated within a temperature of 27 ± 2 ° C and relative humidity of 65 ± 5%. The percentages of mortality were recorded after 10 days from the treatment. The corrected percentages were adopted for Completely Randomized Design (CRD).

Percent corrected mortality was calculated by following formula described by Püntener (1981).

$$\text{Corrected \%} = \left( \frac{\text{Mortality \% in treated plot - Mortality \% in control plot}}{\text{100 - Mortality \% in control plot}} \right) * 100$$



### 2.5 Field experiment:

An experiment were done in the infected rice field by of *B. rehi* Al-Najaf governorate. Rice were cultured for each type of Yassamen, Anbber 33, and Al-Forat rice verities in period extending from June to November 2016. The field were divided to 6 blocks in area of (4×5)m length and width. Spare was done on 30 plants with mealybug infestation by prepared concentration of suspension fungal $6.1\times10^{6}$ and three plants were kept as control without any application of treatments were replicated three times.

Percent corrected mortality was calculated by following formula described by Henderson (1955).

$$\text{Corrected \%} = \left(1 - \frac{\text{n in Co before treatment * n in T after treatment}}{\text{n in Co after treatment * n in T before treatment}} \right) * 100$$

Where : n = Insect population , T = treated , Co = control

### Statistical analysis

For all the obtained data of the mortality of mealybugs at all treatments were subjected to analysis of variance (one way ANOVA) and differences among means were considered significant at a probability  0.05. Statistical package GenStat was used for all these statistical analyses.

# Results

## 3.1 Effect of ash rice in growth of *M. anisopliae* on PSA medium

The result showed as in table(1) the high fungal growth of *M. anisopliae* it found in 1% rice ash when added to solid medium(PSA), the colony area of fungal growth was 81 $cm^2$ after 15 days from the inoculating the medium while the less growth area was in 2,5% ash, it reached to 27.63 $cm^2$ in the same period. Whereas the sporelations of fungus were 29 and $7.7\times10^4$ in 2.5 and 1 % ash treatments respectively.

**Table 2. Effect of ash rice in growth of *M. anisopliae* on PSA medium**



| No. | Concentration | Country area of fungal growth / $cm^2$ | | | | Average | |
|---|---|---|---|---|---|---|---|
| | % | 3 | 7 | 10 | 15 | | |
| 1 | 1 | 23 | 34.2 | 59.5 | 81 | 49.43 | $^{4}10\times7.7$ |
| 2 | 1.5 | 21.5 | 32.5 | 45.2 | 66 | 41.30 | $^{4}10\times2.0$ |
| 3 | 2 | 15.5 | 29 | 43.2 | 58.6 | 36.58 | $^{4}10\times4.5$ |
| 4 | 2.5 | 18.5 | 29 | 43.5 | 59.2 | 37.55 | $^{4}10\times5.2$ |
| 5 | Control | 9 | 20.2 | 34.5 | 46.8 | 27.63 | $^{4}10\times2.9$ |
| Average | | 17.5 | 28.98 | 45.18 | 62.32 | | |
| $L.S.D_{0.05}$ | | Conc.= 3.20 | days =2.87 | Iterance= 6.418 | C.V,%= 11.8 | | |

## 3.2 Efficacy of fungus suspension under laboratory conditions

The result showed as in table(2) the efficacy of *M. anisopliae* at different concentration was evaluated against rice mealybug. The results revealed the concentrations gave significantly difference among themselves in causing mortality of Adult *B. rehi* except at 50% concentration after an exposure of 10 days the mean was 82.97% . For all three cultivars of the rice (Yasamen, Anbber 33, and Furat 1) without significantly difference , there are interference between concentration 50% and rice cultivars were 83.9, 83.9, and 81.1% respectively of the mortality without a significantly difference.

among themselves in causing mortality of Nymph *B.rehi* 50% concentration after an exposure of 10 days the mean mortality was 82.97%. For all three cultivars of the rice (Yasamen, Anbber 33, and Furat 1) without significantly difference. There were the inferences between concentration 50% and Yassamen cultivar numerical increased TO 90% without a considerable difference.

**Table 2. Effect of fungus suspension of *M.anisopliae* on the Mealybug insects on the rice experimentally.**

| No. | Concentration | Adult/ % mortality | | | | Nymphs/ % mortality | | | |
|---|---|---|---|---|---|---|---|---|---|
| | % | Yasemean | Anbber 33 | Furat1. | Average | Yasemean | Anbber 33 | Furat 1 | Average |
| 1 | 10 | 37 | 35.2 | 40.6 | 37.60 | 36.1 | 38.9 | 33 | 36.00 |
| 2 | 20 | 47.2 | 33 | 46.9 | 42.37 | 42.6 | 48.1 | 35.2 | 41.97 |
| 3 | 30 | 59.2 | 58.1 | 61.2 | 59.50 | 67 | 67.3 | 58.6 | 64.30 |
| 4 | 40 | 72.3 | 69.9 | 66.1 | 69.43 | 69.2 | 80.6 | 77.7 | 75.83 |
| 5 | 50 | 83.9 | 81.1 | 83.9 | 82.97 | 90 | 83.8 | 81.1 | 84.97 |
| Average | | 59.92 | 55.46 | 59.74 | | 60.98 | 63.74 | 57.12 | |
| L.S.D$_{0.05}$ | | Conc.=9.36 | For cultivars=7.25 | Iterance= 16.6 | C.V,%=16.6 | Conc.= 12.58 | For cultivars=9.74 | Iterance=21.78 | C.V, %=21.5 |

## 3.3 Efficacy of dry-Biomass of *M. anisopliae* under laboratory conditions

Table 3 shows the results of the effect of dried filtrate of Fungus *M. anisopliae* on the mealybug insects on the rice experimentally. The results that the higher percentage for the adult mortality the mealybug insects on the rice was 75.53% concentration treatment of 25 mg/ml with a considerable difference in comparison with other concentration. At the same time, the results showed that there was not a considerable variation between the three studied cultivars of the rice. It was found the interference between concentration 25 mg /ml and the Furat1, Anbber33, and Yassamen, rice cultivars were 77.1, 75, and 74.5% respectively of the mortality without and a significantly difference**.**

As shown in Table 3, In addition, the fungus filtrate of *M. anisopliae* was appeared a higher influence on the nymphs of mealybug insects at the concentration of 25 mg/ml with a rate of 79.43%. For all three cultivars of the rice, there was not a considerable deference between them at the level of 0.05. the result showed are interference between concentration of 25 mg/ml and cultivars the rice Furat1, Anbber. 33, and Yassamen, were 83.4, 77.7 and 77.2% respectively of the mortality without and a considerable difference.

**Table 3. Effect of dry exudates of *M. anisopliae* on the Mealybug insects on the rice cultivars.**

| No. | Concentration of solution | Adult/ % mortality | | | | Nymphs/ % mortality | | | |
|---|---|---|---|---|---|---|---|---|---|
| | mg/ml | Al-Yasemean | Anbber 33 | Al-Forat No. 1. | Average | Al-Yasemean | Anbber 33 | Al-Forat No. 1. | Average |
| 1 | 5 | 32.5 | 35.2 | 26.8 | 31.50 | 33.9 | 35.2 | 30.9 | 33.33 |
| 2 | 10 | 43.1 | 41.2 | 37.8 | 40.70 | 51.5 | 45 | 42.3 | 41.97 |
| 3 | 15 | 55.8 | 48.8 | 46.3 | 50.30 | 53.3 | 57 | 60.2 | 56.83 |
| 4 | 20 | 57.9 | 57 | 59.5 | 58.13 | 62.4 | 63.9 | 77.1 | 67.80 |
| | 25 | 74.5 | 75 | 77.1 | 75.73 | 77.2 | 77.7 | 83.4 | 79.43 |
| Average | | 52.76 | 51.44 | 49.5 | | 55.66 | 55.76 | 58.78 | |
| $L.S.D_{0.05}$ | | Conc.=9.59 | For cultivars=5.10 | Iterance= 11.41 | C.V,%=13.3 | Conc.= 7.35 | For cultivars=5.35 | Iterance=12.72 | C.V, %=13.4 |

## 3.4 Field studies

The efficacy of suspension $6.1\times10^{6}$ conidia / ml *M. anisopliae*( max with ash rice 1% ) concentration was assessed against *B.rehi*. table 4 revealed that 37.98% mortality was appeared after 14 days in first spare, the mortality in second spare was 48.68% in the same period. The mortality on different cultivars was 38.86% in Anber while the high mean in secondary spraying was 51.66 in Furat cultivar after 14 days.

**Table 4. Efficacy of suspension $6.1\times10^6$ conidia / ml *M. anisopliae*( max with ash rice 1% ) under the field conditions**

| change population reduction% | | | | | |
|---|---|---|---|---|---|
| | days | Furat1 | Anber33 | Yassamen | Average |
| First | 7 | 18.44 | 34.45 | 27.28 | 26.72 |
| | 14 | 25.1 | 43.28 | 45.57 | 37.98 |
| | Average | 21.77 | 38.86 | 36.42 | 32.35 |
| | L.S.D.$_{0.05}$ | 4.547 =Iteranc | 3.21=cultivars | 2.235 days | C.V. 7.9 |
| Second | 7 | 49.6 | 23.58 | 41.55 | 38.24 |
| | 14 | 53.73 | 42.71 | 49.6 | 48.68 |
| | Average | 51.66 | 33.14 | 45.57 | 43.45 |
| | L.S.D.$_{0.05}$ | 6.20= Iterance | Cultivars=4.39 | days=3.58 | C.V.= 8.0 |



# Discussion

The cumulative mortality of Vine mealybug *Planococcus ficus* adults exposed to isolates of the fungus ranged between 52 and 86% , *M. anisopliae* The lowest fungal concentration $5x10^5$ conidia / ml replicated five times with 10 insects per replicate (Ghada, 2016). In line with the present findings, also Ujjan and Shahzad (2007) observed up to 100% mortality of different instars of pink mealy bug, *Maconellicoccus hirsutus* (Green) by various strains of *M. anisopliae*. Similarly.

. Differences in potential of different strain's toxicity depended on physiological and enzymatic properties of each isolate (Leland *et al.*, 2005). The fungal pathogenesis is a complex process and is dependent upon the attributes of both, the pathogen and the host. The cuticle appears to influence all stages of the infection process; adhesion, germination and aspersorium differentiation (Butt *et al.*, 2001).

Integration of entomopathogenic fungi (EPF) in the integrated pest management (IPM) strategy for control of rice mealybug can reduce reliance on synthetic insecticides and increase the levels of control, especially against early season rice mealybug populations. EPF are also ready- made components of IPM because of their complementary or synergistic insecticidal activity with other control elements including predators and parasitoids (Roy and Pell, 2000; and Goettel *et al*., 2010). Commercial products based on *M. anisopliae* are currently in use in some parts of the world like; Europe, United States, Australia ...etc. or under development. Faria and Wraight (2007) gave a comprehensive list with worldwide coverage and international classification of formulation types of the EPF; *B. bassiana* and *M. anisopliae* strains used successfully in controlling different insect pests under field conditions (Lacey *et al.*, 2011).



Based on the results of this study, it was concluded that the EPF products tested may provide viable alternatives to synthetic insecticides used in the control of rice mealybug. As well as in conjunction with other agricultural practices may reduce the use of chemical pesticides and provide an element within an I PM system

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