DOI: https://doi.org/10.26682/ajuod.2020.23.2.2
Journal of University of Duhok., Vol. 23, No.2 (Agri. and Vet. Sciences), Pp 1-6, 2020

PRESERVATIVE MEDIA FOR *Beauveria bassiana* (BALS.) VUILL STORAGE

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(Received: February 16, 2020; Accepted for Publication: July 19, 2020)

ABSTRACT

***Beauveria bassiana* is an important entomopathogenic fungus that used as a biocontrol agent of insect pests. Maintaining and preserving *B. bassiana* cultures is essential for the effective evaluation of its potential as microbial agent against insect pest, for biodiversity studies and also for exchange of fungal material between laboratories. In the present work we evaluated the suitability of four preservation materials based on gelatin and rice to maintain the viability of *B. bassiana* to be used as baits for insect's control. The gelatin amended with sugar recorded the maximum viability after 70 days of storage as 98.3% compared to 63.3% on rice media. Effective control of 80% of the ants was observed after 10 days of feeding on gelatin amended with sugar and rice bait. The results demonstrated that treatment with formulations containing conidia of *B. bassiana* presents insecticidal activity against ant in addition of acting as preservation materials.**

KEYWORDS: *B. bassiana*, gelatin, preservation method, microbial baits, ant**.**

INTRODUCTION

B*eauveria bassiana* (Bals.)Vuill is one of the most common entomopathognic fungi in different kinds of habitats and ecosystems (Meyling and Eilenberg 2007; Akello et al. 2009). This fungus is used to control a wide range of agricultural pests from many different orders (Roy et al. 2010). The benefits of using *B. bassiana* and other fungi as biopesticides are well known, mainly for reducing the side effects related to the use of chemical products, but their application are limited. Such limitations are mostly due to the discrepancy of their performance because of the clear effect of abiotic factors especially the temperature and humidity (Padmini and Padmaja 2010). These factors can significantly reduce conidial viability, speed of germination, hyphal growth rate and spore production (Immdiato 2017). In the other hand, preservation of the fungi as collections and stocks is very important for potential biocontrol of insects. For that, several methods of cultivation and preservation were studied, as long as, the existence of a method that is able to preserve viability, sporulation and pathogenicity of the fungus, were more reliable, and more preferably (Abd-Elsalam et al., 2010; Aparecido and Camilo, 2013). However, most have not proven fully effective, because of the peculiarities of the cultures and even the characteristics of techniques. The gelatin method is the most recent method used for preservation of the entomopathogenic and phytopathogenic fungi (Beloti et al. 2017). Preservation methods for entomopathogenic fungi require effective protocols to ensure uniform processes and to avoid alterations during storage (Ayala-Zermeno et al. 2017).

Previous studies reported that *B. bassiana* is a good biocontrol agent against ants, but the best way for colony exposing is unknown. Different application methods revealed that immersion of adult ants in a conidial suspension was more effective and practical than spraying. Siebeneicher et al. 1992 mentioned that the *B. bassiana* is capable of penetrating insect cuticle, but there was little evidence that infection occurred by the penetration of the cuticle of most areas of the ant's body, with the exception of the tarsi and by oral routes and added that the contamination of soil with conidia did not cause the ants to abandon the soil as a nest media nor was increased ant mortality observed. Although *B. bassiana* has shown great promise in ant control under laboratory conditions, they have been less successful under field conditions (Bextine and Thorvilson 2002, Fuxa and Richter 2004).

In this study we aimed to evaluate the gelatin (never tested for *B. bassiana*) and rice to serve

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as preservation methods as well as a technique (carrier material as microbial bait) to control *Tapinoma* spp ant workers (have symbiotic relationships with aphid) in Duhok city.

MATERIALS AND METHODS

Beauveria bassiana preparation

A local strain of *B. bassiana* was obtained from Mycology bank/ Plant Protection Department/ College of Agricultural Engineering Sciences/ University of Duhok under the No. BEG23 (GenBank MH374538) (Hassan, 2019).

Preservation media

The fungus was cultured on Potato Dextrose Agar (PDA) for 10 days at 25± 1˚C. The concentration of fungal suspension was diluted to 1x 10^7 conidia/ml. in order to provide conidia for the different preservation materials. Four cultures based on gelatin and rice were used as preservation materials; gelatin, gelatin amended with sugar, gelatin amended with rice and rice only.

For gelatin culture, 20g of gelatin was dissolved in 100 ml of distilled water and left to boil. After cooling (culture temperature reach to 40˚C), the culture was inoculated with 10ml of *B. bassiana* suspension at 1x10^7 conidia/ ml. Then, the inoculated culture was poured in small containers and left to solidified (figure 1). The culture blocks were kept in small plastic bags and stored at 4˚C. For rice culture, 40g of rice/ 100 ml water was used. For gelatin amended with sugar, 20g gelatin + 2g sugar was used per 100 ml water. For gelatin amended with rice, 20g gelatin + 20g rice/ 100 ml water was used. The same procedure mentioned for gelatin culture was followed for the other cultures.

Gelatin Gelatin+ Rice Rice Gelatin + sugar

Fig. (1): Culture blocks of preseved materials inoculated with *B. bassiana* suspension at 1x10^7 and stored at 4˚C

Beauveria bassiana conidia viability

To evaluate the viability (germination percentage) of *B. bassiana* conidia, preserved within the material preservation as blocks and stored under 4˚C. A thin layer from each block was cut and put on a clean microscope slide (Figure 2), kept within a petri-dish and incubated at 25±1 C. for 24h (Hassan 2019). An experiment proceeded through three months to count the spore viability which was tested once every ten days. The germination% was estimated by counting 20 spores for each layer/ block using a microscope. Three layers were used/ preservation material/ date and each layer served as a replicate.

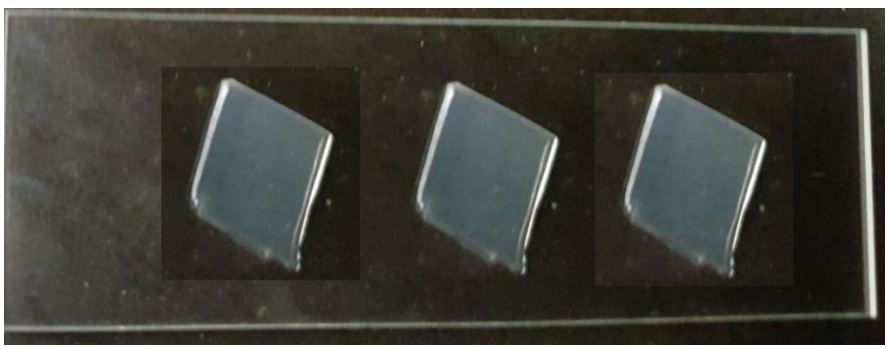

Fig. (2): A technique used to evaluate the germination percentage of *B. bassiana* conidia

Beauveria bassiana virulence

The virulence of *B. bassiana* spores that preseved within the blocks of preservation

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materials as gelatin, gelatin amended with sugar, gelatin amended with rice and rice only was conducted against the ant *Tapinoma* spp and tested twice. For control treatment, sugar suspension 10% was used for feeding.

A total of 200 worker specimens (ants) were collected from the vegetable fields at college of Agricultural Engineering Sciences (University of Duhok, Kurdistan region, Iraq), kept in plastic containers and transported to the Plant protection department labs for bioassay.

Groups of 10 workers kept in a small transparent plastic screw-top jar (10 x 3 cm), and then the containers provided with one gram of the blocks/ preservation materials/ replicate. Three replicates/ preservation material were used and each container act as a replicate. The top face of each container was well tied to prevent ants from escaping. Containers were held under laboratory conditions (25 ± 2˚C) and monitored daily to record the mortality of the ants after 4, 7 and 10 days of treatments.

To confirm that the death of ant workers, were due to mycosis, dead individuals were removed at the assessment period and surface sterilized by washing in 2% sodium hypochlorite (NaOCL) for 2 min. and then rinsed with sterilized distill water. The sterilized dead ants placed either on sterile wet filter paper in Petri dishes or cultured directly on PDA medium and incubated at 25˚C for one week to examine growing fungus. Fungi growing on samples were transferred on standard media for identification (Figure 3).

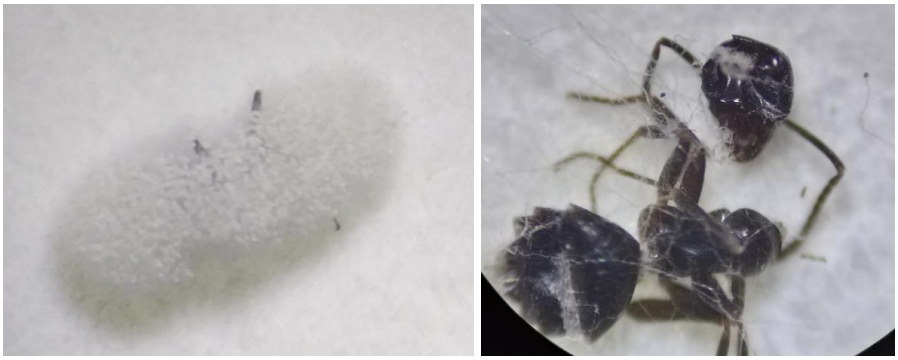

Figure 3: Growing of *Beauveria bassiana* on surface sterilized ants after incubation on moistant filter paper.

RESULTS AND DISCUSSION

Beauveria bassiana conidia viability

The preservation of fungal strains, as reference stocks for ongoing research, requires that the stored cultures remain viable for long time periods without any morphological or physiological alterations. Therefore, in a first attempt, we were Interested in studying the viability over short periods of time (up to 3 months) as a percentage of conidia each 10 days, especially we tested gelatin based media as preservation materials and as control technique for the first time.

The results showed uniform conidia germination after one month amongst different preservation media at 4°C storage temperatures. A conidial viability 100% was retained for the conidia preserved within gelatin, gelatin amended with sugar, gelatin amended with rice and rice only (Figure 4).

After 2 months of storage, conidial preserved within gelatin and gelatin amended with sugar was still able to show the maximum germination of 100 percent while, decreased viability was observed with the conidia preserved within gelatin amended with rice and rice only, respectively. Germination across two preservation material recorded more than 70% at 4˚C after 2 months of storage (Figure 4).

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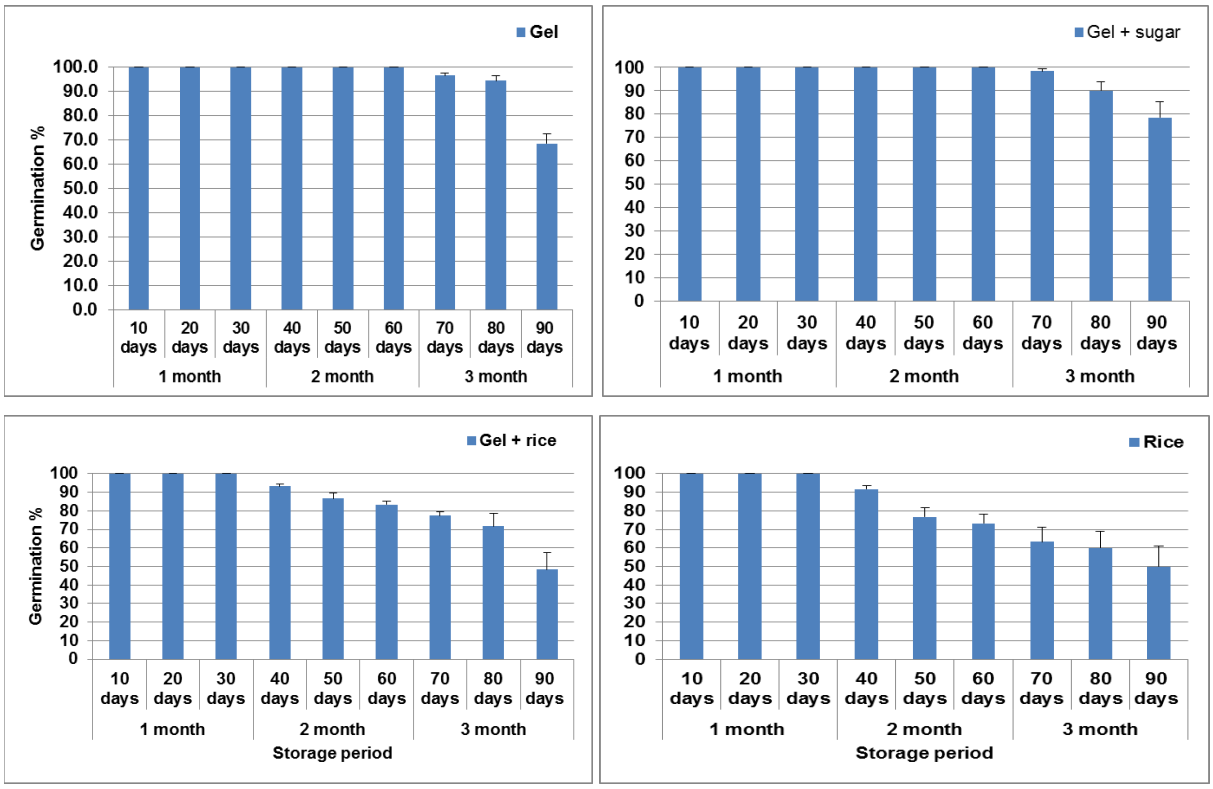

Fig. (4): germination percentage of *Beauveria bassiana* conidia inoculated with preservation materials and sored three months at 4C.

Interaction of storage temperature and preservation material on viable counts of *B. bassiana* after three months at 4˚C revealed that the gelatin based media as gelatin amended with sugar and gelatin showed the maximum viability after 70 days of storage as 98.3 and 96.7%, respectively, which was significantly superior over gelatin amended with rice and rice media as 77.5 and 63.3%, respectively. The minimum germination percentage after 90 days of storage recoded with the *B. bassiana* conidia preserved with gelatin amended with rice as 48.3% which was on par with germination percent of conidia preserved with rice only as 50.0%.

These results agreed with Simkova (2009) who studied the impact of different carriers on *B. bassiana* conidia viability, that the germination rate differed markedly between carriers and were 97.67 % after storage of 90 days at 4˚C. Beloti et al. (2017) who studied the gelatin method remained viability in 10 cultures; reported that gelatin is suitable for preservation of the genera and species of many fungi. Immediato *et al.* (2017) evaluated the viability of *B. bassiana* conidia monthly over a one year period and reported that conidia of *B. bassiana* had a significantly higher rate of survival at 4˚C compared to 22˚C regardless of the other storage conditions.

***Beauveria bassiana* virulence**

The data in Figure (5) shows the mortality percentage of ant workers fed on preservation material blocks (as bait) that previously inoculated with *B. bassiana* suspension as ($1x10^7$) and stored for three months at 4˚C, after 4, 7 and 10 days of treatment. The results showed a positive relationship between the mortality percentage and the period of exposure.

The highest percent of mortality after 4 days of treatment was recorded with the workers that fed on gelatin+ sugar inoculated with *B. bassiana* at $1x10^7$ conidia/ ml which reached to 63.33. % compared to26.66% when fed on gelatin+ rice as bait and significantly differed with mortality percent recorded in control treatment (0.0%).

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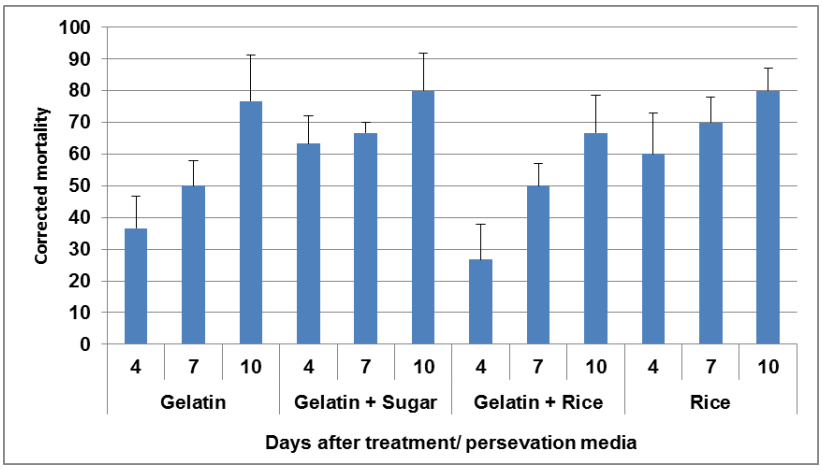

Fig. (5): Corrected mortality % of ants fed on baits of preserved material inoculated with *B. bassiana* conidia

After 7 days of treatment, the mortality gradually increased to reach the highest percent as 70% among the workers fed on rice bait inoculated with *B. bassiana* and significantly not differed with mortality percent with those fed on gelatin amended with sugar bait.

The highest mortality percent after 10 days of treatment risen up to reach 80% when the workers fed on gelatin amended with sugar bait and was on par with mortality percent recorded with workers fed on rice bait compared to 76.67 and 66.67% when fed on gelatin and gelatin amended with rice, respectively. No mortality was recorded in control treatment. Siebeneicher et al. (1992) reported that the liquid baits containing conidia were more effective in causing mortality among ant workers, whereas solid baits containing conidia were more effective in causing mortality among brood.

Li et al. (2016) recorded a cumulative mortality rate as 93.40% at $1\times10^{8}mL^{-1}$conidia among fire ants *Solenopsis invicta* adults after 21 days of treatment. Rojas et al. (2018) recorded a mortality percent of 84.48% after 6 days of *Solenopsis invicta* workers treatment by *B. bassiana.* Daza et al. (2019) mentioned that an effective control of 90% of the nests was observed in the field phase in 60 days when they evaluated a bioinsecticide composed of the spores of *B. Bassiana* and *Trichoderma lignorum* for the control of the leafcutter ant (*Atta cephalotes)* under field conditions.

In the present study, it was demonstrated that formulations containing conidia of *B. bassiana*, in addition of acting as preservation materials, presents insecticidal activity against ant (has a symbiotic relationship with aphid) that in future the ants contaminated with fungus can be a way to serve as a translator of fungus conidia to aphid colony. Also can be considered for the control of soil insects as bait and achieved by controlling/eliminating the food source.

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