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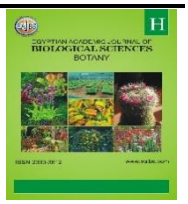

Liquid Culture of Different Grains for Rapid Production of *Beauveria bassiana* Blastospores

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ABSTRACT

This study was carried out at the Laboratory of Plant Protection within the Department of Agricultural Engineering Sciences at the University of Duhok. A significant challenge to the widespread commercial application of fungal biocontrol agents is the lack of affordable production media and efficient manufacturing methods. In this study, an isolate of *Beauveria bassiana* was evaluated for blastospore yield, germination percentage and conidia concentration using fermentation media of four different grains as wheat, whole wheat, rice and corn. The *Beauveria bassiana* mycelium weight, germination % and concentration were differed when cultured on the different grains liquid culture. The data were analyzed statistically using a SAS program following a Completely Randomized Design (CRD).The result indicated that the highest mat weight of *B. bassiana* cultured recorded on the liquid media of rice as to 7.45 grams. The weight of *B. bassiana* mycelia was significantly influenced by the type of medium used, and additionally, the weight decreased with each subsequent drying period. As an average, there is no significant effect of the four grains used as a liquid media on the biomass production of *B. bassiana*, while a significant effect among the drying period was recorded on mycelium weight. In summary, liquid culture fermentation is an essential and economical technique for quickly generating high levels of *Beauveria bassiana* blastospores, which are crucial for effective biological pest management. Using complex organic sources like rice, wheat, and corn enhances blastospore yield, resilience, and shelf life. And Optimizing culture conditions with these substrates is an essential for developing effective, sustainable mycopesticides, and advancing integrated pest management strategies.

INTRODUCTION

Beauveria bassiana (Ascomycota: Cordycipitaceae) is a prominent invertebrate fungal pathogen that plays a significant role in controlling various agricultural arthropods (Mascarin *et al.,* 2016). It is widely utilized worldwide as a biological control agent to manage numerous agricultural pests and vectors of human diseases (Jaiswal *et al.,* 2022). Additionally, this fungus has been recently recognized as an endophyte that offers several advantages, such as promoting plant growth, enhancing drought tolerance, and inducing resistance to diseases (Mascarin *et al.,* 2016; Tomilova *et al.,* 2020).

Various techniques exist for the mass production of entomopathogenic fungi, such as solid substrate fermentation to produce aerial conidia and liquid culture fermentation to generate yeast-like blastospores, microcycle conidia, and microsclerotia (Hegedus & Goettel, 1992; Jackson & Jaronski, 1997; Li *et al.,* 2010; Jaronski & Jackson, 2016).

Typically, Beauveria is applied in large-scale inundative treatments, utilizing substantial quantities of aerial conidia formulated in either dry or liquid forms (Fargues., 1997).

Beauveria, when integrated as one component within a broader pest management strategy rather than used alone, requires further development across various crop systems. Numerous studies offer insights into mass-production techniques, formulation methods, a current list of approved commercial products, significant biocontrol initiatives, and ecological factors influencing the application of Beauveria as a mycoinsecticide (Mascarin *et al.,* 2016). The processes involved in producing and formulating *B. bassiana* are crucial to its effectiveness and success as a commercial biocontrol agent.

This study was conducted to assess grains—including wheat, whole wheat, corn, and rice—as liquid substrates for the large-scale cultivation of *Beauveria bassiana*.

MATERIALS AND METHODS

This research was carried out at the Laboratory of the Plant Protection Department within the College of Agricultural Engineering Sciences at the University of Duhok.

***Beauveria bassiana* Preparation:**

A native strain of *B. bassiana* was obtained from the Mycology Bank / Plant Protection Department at the College of Agricultural Engineering Sciences, University of Duhok, cataloged under No. BEG23 (GenBank MH374538) (Hassan, 2019). This local isolate of *B. bassiana* was harvested from soil samples collected beneath fallen plant litter, which are considered ideal hibernation sites for the sunn pest— a major pest affecting wheat crops in Iraq. The samples were gathered from Gara Mountain (coordinates: N 37° 1.51′, E 43° 23′ 34″) at an elevation of 2,066 meters above sea level.

Preparation of Culture Media:

Liquid media were prepared using four types of grains: wheat, whole wheat, corn, and rice, each consisting of 200 grams. The grains were thoroughly washed, then boiled in distilled water for one hour, after which they were mashed and properly filtered. The resulting filtrate was adjusted to a volume of one liter with distilled water. From each medium, 250 ml portions were transferred into individual 500 ml conical flasks, with three replicates for each medium and dry period. The flasks were sealed using cotton wool and sterilized by autoclaving at 121°C. After cooling, each flask was inoculated with 1 ml of a fungal suspension containing 10 million spores. For the control group, sterilized distilled water was inoculated with the same fungal suspension. All procedures took place inside a laminar airflow cabinet. The flasks were then incubated at 25°C for a period of 21 days. Following incubation, the flasks were shaken and filtered through filter paper. The resulting fungal mats were dried at room temperature to evaluate their growth.

1. The weight (gm) of fungal mat directly after filtering (wet weight) and after 1, 5 and 10 days of drying using a sensitive balance.
2. Spore concentration after drying using the hemocytometer slide.
3. The percentage of spore germination was estimated by culturing a fungal suspension (0.1 ml containing 1×10^5 conidia) on a PDF thin-layer slide. The viability of the conidia was calculated using the formula: R = (N / T) × 100%, where R represents the germination rate, N is the number of conidia that germinated, and T is the total number of conidia observed. This procedure was performed three times to ensure accuracy.

The data were analyzed statistically using the SAS software, employing a Completely Randomized Design (CRD) with three replicates. The means were compared through Duncan's multiple range test at a significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Effect of Culture Media on *B. bassiana* yield:

1-Wet Weight:

Wet weight refers to the weight of the fungal mat after filtration including the water content. It is typically higher than dry weight because it includes both the solid material and the water content (Ferron., 1978). The data in Figure (1), demonstrated that the mat weight of *B. bassiana* cultured on the liquid media of rice was the highest as 7.45 grams. Whole wheat and Corn have relatively similar wet weights, with Whole wheat slightly higher as 6.025 gram compared to Corn 5.9925 gram. Wheat has the lowest wet weight as 4.68 grams.

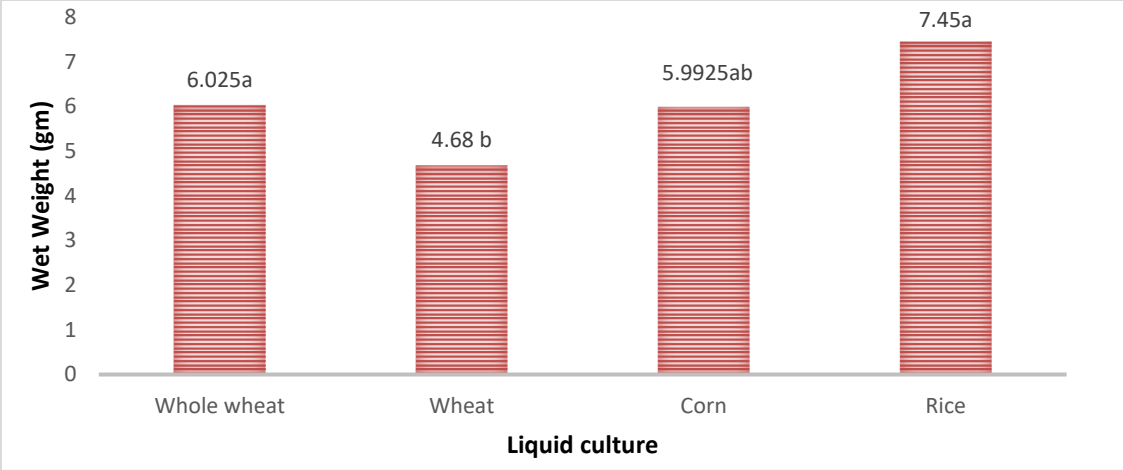

Fig.1: Biomass production (wet weight) of *Beauveria bassiana* on different grains as liquid medium**.**

2-Dry Weight:

The growth of *B. bassiana* cultured on four different liquid media was calculated on the basis of weight in grams after 1, 5 and 10 days after drying (Hassan., 2019). The data in Table (1), showed the effect of different media over three different drying time periods (1, 5 and 10 days).

The findings indicated that the mycelial weight of *B. bassiana* was significantly influenced by the type of growth medium in (Table 1), and the biomass decreased after each drying period. Among the four liquid media tested, whole wheat medium yielded the highest biomass, measuring 2.755 grams per 250 ml of medium after one day of drying. This value was significantly higher than those obtained with rice, wheat, and corn media, which recorded weights of 2.048, 1.898, and 1.070 grams, respectively, after the same drying period. Generally, wheat and rice contain higher levels of proteins and carbohydrates compared to corn, nutrients that are vital for fungal growth and thus promote greater biomass production (Liu *et al.,* 2021). Additionally, Ibrahim (1993) reported that the dry weight of *B. bassiana* biomass produced on wheat and rice grains was 0.378 g and 0.555 g per 100 grams of grains, respectively.

The highest yield of *B. bassiana* after five days of drying was observed with rice medium, reaching 0.923 grams, which was significantly not differ from the lowest weight of 0.443 grams recorded with whole wheat medium. Over the course of time, the biomass weight decreased across all media; after ten days, the weights ranged from 0.153 grams for corn to 0.188 grams for wheat. According to Hassan (2019), the maximum biomass of *B. bassiana* was achieved on rice medium, measuring 1.01 grams per 250 ml after one day of drying. This value was significantly different from the weights recorded after five and ten days, which were 0.78 and 0.77 grams per 250 ml, respectively. Similarly, on wheat medium,

the biomass was 0.72 grams initially, decreasing to 0.68 grams after five days and 0.67 grams after ten days. The overall results indicated that, on average, the four types of grains used as liquid media did not have a significant impact on *B. bassiana* biomass production. However, there was a significant effect of the drying period on the mycelium weight.

Table 1: Biomass production of *Beauveria bassiana* on four grains as liquid medium

Media	Weight g/ drying period			Effect of media
	After 24 hours	After 5 days	After 10 days	
Whole wheat	2.755a	0.443d	0.183de	1.127a
Wheat	1.898b	0.748cd	0.188de	0.944b
Corn	1.070c	0.903c	0.153e	0.708b
Rice	2.048ab	0.923c	0.175de	1.048ab
Effect of period	1.943a	0.754 b	0.175 c	

Means followed by the same common letter are significantly not differed at 5% level by DMRT

3-Spore Germination:

The data in Figure (2), illustrated the effect of media type on *B. bassiana* spore germination%. The percentage for Whole wheat, Corn, and Rice is 100%, while Wheat has a lower percentage of 66.66%.

The 66.66% for Wheat could indicate that this medium is used or effective at a lower capacity or quantity relative to the others (Geetha &Varalakshmi, 2001). This could reflect differences in nutrient availability, water absorption, or another characteristic that makes Wheat less effective or potent in this context.

The rate at which conidia germinate is important in the infection process, since those that germinate faster are generally more likely to lead to infection. However, germination rates seen on the cuticle can differ from those on artificial media because the cuticle contains antifungal compounds that may inhibit germination (Charnley, 1989).

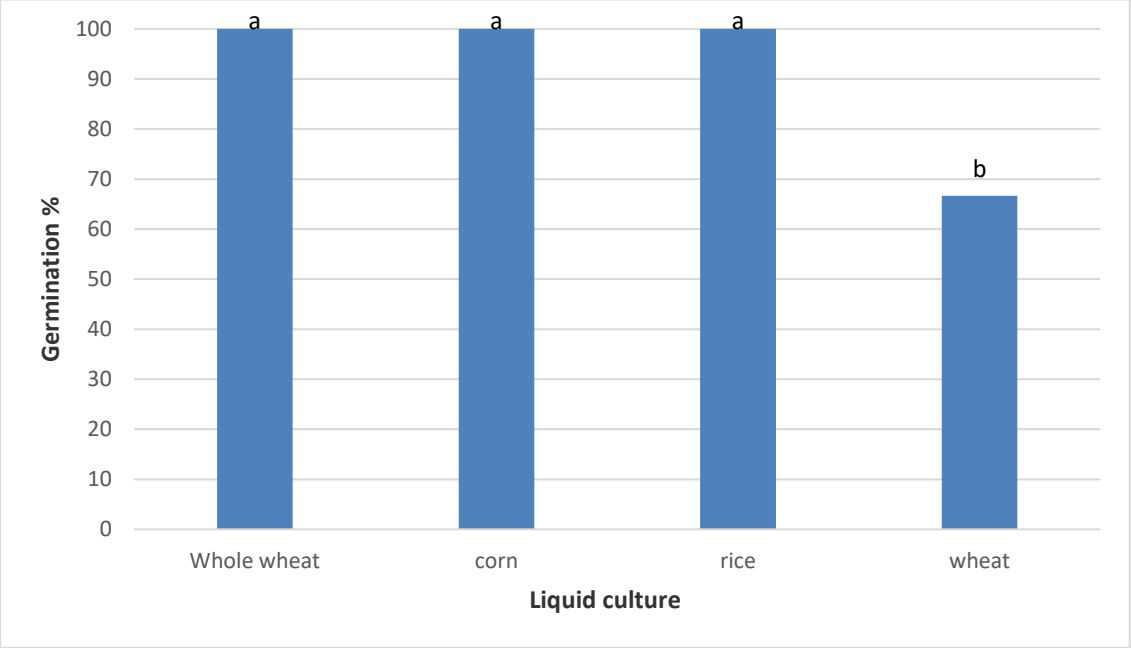

Fig. 2: Spore concentration % of *Beauveria bassiana* on different grains as liquid medium.

Spore Concentration:

The concentration of conidia/ ml of fungal suspension was significantly affected by the type of media. The highest spore production was recorded with *B. bassiana* cultured on

whole wheat grains as 6.64 x 10^6 conidia/ ml followed by 3.83x10^6 conidia / ml for *B. bassiana* cultured on wheat which is significantly differ (Fig 3). Hassan *et al.* (2020) mentioned that the highest spore production was recorded with *B. bassiana* cultured on wheat grains as 5.0 x 10^6 conidia/ ml after 10 days drying compared to 8.3 x 10^5 when cultured on rice grains for a solid .The lowest concentration was reported with *B. bassiana* cultured on rice and corn liquid culture as 3.13 x 10^6 and 2.66 x10^6, respectively. Hassan (2019) stated that the concentration of spore production reached to 6.1 × 10^6 and 4.1 × 10^5 conidia/ ml after 10 days drying which recorded with *B. bassiana* cultured on rice and wheat liquid medium, respectively.

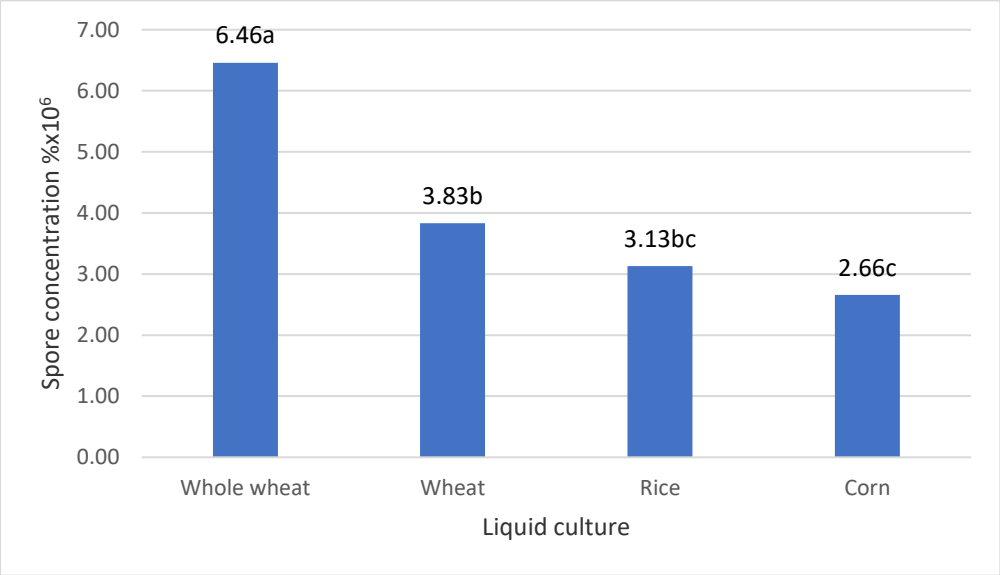

Fig. 3: Spore concentration % of *Beauveria bassiana* on different grains as liquid medium

CONCLUSION

Liquid culture fermentation has emerged as a pivotal method for the rapid and cost-effective production of *Beauveria bassiana* blastospores, which are instrumental in biological pest control. This technique enables the generation of high concentrations of stable and infective blastospores within a short timeframe, typically around three days. The source in the culture medium significantly influences the yield. Studies have demonstrated that utilizing complex organic sources, such as Rice, wheat and Corn not only enhances blastospore production but also improves their resilience to environmental stresses and prolongs shelf life. These findings underscore the importance of optimizing culture conditions to produce high-quality blastospores, thereby advancing the development of effective and sustainable mycopesticides for integrated pest management programs

Declarations:

Ethical Approval: Not applicable.

Conflict of interest: The author declares no conflict of interest.

Author's Contributions: I hereby verify that the author mentioned on the title page has Contributed significantly to the idea and planning of the research, has carefully read the work, attested to the veracity and correctness of the data and its interpretation, and has given their approval for submission.

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REFERENCES

Chamley, H. (1989). *Clay Sedimentology*. Berlin: Springer-Verlag. ISBN: 978-3-540-50889-2

Fargues J, Goettel MS, Smits N, Ouedraogo A, Rougier M. (1997). Biological control of insect pests using entomopathogenic fungi: a review. *Biocontrol Science and Technology.*7(4):487-502. doi:10.1080/00275514.1997.12026797.

Ferron P. (1978). Biological control of insect pests by entomogenous fungi**.** *Annual Review of Entomology,* 23:409-423. doi:10.1146/annurev.en.23.010178.002205

Geetha T, Varalakshmi P. (2001) .Anti-inflammatory activity of lupeol and lupeol linoleate in rats.*The journal of Ethnopharmacol,* 76(1):77-80.

Hassan FR, Abdullah SK, Assaf LH. (2020) .Molecular identification and biomass production of an endophytic *Beauveria bassiana* isolated from cucumber leaves in Iraq. *Journal of University of Duhok*, 22(2):38-47. doi:10.26682/cajuod.2020.22.2.5

Hassan FR. (2019). Selective Isolation and Biomass Production of *Beauveria bassiana* and its Virulence to Squash Beetle *Epilachna chrysomelina* F. Ph.D dissertation, College of Agricultural Engineering Sciences, University of Duhok, Iraq, 165 pp.

Hegedus D, Goettel MS. (1992) .Fungal biological control agents in the management of insect pests. *Biologycal Control,* 2(1):1-10.

Ibrahim YB, Low W. (1993). Potential of mass production and field efficacy of isolates of the entomopathogenic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* on *Plutella xylostella*. *Journal of Invertebrate Pathology*,39:222-232. doi:10.1080/0967087930937180.

Jackson MA, Jaronski ST. (1997) .Mass production of entomopathogenic fungi. In: Fungal Pathogens of Insects. p. 1-15. doi:10.1016/B978-0-12-386899-2.00008-7.

Jaiswal DK, Gawande SJ, Soumia PS, Krishna R, Vaishnav A, Ade AB. (2022). Biocontrol strategies: an eco-smart tool for integrated pest and diseases management. *BioMed Central (BMC)Microbiology,* 22(1):324. doi:10.1186/s12866-022-02744-2.

Jaronski ST, Jackson MA. (2016). Mass production of entomopathogenic fungi: a review. *Mycological Research*, 116(1):1-12. doi:10.1016/B978-0-12-803527-6.00009-3.

Li Y, Han D, Hu G, Sommerfeld M, Hu Q. (2010). Inhibition of starch synthesis results in overproduction of lipids in *Chlamydomonas reinhardtii*. *Biotechnology and Bioengineering,* 107(2):258-268. https://doi.org/10.1002/bit.22807.

Liu J, Wang ZK, Sun HH, Ying SH, Feng MG. (2021). Nutritional requirements and growth characteristics of *Beauveria bassiana*. *Journal of Fungal Biology,* 12(3):45-56.

Mascarin GM, Jackson MA, Behle RW, Kobori NN, Júnior ÍD. (2016) .The role of *Beauveria bassiana* in integrated pest management.*pest Management Science,* 72(1):1-10. doi:10.1007/s11274-016-2131-3.

SAS Institute Inc. SAS/STAT Software, Version 13. Cary, NC: SAS Institute Inc.; 2013.

Tomilova OG, Shaldyaeva EM, Kryukova NA, Pilipova YV, Schmidt NS, Danilov VP. (2020). Endophytic fungi: a new frontier in plant growth promotion and disease resistance.*Fungal Biology Reviews,* 34(1):1-10. doi:10.7717/peerj.9895.

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