

Production of entomopathogenic fungi in large quantities *Beauveria bassiana* (Balsamo) by controlling date palm pests with agricultural goods based on the liquid-solid diphasic technique

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ABSTRACT: The best media for producing *Beauveria bassiana* in both liquid and solid phases to create clamidospores and conidiosopres was determined to be fungi in this investigation. The solid phase of plant materials, such as sugar cane, maize, barley, rice, millet, and sorghum, and the liquid phase of plant meals, such as potatoes, wheat flour, rice flour, corn flour, and sugar cane molasses, were assessed. The liquid phase's performance parameters were compared by the percentage of clamidospores that germinated and the concentration of spores. Wet weight, dry weight, germination, and conidia concentration were used to compare the solid phase's performance characteristics. The results demonstrated that there were substantial differences at a 1% probability between the various plant extracts employed as liquid and solid media to grow *B. bassiana* clamidospores and conidia. Due to their greatest yield and cost-effectiveness, rice and sugar cane molasses extract were ultimately chosen to manufacture *B. bassiana* conidia and clamidospores, respectively.

Keywords: Mass production, liquid and solid medium, *B. bassiana*,

INTRODUCTION

Over the last several decades, there has been a worldwide surge in interest in the use of entomopathogenic fungi as biological control agents for insect species. To manage a variety of insect pests, myco-insecticides based on *Beauveria bassiana* (Balsamo) Vaillem (Babu et al., 2001; Sharma, 2004), *Paecilomyces fumosoroseus* (Wize) Brown and Smith (Alter and Vandenberg, 2000; Avery et al., 2004), and *Verticillium lecanii* (Zimm.) Viegas (Butt et al., 2001). A crucial part of the biocontrol program is the production of sufficient amounts of high-quality inoculums. There are two ways to produce

entomopathogens: either a relatively small amount of inoculums for field testing and laboratory experimentation during mycopesticide development, or the development of a basic production system for large-scale production by using labour-intensive and financially feasible methods for relatively small-sized markets. Fungal biocontrol agents were sufficiently provided by China (Feng et al., 1994) and America (Alves and Pereira, 1989) for local niche markets. The basic multiplication processes of submerged liquid fermentation for the production of short-lived blastospores and hydrophilic (Rombach, 1989) or solid state fermentation (Rousson et al., 1983) for the production of aerial conidia are followed in the development of a straightforward and dependable production system. The most practical mass production techniques, however, include using a diphasic approach, whereby the fungal inoculums are created in liquid culture and then used to inoculate the solid substrate or substrates for the formation of conidia (Burgess and Hussey, 1981). The first prerequisite for cultivating microorganisms using any culture method is an understanding of their nutritional needs. Microelements like carbon, hydrogen, nitrogen, sulphur, and phosphorus make up carbohydrates, proteins, lipids, and nucleic acids. These elements are involved in host-pathogen interaction and self-defence systems. The primary component is carbon, and carbon molecules also contribute to hydrogen and oxygen. The impact of nutrition sources on microorganism growth and development is examined. Mycelia growth and spore output are caused by the fungus and medium components utilised. Saprophytic fungus use a variety of nutrition sources, but for bulk production and Simple, inexpensive media are required for commercialisation (Raimbault, 1998). The macronutrients carbon, hydrogen, oxygen, sulphur, phosphorus, and nitrogen—which make up proteins, carbohydrates, and nucleic acids—are necessary for the complete development of microbes. The tolerance's election studies benefit from both growth traits and growth chemicals. Microorganisms may be grown, stored, and transported using either a liquid or solid preparation (Jenkins, 1995). All of the nutrients needed for microbe development should be present in

the medium. The development and sporulation of filamentous fungus, including *B. bassiana*, *M. anisopliae*, and *I. fumosorosea*, have been the subject of several nutritional studies (Kumar et al., 1996). In Colombia, a simple sterilisation method based on cooked rice put inside bottles is used to produce the majority of *B. bassiana* spores for coffee berry borer biocontrol. Field spray treatments are the primary usage for the spores (Posada 1993; Bustillo and Posada 1996). Using a 1% oil-water solution, the spores are extracted from the rice medium (Antía et al. 1992). To prevent spore germination, this aqueous spore solution has to be utilised right away after preparation. Additionally, since high moisture content quickly reduces spore viability, spores maintained in bottles have a limited lifespan. It takes time to harvest the spores from the bottles, and drying the rice with spores is challenging if the cultures generated in the bottles are to be utilised to make a spore powder (Posada 1993; González et al. 1993). Over the last ten years, Iran has conducted a significant amount of research on entomopathogenic fungi that suppress date palm pests. Fungal pathogens have been shown in several studies to be successful in controlling *Oryzaephilus surinamensis* (Latifian & Rahkhodaei, 2012). *B. bassiana* and *Metarhizium anisoplia* have been used in the majority of this study (Latifian and Rad, 2012). However, research on producing locally isolated Entomopathogenic fungi on appropriate medium for widespread use is still lacking. In order to produce *B. bassiana* in large quantities, the current research was conducted to assess grains such as rice, wheat, ragi, sorghum, pearl millet, and maize as well as liquid media such Potato Dextrose Broth and Sabouraud's Dextrose Broth.

MATERIAL AND METHODS

Preparation of fungi

Beauveria bassiana strain Iran441c was acquired from the Iranian Research Institute of Plant Protection's fungus collection. After the isolate was cultivated in SDA medium for ten days at 25±1 °C, 100% relative humidity, and a 12:12 (L:D) photoperiod, the conidia were collected, suspended in Tween 80 (0.2 ml/l) in sterile distilled water, and vortexed for three minutes to create a homogeneous suspension. After that, the suspension was filtered through many cheesecloth layers to get rid of dirt and mycelia. The spore concentration was calculated and corrected to 104 conidia/ml-1 using a Haemocytometer.

Liquid media

The growth and sporulation of three tested fungi were assessed in liquid medium, including potatoes, wheat flour, rice flour, maize flour, and sugar cane molasses. Conical

flasks with a 250 ml capacity were filled with 100 mg of each medium and autoclaved for 20 minutes at 15 psi of pressure. One millilitre of each fungus's spore suspension was added to five flasks of each medium, and the flasks were then incubated for three days at 25 degrees Celsius. Spore counting was performed on the spore suspension using the method outlined in the preceding section.

Solid media

Tests were conducted on non-synthetic solid media, including sugar cane, maize, barley, rice, millet and sorghum. One millilitre of spore suspension was added to 150 grams of each solid substance in one-litre conical flasks, which were then incubated for twelve days at 25 degrees Celsius in a BOD incubator. As previously stated, a spore count was conducted.

Spore harvesting and drying

To assess spore yield, the spores were collected three and twelve days after the inoculation for liquid and solid medium, respectively. The fungus has to be dried in order to lower the moisture content and enable the spores to detach from the substrate in order to collect the spores as powder. This method allows for the long-term preservation of spores without compromising their germinative potential or pathogenicity (Bateman, 1995). The plastic bags were left to air dry in a room with an average relative humidity of 50±5% and a temperature of 20 ± 5°C in order to dry the cultures. For twenty minutes, harvesting was done both manually and mechanically. A shaker Ro-Tap, which employs horizontal circular motion, was used in the mechanical harvest. Particles are stratified and screened using a vertical taping action. The sieve was moved back and forth during the hand harvest. After filtering, the recovered spore powder was weighed and stored in different sterile vials for quality and moisture content evaluations.

Culture moisture content assessment

Following incubation times, each sample was weighed to track the cultures' air drying. Simultaneously, subsamples were obtained every day from separate samples that were maintained under experimental circumstances in order to measure the humidity content using oven drying, where the subsamples were dried for 24 hours at 105°C (Rao et al. 2006). Determining the fungal culture's moisture content was the procedure's goal.

Quality control of spores

Using a quality control approach developed at CENICAFÉ, the collected spores were analysed for moisture content, germination, and spore concentration (Vélez et al. 1997).

Statistical analysis

The relevance of medium on the formation of high-quality spores of both liquid and solid fungal pathogens was analysed using ANOVA, and the "STATISTICA" computer program was utilised to distinguish the means of the cultures across the media using the SNK ($P = 0.05$) test.

substrates of both solid and liquid media were tested for mass multiplication of *B. bassiana*. The success of microbial control of insect pests depends not only on the isolation, characterization and pathogenicity, but also on the successful mass production of the microbial agents in the laboratory. Large-scale availability of the pathogen is a primary requirement in the bio-control program. For a successful integrated pest management program, the agents like the entomopathogenic fungi should be amenable to easy and cheap mass multiplication.

The results (Table 1) showed that there were significant differences among the culture Medias tested, *B. bassiana* spore production on both media.

RESULTS AND DISCUSSION

In the present study, several naturally available

Table1. Results of different characteristic of *B. bassiana* spore production of liquid and solid phases

Phase	MS				
Liquid	Df	Concentration		Germination	
	4	104.69 **		0.185**	
Solid	Df	Ww	DW	Concentration	Germination
	5	12.07 **	9.44**	21722.9**	67.2**

** showed that there was singnificant difference at percent WW=Wet Weight, DW=Dry Weight

There were significantly statistically significant differences between available cultural nutritive media for the mass multiplication of *B. bassiana*.

Table 2. comparison the mean of spore quality on liquid phase

Culture media	Clamidospore concentration	Germination
Potato	1.2×10^e	84.1 e
Wheat	5.1×10^d	87.6 d
Rice	$3.3 \times 10^7 b$	96.7 c
Maize	$8.3 \times 10^5 c$	98.4 b
Sugarcane melees	$2.4 \times 10^8 a$	99.5 a

Liquid media

Sugar cane melees generated significantly more spores than the other liquid medium. (2.4×10^8 spores/ml) and 99.5% of germination was seen in *B. bassiana*; all examined fungi also produced spores in the other medium (Table 2). Potato culture medium had the lowest spore output (12 spores/ml) and 84.1 percent germination. Similar results were also noted in *M. anisepliae* by Dangar et al. (1991). Fungal growth and spore formation may be boosted by an abundance of glucose and minerals in the culture medium. All of the examined fungi were also able to grow and sporulate in rice and maize-washed water. The cleaned rice wash water produced the highest spore count in *M. anisepliae*, according to Patel et al. (1990).

Solid media

B. bassiana produced the most spores (6.24×10^4 spores/ml) on rice out of all the solid wastes examined, followed by sorghum seeds (4.34×10^4). Maximum spore and biomass generation was likewise encouraged by rice trash (Table 3). According to Gopalakrishnan and Mohan (2001), Gopalakrishnan et al. (1999), and Tincilley et al. (2000), carrots are the most affordable and ideal medium for producing Deuteromycete fungus on a big scale. According to Puzari et al. (1997), *M. anisopliae* sporulation increased when rice husk was added with a 2% dextrose solution. It was evident from the research that the examined fungus could grow on a broad range of agricultural goods and byproducts in both solid and liquid states, which might help farmers cultivate these fungi with ease.

Table 3. comparison the mean of spore quality on solid phase

Culture media	WW	WD	Concentration ($\times 10^4$)	Germination
Sugarcane	1.69 f	0.36 f	326 d	92.5 b

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Maize	2.29 e	1.64 c	3.6 e	89.5 c
Barley	4.94 b	2.29 b	194.8 f	87.6 e
Rice	5.21 a	3.83 a	624.2 a	95.5 a
Millet	3.05 d	1.25 e	386 c	86.5 f
Sorghum	3.36 c	1.47 d	434.2 b	88.6 d

Ibrahim and Low (1993) and Sharma et al. (2002) reported that rice was determined to be an appropriate medium for *B. bassiana* bulk culture. Other Deuteromycete fungus were also produced in large quantities using this wheat. According to Gopalakrishnan et al. (1999), sorghum was the best crop for producing *Paecilomyces farinosus* in large quantities. Sorghum was determined to be the best crop for mass production in the instance of *V. lecanii*, which is consistent with the results of Lakshmi et al. (2001). Due to their greatest yield and cost-effectiveness, rice and sugar cane molasses extract were ultimately chosen to manufacture *B. bassiana* conidia and clamidospores, respectively.

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