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RESEARCH

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Screening of *Beauveria bassiana* (Bals.) (Hypocreales: Cordycipitaceae) strains against *Megalurothrips usitatus* (Bagnall) (Thysanoptera: Thripidae) and conditions for large-scale production

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Abstract

Background: *Beauveria bassiana* (Bals.) (Hypocreales: Cordycipitaceae) is an entomopathogenic fungus that has potential as a biological control agent against many insect pests. This study was conducted to optimize the conditions for large-scale production on rice of *B. bassiana* and evaluate its virulence against the bean flower thrips *Megalurothrips usitatus* (Bagnall) (Thysanoptera: Thripidae).

Results: The optimal substrate quantity for conidia production was 200 g of rice per container (2.1 l), with substrate having a 52% moisture content, being supplemented with 2% cooking oil. In stage production of a batch, 150 ml of conidia suspension (at 24.33×10^7 spores/ml) was incubated for 10 days in SDA liquid medium amended with 4% glucose and 1.5% yeast extract. The SDA medium was then used to inoculate one container of the rice substrate. The optimum ratio of conidial suspension to substrate (v/v) was 20–25%. Virulence of the spore powder harvested from the rice substrate was same as that of produced on the SDA solid media against thrips. The level of control from an application of spore powder of the thrips *M. usitatus* was similar to that from chemical the pesticide spinetoram when applied in a cowpea (*Vigna sinensis* Endl) field.

Conclusions: The optimized rice substrate system can be used for a large-scale production of *B. bassiana* spores, which can be used for field control of thrips.

Keywords: Entomopathogenic fungi, Mass production, Rice substrate, Virulence, Thrips

Background

The bean flower thrips, *Megalurothrips usitatus* (Bagnall) (Thripidae: Thysanoptera), has always been an important pest of legumes in Hainan Province, China (Tang et al. 2015). Chemical insecticides are the principal method for controlling this thrips, but their use is not always effective due to some of the thrips' biological characteristics such as sheltering inside flowers and having a short life cycle (Liu et al. 2018). Biological control, especially the use of

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EPFs, has attracted widespread attention as a potentially effective control strategy.

Hyphomycete fungi such as species of *Metarhizium* or *Beauveria* are widely used as EPFs because of their low cost (due to efficient local mass-production), and some species or strains are already commercially available (Islam et al. 2021). *Beauveria bassiana* is a globally distributed, anamorphic fungus that can infect hundreds of insect species in various orders (Wang et al. 2021). Because many EPNs occur naturally in many countries, their use as mycoinsecticides is generally considered safe (Biryol et al. 2021). For a mycoinsecticide to be successful, it must not only effectively control the target pest, it must also be easy and cheap to mass produced good quality conidia.

EPFs may be reared in in relatively small quantities for laboratory experiments and field-tests during the development of a mycopesticide or it may be grown on a large-scale using methods that are often labor intensive, variable economic costs (Seema et al. 2013). The successful development of a commercial product based on a microbial control agent for control of pest insects depends not only on the isolation, characterization and pathogenicity of an effective species or strain, but also on its successful mass production in the laboratory. For biocontrol or IPM programs using EPFs to be successful large-scale production of the pathogen must be feasible and relatively cheap (Bich et al. 2018).

EPNs may be mass produced on solid media (PDA, SDA), in diphasic liquid–solid fermentation systems, or on a variety of less well defined solid substrates. Diphasic liquid–solid fermentation has many advantages including reducing the risk of colony contamination and rapid colonization and conidiation during the solid substrate phase. Efficient cultivation of EPNs is influenced by the fungus used and the exact components of the rearing media as both factors affect mycelia growth and spore yield (Bich et al. 2018).

Mass production of *B. bassiana* has been done using various solid substrates (e.g., white rice, wheat, rye, corn, sorghum, rice powder, rice bran, and sorghum grain) (Chałańska et al. 2017) or in diphasic liquid–solid fermentation systems based on solid media such as grains, vegetable wastes, maize, bran, cotton seed, rice husk, or wheat and liquid media such as coconut water and cooked rice (Seema et al. 2013). Culture conditions may be varied to increased conidial production or quality, or conidial efficacy for pest control (Sala et al. 2019). For the mass production of *B. bassiana* using a diphasic liquid–solid phase production system, temperature, pH, moisture content of the solid substrate, and the composition of the liquid medium all affect the quality and the quantity of the inoculum produced. Our objectives in this study were: (1) to evaluate the virulence of five strains of *B. bassiana* against bean thrips and (2) to optimize conditions for large-scale production of the most virulent strain.

Methods

Thrips and microorganisms

The pest thrips, *Megalurothrips usitatus* (Bagnall) (Thysanoptera: Thripidae), was collected from Hainan Province of China (18.7851°N, 110.3910°E) and reared for more than 50 generations in the Laboratory of Biological Control of Agriculture Pests, at China Agricultural University (Beijing, China). The colony of *M. usitatus* was reared on kidney bean pods (*Phaseolus vulgaris* L.; Fabaceae) in 2 l glass jars at 26 ± 1 °C, 65% RH, and 16:8 h (L:D) (Liu et al. 2018).

We tested five strains of *B. bassiana* isolated from infected chinch bugs (*Nysius* spp., Lygaeidae) in maize fields or the associated soils. After isolation, these strains were stored in the lab mentioned in the previous paragraph. These five strains were (1) Bbi, isolated from host bugs using SDA media, (2, 3) Bcmdrbc1 and Bcmdrbc3 isolated from DRBC (Dichloran Rose-Bengal Chloramphenicol) agar-based medium using carbendazim (BCM), and (4, 5) Doddrbc1 and Dodsda4 isolated from DRBC and SDA media using dodine (DOD).

Laboratory virulence assay

To evaluate the virulence of five strains of *B. bassiana*, adult thrips were exposed, in a laboratory trial with 3 replicates of 20 thrips each. The concentrations of all 5 strains of *B. bassiana* were adjusted to 1.8×10^6 spores in 50-ml tubes by adding sterilized distilled water containing 0.05% Tween 80. The pods of soya beans were placed in tubes for 30 s, removed, and dried for 2 min before placing them in Petri dishes with the thrips to be tested. The number of dead thrips was recorded daily for 4 days, and fresh pods, which had been immersed in the same concentration of *B. bassiana* spores for 30 s, were substituted for the original pods on day 2.

Preparation of artificial solid media and measurement of conidia concentration

To assess mass production efficiency of various media, we tested four synthetic solid diets for *B. bassiana* production: (1) PDA (potato dextrose agar), (2) SDA (Sabouraud dextrose agar), (3) DRBC (Dichloran Rose-Bengal Chloramphenicol), and (4) YES (yeast extract with supplements). The media were prepared, autoclaved at 120 °C for 20 min, and when cooled, 20 ml aliquots were placed in each petri dish. PDA, SDAY, and YES were also prepared as liquid media. Culture plates were inoculated with 0.1 ml of a *B. bassiana* suspension of 10^6 conidia/

ml and then incubated at 27 °C. The quantity and quality of conidia produced were checked at 10 and 15 day after inoculation. After incubation, 0.1 g of fungal growth was harvested from the surface of each medium and placed into 50 ml tube. To these tubes, we added 10 ml of sterilized distilled water containing 0.05% Tween 80. Tubes were shaken for 5 min, diluted fourfold, and then spores were counted under the microscope to estimate the number of spores/gram harvested fungal growth (Sun et al. 2011).

Preparation of liquid media calculation of spore count

Three liquid media were tested for their effect on growth of *B. bassiana*: (1) PDA, (2) SDA, and (3) YES. For each liquid medium tested, 150 ml was put in a 250-ml flask and autoclaved for 20 min at 121 °C. When the medium had cooled, it was inoculated with conidium harvested from the solid SDA medium and then incubated at 26 °C. These inoculated liquid media (PDA, SDA, and YES) were then shaken at 180 rpm for 72 h. After 72 h, the spore concentration in the flask was determined by putting 1 ml of the spore suspension in a new 250-ml flask and adding 99 ml 0.05% sterile Tween solution and attenuated in gradient. The final solution was examined with a microscope and the number of conidia counted.

Effects of rice quantity, moisture level, oil versus potassium nitrate (KNO_3) supplement on conidial production

The effect of three different quantities of rice (200, 250, and 300 g) in a rearing container (30 × 20 × 3.5 cm) (2100 cm^3) (filling approximately 9.5, 11.9, and 14.3% of the volume of the container) was examined. We also examined three different moisture levels of the rice medium (53.6, 51.5, and 49.8%), the addition of 2% cooking oil (peanut oil or soybean oil versus the addition of potassium nitrate KNO_3 (0.2%).

Rice moisture levels were determined as follows:

$$\%\ \text{moisture content} = \frac{(W - B) - (D - B)}{W - B} \times 100$$

where W = weight of bottle plus wet rice, D = weight of bottle plus dry rice and B = weight of bottle.

After incubation for 10 d at 26 °C and 60–80% r.h., the quantity of spore powder and the spore concentration were estimated for each combination of media components.

Effects of the conidia number and the level of glucose and yeast in the media on conidial production in a rice medium

The effects of different conidial ratios (15, 20, 25, and 30%), and the concentration of glucose (1, 2, 3, and 4%) and yeast (0.5, 1, 1.5, and 2%) in the suspension were evaluated. The SDA (Sabouraud dextrose agar) medium was prepared with all the above concentrations and autoclaved for 20 min at 120 °C. The cooled media were then inoculated with a plug 9 cm in diameter from the solid SDA medium that had been incubated for 10 days after its inoculation with *B. bassiana*. The inoculated media were then incubated for 72 h. The rice was prepared, inoculated, and incubated as described above.

Isolation and purification of spores from white rice media

Spores were harvested and counted 10 d after inoculation of the medium. The rice medium was dried at 35 °C for 5 h, followed by 28–30 °C for 5 days, in an oven dryer. The dried rice medium was ground (motor speed 32,000 revolutions/min)for 5–8 min and interval time 8–10 min, and the dry media were sieved (screen openings 150 µm) to separate spores from the rice.

Field efficacy trial

For our field trial, there were four treatments, each with 3 replicates. The 4 treatments were: (1–3) a suspension of 10^5, 10^6, or 10^7 spores/ml *B. bassiana* spores (Dodsda1); (4) the chemical check (positive control), consisting of 60 g/l of spinetoram 1000 times diluent. A 7 m long row of cowpeas was the crop unit to which treatments (and replicates) were applied. The crop units were arranged randomly within the test field. A knapsack electric sprayer was used to apply the 5 treatments, with an application volume of 1 kg of solution per 10 m^2. Applications were completed by 10 a.m on the day of treatment.

Data analysis

All data were analyzed using one way ANOVA, followed by Turkey's mean separation tests, using a P value of < 0.05 to indicate significant difference. SPSS 24.0 was used for all analyses.

Results

Virulence of five *Beauveria bassiana* strains to bean flower thrips

Five fungal strains of *B. bassiana* were tested against this thrips. Three strains (Bcmdrbc1, Bcmdrbc3, and Doddrbc1) caused 75–80%, mortality 4 days after inoculation exposure to *B. bassiana* spores. The other two strains (Dodsda4 and Bbi) caused 53.3–63.3% (Fig. 1).

Effects of artificial media on the growth of *B. bassiana*

The four solid media examined (SDA, PDA, YES, DRBC) yielded similar numbers of conidia (0.73×10^8, 0.66×10^8, 0.56×10^8, 0.46×10^8) per gram of medium 10 days after inoculation of the media, and there values were not significantly different (Fig. 2A). After 15 days of incubation, the SDA solid media produced the most

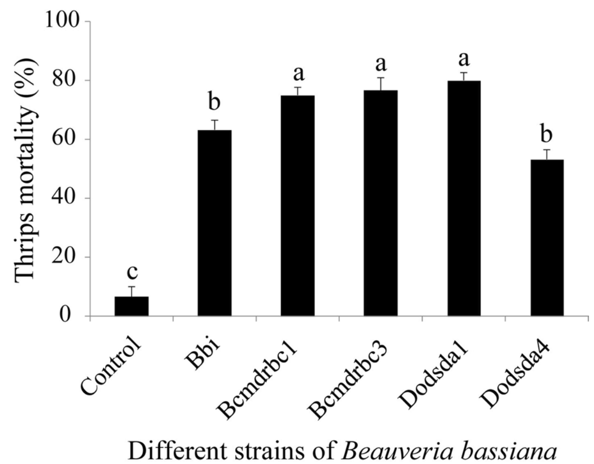

Fig. 1 Mortality rates of *Megalurothrips usitatus* different *Beauveria bassiana* strains 4 days after exposure to fungal spores. The bars marked with the same letter were not significantly different according to Turkey at $p > 0.05$. Bars with different letters are significantly different

conidia (71×10^8) per gram of media (Fig. 2B). SDA solid media also produced highest weight of spores per rearing container 0.33 and 0.34 g/plate at 10 and 15 days of inoculation, respectively (Fig. 2C, D). When *B. bassiana* was cultured in three liquid media (SDA, PDA and YES), spore production after 72 h of incubation was 24.33×10^7, 8×10^7, and 16.67×10^7 spores/ml of media, respectively (Fig. 2E).

Effects of volume, moisture level, oil level, and potassium nitrate (KNO_3) level of a white rice medium on conidial production

When the fungus was produced in 2.1-l flasks on solid fermentation media, the amount of rice affected sporulation efficiency. The weight of spore powder produced from 200 g of rice medium was significantly higher than for the 300 g rice medium, and the conidia concentration from the 200 g rice medium was significantly higher than other two media (Fig. 3A, B).

The moisture content of the rice media also affected the weight of spore powder produced per container, with the highest level of spore weight being produced at 51.5% moisture compared to media with 53.6% moisture but the highest level was non-significantly different from rice with 49.8% content. The conidial concentration of the spore powder produced on rice with 51.5% moisture was significantly higher for either 53.6 or 49.8% moisture levels (Fig. 3C, D).

The weight of spore powder and the conidial concentration of the spore powder from the rice supplemented with 2% oil were significantly higher than for rice supplemented with 0.2% KNO_3 or without any additives. The effect of the addition of 0.2% KNO_3 on the production of *B. bassiana* was non-significant (Fig. 3E, F).

Effects of conidial solution concentration, glucose concentration, and yeast concentration on spore powder weight and conidial production after incubation

The conidia solution quantity that resulted in the highest spore powder weight was the 20% amount (Fig. 4A), while the smallest spore powder weight occurred at 15%. In contrast, all conidia solution quantities resulted in similar conidial concentrations in the final product, with the exception of the 30% inoculation quantity, which resulted in lower conidial concentrations in the final product (Fig. 4B).

Glucose concentration (1, 2, 3, and 4%) had no significant effect on spore powder weight (Fig. 4C), but strongly affected the conidia concentration of the final spore powder with 4% glucose producing the highest spore concentration (Fig. 4D).

Similarly, yeast concentration had little effect on spore powder quantity (Fig. 5E), but strongly affected spore concentration in the final product (Fig. 4F), with the 1.5% yeast concentration resulting in the highest spore concentration.

Field efficacy trail

The Dodsda1 strain was chosen for the field trial. It was cultured on a solid medium (200 g rice/2.1 l, with 51.5% moisture content) that had been inoculated with a 25% spore suspension. The medium was supplemented with 4% glucose, 1.5% yeast, and 2% oil, and was incubated for 3 days. In a pre-trail laboratory assessment, we found that spores of the Dodsda1 strain from two different rearing media (white rice spore powder and SDA spore powder) were equally infective to thrips (causing 88.3 and 91.7% mortality 4 d after inoculation, levels that were not significantly different (Fig. 5).

In our field trial, at 3 days post-application, spinetoram (at 60 μg/ml) gave significantly higher levels of thrips control than did the Dodsda1 strain of *B. bassiana* (Table 1). However, at 5 and 7 days post-application there was no statistical difference in thrips mortality among the three concentration of the fungus or with spinetoram, with mortality of thrips being in the 25–34% range by day 7 (Table 1).

Discussion

Beauveria bassiana has been used for the control of many species of insects (Fabrice et al. 2020). However, the selection of highly virulent isolates of *B. bassiana* strains is a key factor for success. Yang et al. (2020)

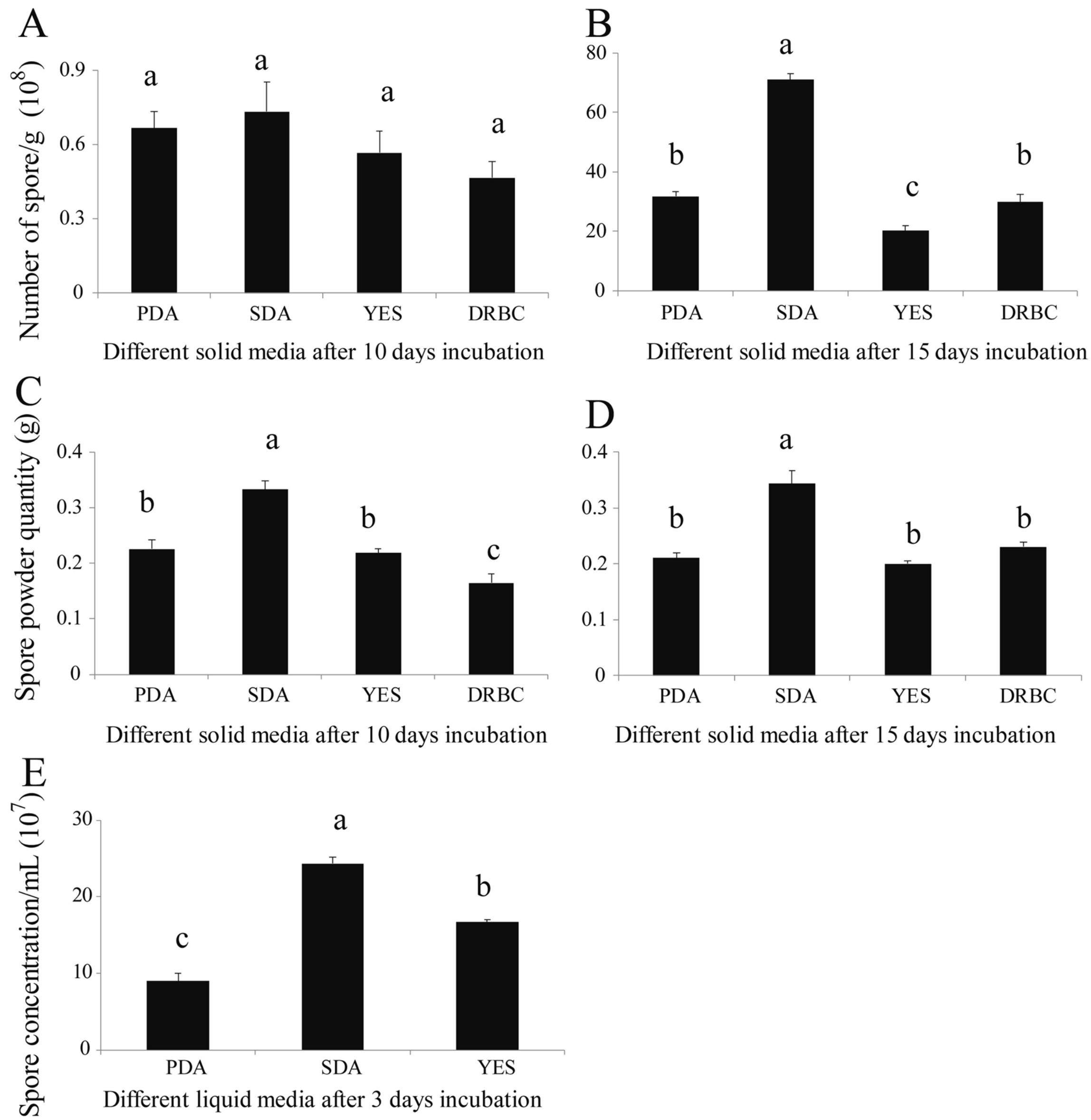

Fig. 2 Effects of various artificial media on sporulation of *Beauveria bassiana*. Bars with the same letters were not significantly in Duncan's test

identified six fungal strains from soil samples, and one of the three *B. bassiana* strains was pathogenic to *M. usitatus*. We found that that all five of the isolates of *B. bassiana* tested in this study were pathogenic to thrips, but only three were highly virulence. Indeed, the strains isolated from soil (rather than host insects) were more virulent to thrips in our study than the one strain isolated from chinch bugs (strain Bbi). The strains tested that had been isolated from the soil caused high mortality to *Galleria mellonella* (L.) (Lepidoptera: Galleriinae: Pyralidae) larvae, which did the Bbi strain from chinch bugs (Baki et al. 2021).

Mass production of entomopathogenic fungi requires media that provide suitable nutritional conditions for the fungal strain being produced. Different fungal strains may vary in their nutritional requirements for growth. Theoretically, diets must be optimized for each strain of entomopathogen (Teja and Rahman 2017). In this study, the *B. bassiana* strains assessed showed different responses to the nutritional compositions of the different media tested. Use of the SDA medium, however, provided the best level of spore yield and the highest conidia concentration per gram of spore powder

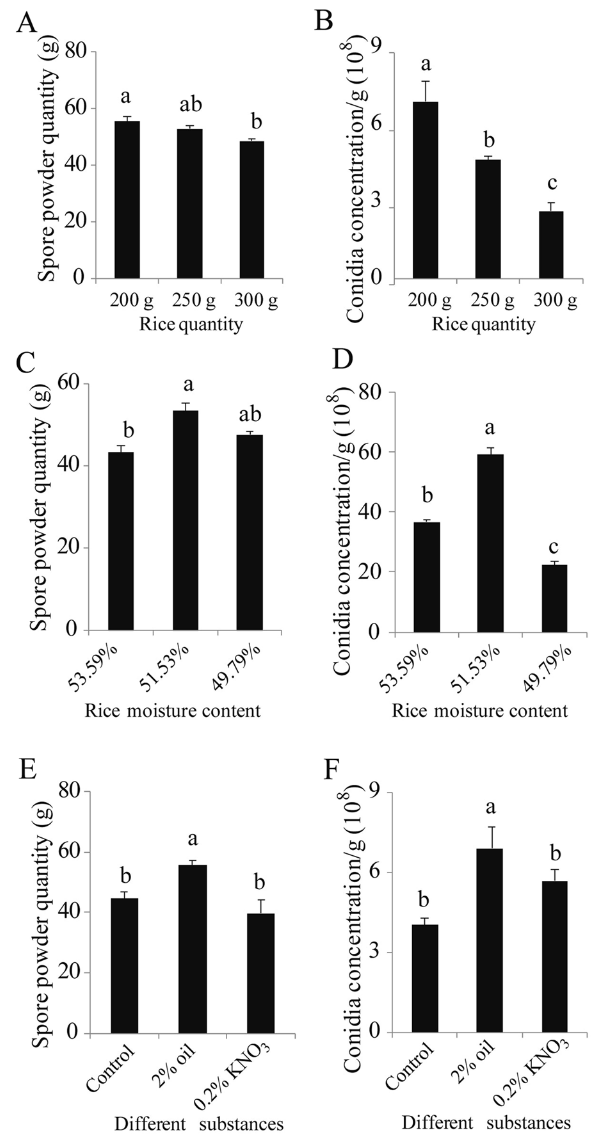

Fig. 3 Effects of rice quantity (**A**, **B**), media moisture level (**C**, **D**), and supplementation with 2% cooking oil or KNO_3 (**E**, **F**) on conidia production. Bars with same letters were not significantly different in Duncan's test

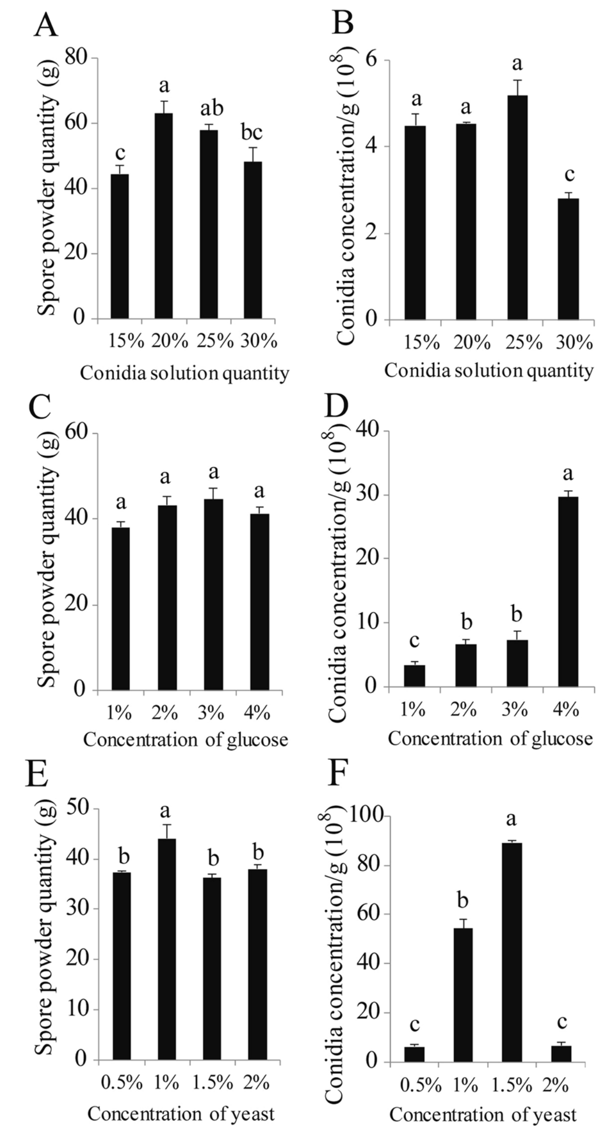

Fig. 4 Effects of conidial solution quantity (**A**, **B**), glucose concentration (**C**, **D**), and yeast concentration (**E**, **F**) in liquid media on spore powder quantity (**A**, **C**, E) and conidial concentration (**B**, **D**, **F**) in the final product. Bars with same letters were not significantly different according to Duncan's test

among the four media tested, making it more economical for fungal production.

Hypoxia is one of the more important environmental stresses affecting the growth and development of fungi (Ortiz-Urquiza and Keyhani 2015). In the mass production of EPF, aeration plays an important role because all monosporic fungi are aerobic and require the oxygen for growth and aerial conidia production (Muñiz-Paredes et al. 2017). With high amounts of rice per rearing container, the circulation of the air is proportionally reduced, which can affect the growth and the conidiation of the fungus. In the current study, it was found that 200 g rice/2.1 l container was the optimal less of rice and more rice per container did not result in proportionally more production of conidia.

The moisture less of the media also affects conidia yield. The optimal moisture level needs to be identified for each production system. Most monosporic fungi prefer humid environments (Sala et al. 2019). The optimum moisture level of the rearing substrate for large-scale production depends not only on the fungal strain

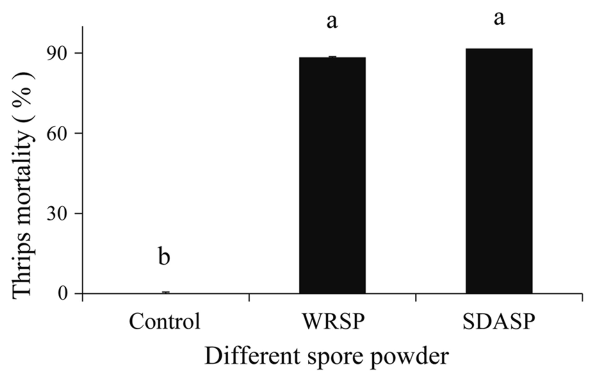

Fig. 5 The mortality (mean ± SE) of thrips caused by the spore powder from white rice and SDA solid media after inoculation 4 days. WRSP: white rice spore powder; SDASP: SDA spore powder. The bars marked with the same letter are not significantly different according to Tukey' test at $P < 0.05$. Bars with different letters are significantly different

Table 1 Corrected mortality rates of bean flower thrips, *Megalurothrips usitatus,* treated with the Dodsda1 strain of *Beauveria bassiana* in a field of cowpeas

Treatments (spores/ml)	Days after application		
	3	5	7
B. bassiana 10^5	14.98b	17.87a	25.13a
B. bassiana 10^6	16.69b	17.50	26.77a
B. bassiana 10^7	31.46ab	36.21a	28.83a
Spinetoram	40.67a	41.67a	34.02a

Values with the same lowercase letters were not significantly difference according to Tukey' test, $a = 0.05$

but also on the type of substrate used (Muñiz-Paredes et al. 2017). In the present study, the optimum moisture level of the rice substrate was 51.5%. Sala et al. (2020) found that 65–70% moisture was optimal for rearing on a rice husk substrate. In other rearing systems 66% moisture was optimal (for wheat) (Nuñez-Gaona et al. 2010) and 40% (for rice) (Pham et al. 2010).

The successful use of any EPF depends on finding an economical method for its mass production (Abreo et al. 2019). In this process, many nutrients might conceivably affect a medium's suitability for growth and sporulation of an EPF. In the present study, spore powder weight and conidia concentration were high when oil was added to the rearing substrate. The addition of oil to the rearing medium not only increased *B. bassiana* sporulation, but also reduced the cost of preparation of the medium.

The carbon/nitrogen balance of a rearing medium has also been reported to affect EPN production (e.g., Cojanu and Luminare 2021). However, in our study, the addition of a nitrogen source (0.2% KNO_3) had no significant effect on *B. bassiana* production.

The two other supplemental dietary materials we tested (glucose and yeast) both increased the sporulation, with 4% glucose and 1.5% yeast being the most beneficial, confirming the general notion that a carbon source can enhance fungal production (e.g., Teja and Rahman 2017).

The quantity of fungal inoculum (i.e., the number of conidia in the solution used to start rearing) also affected the level of sporulation of the entomopathogen we studied, with a 20–25% level of inoculum solution leading to a larger production of spore powder and a high conidia concentration compared to 15 or 30% of inoculum solutions, after 10 days of incubation. However, in another study, Pham et al. (2010) found that after 15 days of incubation, a 10% inoculum solution was optimal.

Conclusions

The five tested strains of *B. bassiana* were all pathogenic to thrips, but three strains were highly virulent. A rice substrate with optimal moisture and supplements of glucose, oil, and yeast was the most suitable for large-scale production of *B. bassiana* spores of the studied strains. The EPN could be used for field control of thrips. Spores produced using the diet and the discussed methods had the same level of virulence to thrips as those cultivated in SDA solid media, and their control effectiveness was close to that of chemical pesticides (Spinetoram) in the field.

Abbreviations

PDA: Potato dextrose agar; SDA: Sabouraud dextrose agar; YES: Yeast extract with supplements; DRBC: Dichloran Rose-Bengal Chloramphenicol; BCM: Carbendazim; DOD: Dodine; WRSP: White rice spore powder; SDASP: SDA spore powder.

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Author contributions

ST and WS were involved in the conceptualization; IC, KC, and RS contributed to the methodology; ST and IC helped in the formal analysis; IC, PW, and RS were involved in the investigation; ST and WS contributed to resources; IC and ST contributed to writing—original draft preparation; ST was involved in the visualization; WS and ST were involved in the supervision; WS contributed to the project administration; WS and ST acquired the funding. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
The manuscript has been approved for publication in ***Egyptian Journal of Biological Pest Control*** after review.

Competing interests
The authors declare no competing interest.

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