ORIGINAL PAPER
Polish Journal of Microbiology
2023, Vol. 72, No 4, 377–389
https://doi.org/10.33073/pjm-2023-033

Biological Characteristics of *Beauveria majiangensis* Strain MJ1015 and Optimization of Solid Medium Technology for Sporulation

XINGHONG WANG[1], ZHENXING HUANG[1], CONGCHUN LI[2], MAN LIU[1]*, GUANGMING YANG[1], LILIN LUO[1], YONGCHAO RAO[1], YAN SHEN[1] and JING WANG[1]

[1] Guizhou Institute of Biology, Guiyang, Guizhou, China
[2] Guangrao Vocational School of Secondary Technical Education, Dongying, China

Submitted 22 May 2023, accepted 24 July 2023, published online 10 October 2023

Abstract

The entomopathogenic fungus *Beauveria majiangensis* strain MJ1015, recently isolated from white grubs on a blueberry farm in Guizhou, China, could be used as a biocontrol agent. As a first step toward determining the effect of different solid culture media, temperature, and pH on colony growth rate and sporulation, we evaluated the optimum solid medium for mycelial growth and conidia production on a commercial scale. Subsequently, we also used single-factor analysis and response surface optimization to optimize the composition of the solid culture medium. On potato dextrose agar (PDA) medium, MJ1015 grew fastest and produced the highest spore yield at 29°C and pH 5. The best solid medium for the growth and sporulation of strain MJ1015 comprised 64.70 g/l of rice, 13.00 g/l of wheat, 0.30 g/l of $NaNO_3$, 0.36 g/l of $K_2HPO_4 \cdot 3H_2O$, and 1.00 g/l of $CaCO_3$. Rice, $NaNO_3$, and $K_2HPO_4 \cdot 3H_2O$ were the main influencing factors. The predicted value of cultured spores using the optimal medium was 4.56×10^{10} conidia/l. The validation test results showed that the average growth rate of strain MJ1015 on the optimal medium was 85% and 96% faster than that on Sabouraud dextrose agar with yeast extracts medium (SDAY) and PDA, respectively. Sporulation was 43.90 times and 9.65 times of that produced on SDAY and PDA, respectively. Our findings provide a theoretical basis for the commercial production of *B. majiangensis* to control white grubs.

K e y w o r d s: *Beauveria*, biocontrol agent, biological characteristics, solid media, growth rate, spore sporulation

Introduction

Entomopathogenic fungi are widely used as biocontrol agents against many insect pests like lepidopteran larvae (Wraight et al. 2010; Fite et al. 2020; Gielen et al. 2022), thrips (Ugine et al. 2005; Bara and Laing 2020) and aphids (Castillo Lopez et al. 2014; Mantzoukas et al. 2022). In 1879, Metchnikoff used *Metarhizium* to control *Anisoplia austriaca*, a pest of cereal crops (Pu and Li 1996), demonstrating that entomopathogenic fungi could be used against white grubs. Subsequently, many studies have focused on the biological characteristics, production, and application of entomopathogenic fungi (mainly *Metarhizium* and *Beauveria*) against white grubs (Erler and Ates 2015; Kim et al. 2020). The use of *Beauveria* to control grubs on peanut (Nong et al. 2011), sugarcane (Visalakshi et al. 2015; Nozipho 2016), maize (Tamayo-Sánchez et al. 2022) and potato (Soni et al. 2018) has achieved remarkable results. Therefore, as an environmentally friendly fungal insecticide, *Beauveria* has a broad application potential and significant commercial value as a biological pest control agent for white grubs.

Beauveria majiangensis (Ascomycota: *Cordycipitaceae*) was first isolated from the larva of *Holotrichia scrobiculata* on a blueberry farm in Guizhou, China (Chen et al. 2018). The fungus shows a high level of infectivity and virulence toward the larvae of three species of beetle: *H. scrobiculata*, *Holotrichia parallela*, and *Oxycetonia bealiae* (Liu et al. 2020). However, not all members are suitable for mycoinsecticide formulations for controlling scarab beetles (Wang et al. 2022). Although *B. majiangensis* has shown potential as a biological control agent and host specificity, further investigations are needed to determine the optimum culture conditions and to identify a suitable growth medium for the commercial-scale production of stable *B. majiangensis* spore powder for the large-scale control of underground pests.

* Corresponding author: M. Liu, Guizhou Institute of Biology, Guiyang, Guizhou, China; **e-mail: freemyself516@hotmail.com**

Liquid fermentation, solid fermentation, and liquid-solid two-phase fermentation systems are used for the large-scale production of biopathogenic fungi. Earlier studies (Ibrahim and Low 1993; Ovruski et al. 2003; Feng et al. 2004) have suggested that fungi could be economically and efficiently mass-produced on different solid substrates, such as sorghum, rice, rice straw, wheat, wheat bran or Italian millet (Sikura and Primak 1970; Ibrahim and Low 1993; Machado et al. 2010; Kankale et al. 2017; Hassan et al. 2020; Yu et al. 2020), to produce stable and easily preserved conidia (Pandey 1994; Soccol et al. 2017). Therefore, the solid fermentation method became the medium of choice in the mass production of *Beauveria*. A strain's growth rate and sporulation yield differ on different culture substrates (de Farias et al. 2010; Bhadauria et al. 2012; Ibrahim et al. 2015; Song et al. 2019). Similarly, the sporulation yield of different strains on the same solid medium also differs (Goffré et al. 2018; Song et al. 2019). Optimization studies are therefore needed to establish the optimal production conditions by different strains.

In this study, the biological characteristics of *B. majiangensis* strain MJ1015 were studied, and colony growth and conidial production on different solid media were assessed to provide a theoretical basis for expanding this research into other fields and for the large-scale industrial production of this strain.

Experimental

Materials and Methods

Test strain. *B. majiangensis* strain MJ1015 was isolated from the larva of *H. scrobiculata* on a blueberry farm in Guizhou, China (Chen et al. 2018), and is stored in the Species Preservation Room at Guizhou Institute of Biology (preservation number CGMCC No. 15090).

Preparation of spore suspension. Aerial conidia of MJ1015 were obtained from a stock culture growing on a potato dextrose agar (PDA) plate by suspending them in sterile water containing 0.05% Tween-80. The number of conidia in the suspension was counted using a hemocytometer (Yue Cheng Trading Co., Ltd., China) and then diluted to 1.0×10^{10} conidia/l with 0.05% Tween 80 for use in subsequent experiments.

Inoculation and cultivation of strain MJ1015. Media plates were inoculated in the center with 1 µl of spore suspension using a pipette and then incubated in the dark in a climate chamber with 90 ± 5% relative humidity for 14 days. The colony growth rate and spore production were measured.

Effect of different media on colony growth rate and sporulation. To assess the effects of different media on colony growth rate and sporulation, four different media were inoculated with MJ1015 spore suspension: Sabouraud dextrose agar with yeast extract (SDAY), which comprised 10 g/l of tryptone, 40 g/l of dextrose, 20 g/l of agar, 10 g/l of yeast extract, and, with a pH 6.0 ± 0.1; PDA medium; ¼ Sabouraud dextrose agar with yeast extract (¼SDAY, 2.5 g/l of tryptone, 10 g/l of dextrose, 20 g/l of agar, 5 g/l of yeast extract); and base medium (CZM), which comprised 30 g/l of dextrose, 2 g/l $NaNO_3$, 0.5 g/l $MgSO_4 \cdot 7H_2O$, 1 g/l K_2HPO_4, 0.5 g/l KCl, 0.001 g/l $FeSO_4 \cdot 7H_2O$, 20 g/l of agar. The inoculated plates were cultivated in a climate chamber at 26.5 ± 1°C, with at least three replications of each treatment.

Effect of different temperatures on colony growth rate and sporulation. To assess the effects of different temperatures on colony growth rate and sporulation, plates of PDA medium were inoculated with spore suspension and then incubated in the climate chamber at 20 ± 1°C, 23 ± 1°C, 26 ± 1°C, 29 ± 1°C, or 30 ± 1°C, with at least three replications of each temperature treatment.

Effect of pH on colony growth rate and sporulation. To assess the effects of different pH values on colony growth rate and sporulation, plates of PDA medium were prepared with pH values adjusted to pH 4, 5, 6, 7, 9, 11, or 13 with 0.1 mol/l HCl or 0.1 mol/l NaOH under sterile conditions. The PDA plates were inoculated with spore suspension and cultured at 29°C, with at least three replications of each pH value.

Solid medium single-factor screening. ***Carbon source.*** To assess the effects of media with different carbon sources on colony growth rate and sporulation, the 40 g/l of glucose in SDAY medium was replaced with 40 g/l of sucrose, fructose, brown sugar, corn meal, corn cob meal, wheat bran, wheat meal, rice stalk meal, bran, rice meal, potato meal, or sweet potato meal, or with a mixture of rice and wheat (1 : 1 mass ratio). The plates were inoculated with spore suspension and cultured at 29°C. SDAY was used as a control. Each medium treatment comprised at least three replications, and three solid medium plates were used for each biological replicate experiment.

Proportion of rice and wheat. To assess the effects of media with different proportions of rice and wheat as the carbon source on colony growth rate and sporulation, the 40 g/l of glucose in SDAY medium were replaced with rice and wheat mass ratios of 1 : 1, 2 : 1, 3 : 1, 5 : 1, 7 : 1, 9 : 1, 1 : 2, 1 : 3, 1 : 5, 1 : 7, or 1 : 9. Plates of yeast extract plus tryptone, rice or wheat as the carbon source and SDAY were used as control groups. The culture conditions and the number of replicates were the same as those used in the carbon source screening experiment.

Nitrogen source. To assess the effects of different nitrogen sources on colony growth rate and sporulation, plates of medium were prepared with a 2 : 1 ratio of

Table I
Range of different factors investigated with Plackett-Burman design.

Symbol	Variables	Experimental value	
		Low (−1)	High (+1)
X_1	Rice (g/l)	27	33.75
X_2	Wheat (g/l)	13	16.25
X_3	Virtual 1	−1	1
X_4	$NaNO_3$ (g/l)	1	1.25
X_5	$CaCO_3$ (g/l)	1	1.25
X_6	Virtual 2	−1	1
X_7	$K_2HPO_4 \cdot 3H_2O$ (g/l)	1	1.25
X_8	Virtual 3	−1	1

rice and wheat as the carbon source and 20 g/l of yeast extract, yeast paste, peanut powder, cottonseed powder, silkworm pupal powder, soybean powder, or fish meal, or 1 g/l of ammonium sulfate, sodium nitrate, or urea. Plates of SDAY and rice: wheat ratio of 2 : 1 as the carbon source without the nitrogen source was used as control groups. The culture conditions and the number of replicates were the same as those used in the carbon source screening experiment.

Inorganic salt. To assess the effects of different inorganic salts on colony growth rate and sporulation, agar plates were prepared with rice: wheat ratio of 2 : 1 and $NaNO_3$ as the carbon and nitrogen source, respectively. To this medium, 1 g/l of KH_2PO4, $K_2HPO_4 \cdot 3H_2O$, NaH_2PO_4, KNO_3, $MgSO_4 \cdot 7H_2O$, $CaSO_4 \cdot 2H_2O$, $ZnSO_4$, $FeSO_4$, $MgCl_2 \cdot 6H_2O$, KCl, $CaCl_2$, NaCl, or $CaCO_3$ were added. Medium without the addition of an inorganic salt acted as the control group. The culture conditions and the number of replicates were the same as those used in the carbon source screening experiment.

Plackett-Burman design. Based on the results of the single-factor screening experiments, we used a Plackett-Burman design ($N = 12$) to assess five factors: two carbon sources, a nitrogen source, and two inorganic salts. Three dummy variables were used to estimate the error. For each factor, two levels were assessed: high (+1) and low (−1) (Table I). The high level was 1.25 times that of the low level. Colony growth rate and sporulation measurements were taken as response value *Y*. The design code and the coding values are shown in Table I. The culture conditions and the number of replicates were the same as those used in the carbon source screening experiment.

Steepest ascent path. Based on the main factors promoting sporulation identified by the Plackett-Burman method, the steepest ascent method was designed using rice, $NaNO_3$, and $K_2HPO_4 \cdot 3H_2O$. The step size and the direction of each significant factor were determined by the coefficient of sporulation regression equation of the Plackett-Burman design model. The culture conditions and the number of replicates were the same as those used in the carbon source screening experiment.

Response surface optimization experiment. Based on our analysis of the significance factors and levels obtained using the Plackett-Burman test and steepest ascent method, we performed a response surface analysis experiment comprising three factors and five levels on a solid medium, which was designed using a central combination experimental design. The design is shown in Table II. The culture conditions and the number of replicates were the same as those used in the carbon source screening experiment.

Statistical analysis. Colony growth rate (mm/d) and sporulation (conidia/l) data are presented as means ± the standard error (SE) and were statistically analyzed by performing a one-way analysis of variance (ANOVA) or Welch's ANOVA using SPSS 21.0. SigmaPlot 14 software was used to plot the experimental results. Calculation of growth rate – colony growth diameter was measured with vernier caliper crossovers after 14 days of culture and recorded:

$$Colony\ growth\ rate\left(\frac{mm}{d}\right) = \frac{Colony\ growth\ diameter\ (mm)}{culture\ time\ (d)}$$

Calculation of spore number – the sterilized hole punch of 1 cm^2 was used to take bacteria cakes from the center of the colony ½ distance from the edge, and three bacteria cakes were taken from each treatment. The cakes were placed in 20 ml sterile water containing 0.05% Tween-80 and fully oscillated and mixed on the vortex oscillator to make spore suspension.

Table II
Factors and levels of response surface central composite design.

Symbol	Variables	Code level				
		−1.6828	−1	0	1	1.6818
A	Rice (g/l)	58.4489	60.75	64.125	67.50	69.8011
B	$NaNO_3$ (g/l)	0.1755	0.23	0.31	0.39	0.44459
C	$K_2HPO_4 \cdot 3H_2O$ (g/l)	0.2389	0.29	0.365	0.44	0.49119

The spore concentration was measured with the blood cell counting plate and the number of spores (spores/l) was calculated:

$$\frac{Spore\ number}{l} = \frac{N}{5} \times 25 \times 10 \times 10^6 \times dilution\ ratio$$

where N is the total number of spores in the 5 squares in the middle.

Results

Effect of different media on colony growth rate and sporulation. The colony growth rate of strain MJ1015 on PDA was significantly faster (3.37 mm/d; Fig. 1a), and sporulation was significantly higher (7.73×10^{10} conidia/l; Fig. 1b) than on the other three media. Although there was no significant difference in

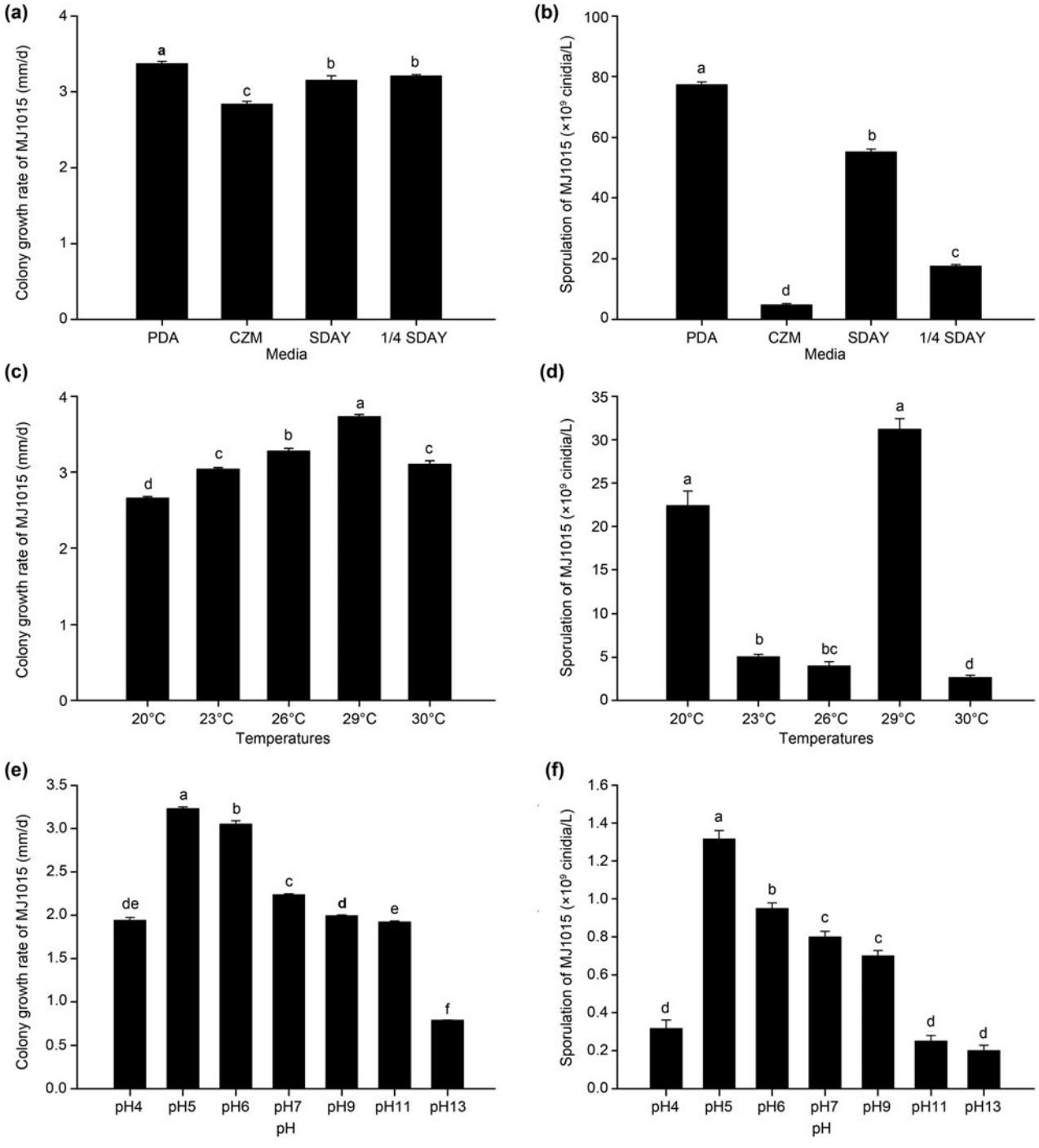

Fig. 1. Effect of different culture conditions on colony growth rate and sporulation. Effect of different media (a–b), temperatures (c–d), and pH values (e–f) on colony growth rate and sporulation of *Beauveria majiangensis* strain MJ1015. Values shown are means ± the standard error. Different lowercase letters indicate significant differences between values ($p < 0.05$).

PDA – potato dextrose agar medium, CZM – base medium, SDAY – Sabouraud dextrose agar with yeast extract medium, ¼ SDAY – ¼ Sabouraud dextrose agar with yeast extract medium.

the colony growth rate on SDAY and ¼ SDAY, sporulation was significantly higher on SDAY than on ¼ SDAY. The colony growth rate on CZM was significantly slower (2.84 mm/d), and sporulation was significantly lower (4.80×10^9 conidia/l) than on the other media.

Effect of different temperatures on colony growth rate and sporulation. Colony growth and sporulation were observed under all the different temperature treatments (i.e., from 20°C to 30°C; Fig. 1c). The growth rate increased with increasing temperature up to 29°C. The optimal temperature for mycelial growth was 29°C and spore production was significantly higher than at 23°C, 26°C, or 30°C, but was not significantly different from that at 20°C (Fig. 1d). Moreover, the lowest level of sporulation occurred at 30°C, and the growth rate was significantly lower than at 26°C or 29°C.

Effects of different pH on colony growth rate and sporulation. Colony growth and sporulation were observed under all the different pH treatments (i.e., from pH 4 to 13; Fig. 1e and 1f). However, the optimal pH for mycelial growth (3.23 mm/d) and sporulation (1.32×10^9 conidia/l) was pH 5.

Effect of different carbon sources on colony growth rate and sporulation. When we replaced the 40 g/l of dextrose in the SDAY medium with one of 13 different carbon sources, the colony growth rate of strain MJ1015 was either not significantly different or was better than that on SDAY (Fig. 2a and 2b). It appears that the growth rate on a 1:1 ratio of rice and wheat (3.26 ± 0.03 mm/d) was not significantly different from that of corncob, bran flour, wheat, rice straw powder, chaff, rice, and potato. Moreover, a 1 : 1 ratio of rice and wheat was the optimal carbon source for promoting sporulation ($(4.11 \pm 0.22) \times 10^9$ conidia/l), significantly higher than that produced on media with other carbon sources.

Effect of different proportions of rice and wheat on colony growth rate and sporulation. To determine the optimal proportion of rice and wheat in the medium for colony growth and sporulation, we assessed 11 different rice: wheat ratios (Fig. 2c and 2d). A significantly higher growth rate (3.02 ± 0.03 mm/d) and greater sporulation ($(1.66 \pm 0.02) \times 10^{10}$ conidia/l) were achieved on medium with rice: wheat ratio of 2 : 1 compared with the other treatments.

Effect of different nitrogen sources on colony growth rate and sporulation. When we replaced the yeast extract powder and tryptone in the SDAY medium with one of 11 different nitrogen sources, the colony growth rate of strain MJ1015 was significantly better than that on SDAY (Fig. 2e and 2f). When strain MJ1015 was grown on media containing $NaNO_3$, the mycelial growth rate (3.81 ± 0.04 mm/d) was significantly faster, and sporulation was significantly higher ($(25.83 \pm 0.80) \times 10^9$ conidia/l) than on other media, suggesting that $NaNO_3$ was the optimal nitrogen source for the growth and sporulation of strain MJ1015.

Effect of inorganic salts on colony growth rate and sporulation. When strain MJ1015 was grown on media containing rice: wheat ratio of 2 : 1, $NaNO_3$, and either 0.10% $K_2HPO_4 \cdot 3H_2O$ or $CaCO_3$, the average mycelial growth rate (4.34 ± 0.03 mm/d or 4.12 ± 0.04 mm/d, respectively) was significantly higher than that on other media (Fig. 2g and 2h). In addition, significantly higher sporulation ($(41.01 \pm 1.56) \times 10^9$ conidia/l) was achieved on media containing $CaCO_3$ than on other media.

Plackett-Burman design. Based on the test results shown in Table III, multiple regression equation fitting and ANOVA of sporulation by MJ1015 colonies were performed using Design-Expert 12 software (Table IV). The regression equation for colony sporulation was:

$$Y_{(\ln(\text{sporulation}))} = 22.64 + 0.7365 \times X_1 + 0.0313 \times X_2 - \\ - 0.4789 \times X_4 + 0.0448 \times X_5 - 0.4468 \times X_7$$

Regression analysis showed that the Plackett-Burman model (Table IV) was highly significant ($p < 0.01$), indicating that the model could be used to analyze significant factors of *B. majiangensis* MJ1015 sporulation. The results of the regression analysis (Table IV) showed that rice was positively correlated with sporulation ($p < 0.01$), increasing its content promoted the sporulation of strain MJ1015, whereas $NaNO_3$ and $K_2HPO_4 \cdot 3H_2O$ were negatively correlated with sporulation ($p < 0.05$). Increasing the $NaNO_3$ and $K_2HPO_4 \cdot 3H_2O$ content of solid media could inhibit sporulation. However, the other factors were not significant (Table IV). Because the added reagents did not affect the colony growth rate in our laboratory, the colony growth rate was not analyzed here or in subsequent experiments.

Steepest ascent path. Steepest ascent tests of rice, $NaNO_3$, and $K_2HPO_4 \cdot 3H_2O$ were carried out with sporulation as the main factor from Plackett-Burman result. Based on the coefficient of the sporulation regression equation of the Plackett-Burman design model, the step size and direction of each significant factor were determined, as shown in Table V. Colony spore production peaked at the "origin + 10Δ" factor level and then decreased. The response interval of the maximum value was around factor level 10, which was selected as the center point.

Response surface optimization experiment. According to the Plackett-Burman test results and steepest ascent path, a three-factor and five-level response surface analysis was conducted on rice, $NaNO_3$ and $K_2HPO_4 \cdot 3H_2O$ using a central combination design. The central combination design and its levels are shown in Table II, and the experimental results are shown in Table VI.

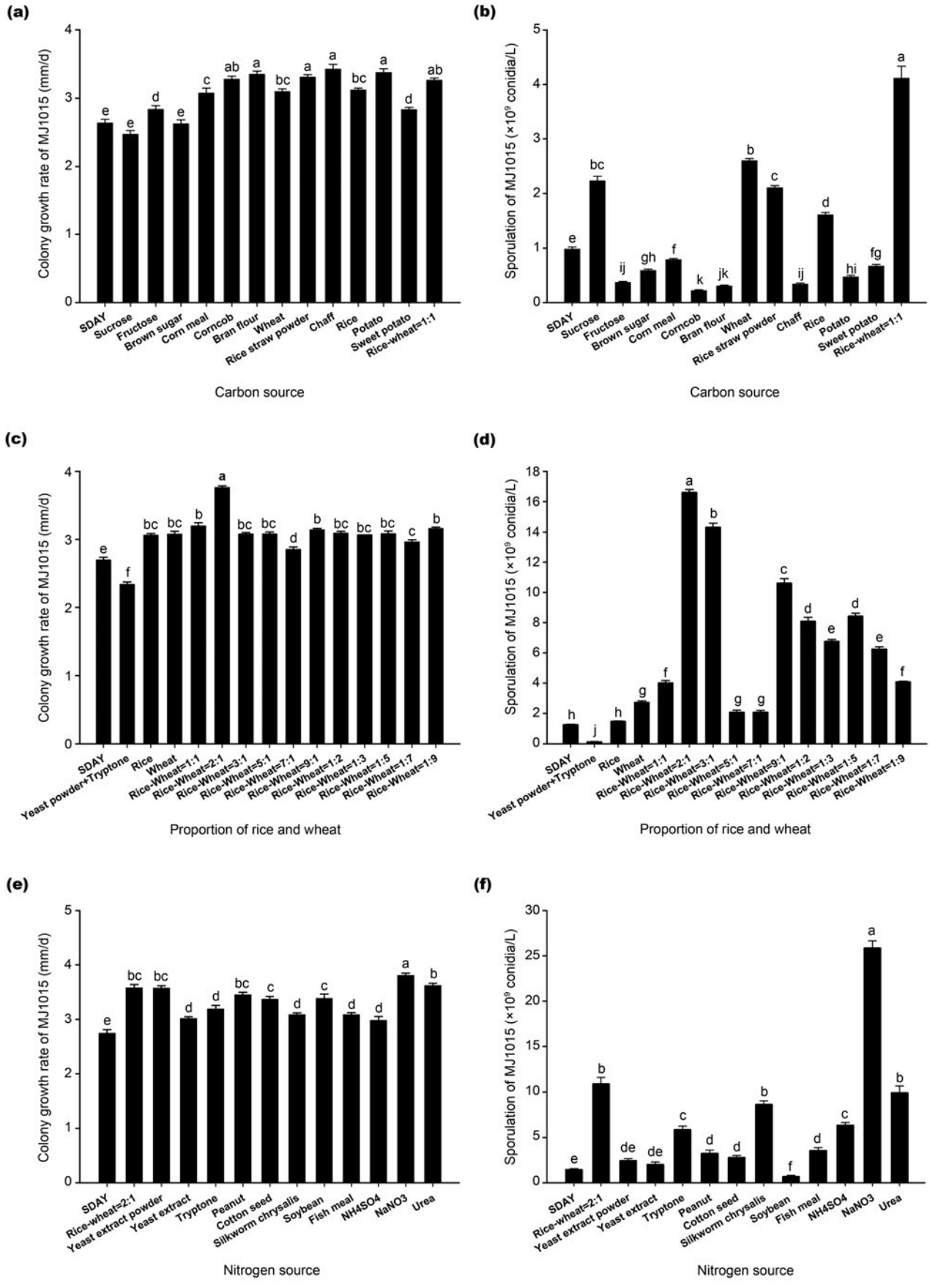

Fig. 2 a–f. Solid medium single-factor screening. Effect of different carbon sources (a–b), proportions of rice and wheat (c–d), nitrogen sources (e–f), and inorganic salts (g–h) on colony growth rate and sporulation of *Beauveria majiangensis* strain MJ1015. Values shown are means ± the standard error. Different lowercase letters indicate a significant difference between values ($p < 0.05$).

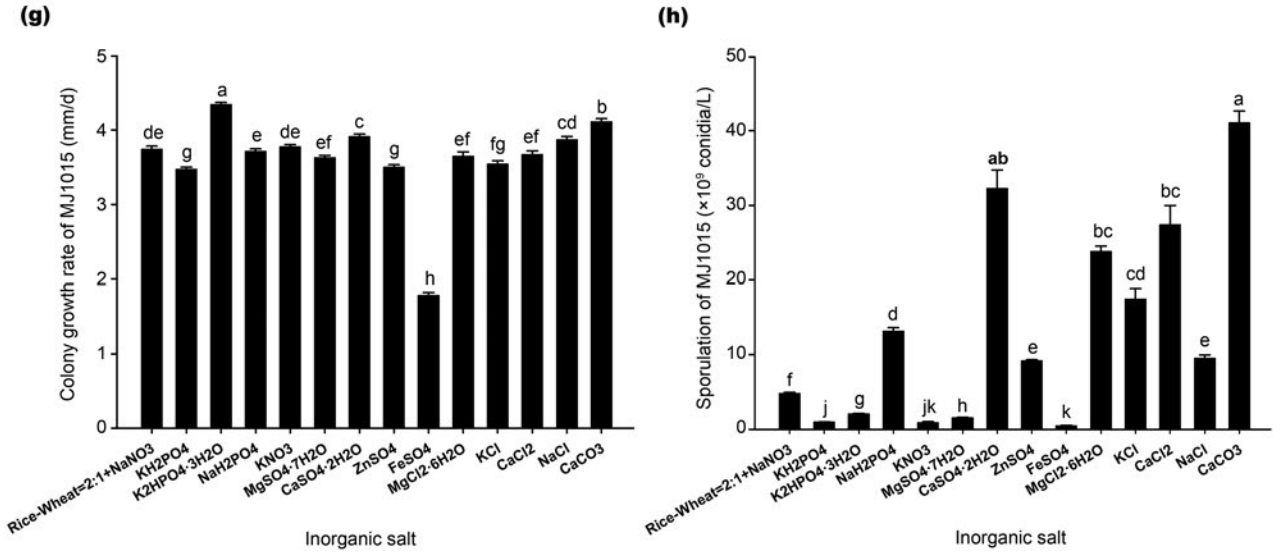

Fig. 2 g–h. Solid medium single-factor screening. Effect of different carbon sources (a–b), proportions of rice and wheat (c–d), nitrogen sources (e–f), and inorganic salts (g–h) on colony growth rate and sporulation of *Beauveria majiangensis* strain MJ1015. Values shown are means ± the standard error. Different lowercase letters indicate a significant difference between values ($p < 0.05$).

Multiple regression equation fitting and ANOVA with Table VI test data using Design-Expert 12 software yielded the regression equation:

$$Y_{(\text{Sporulation (conidia/l)})} = (4.508 + 0.246 \times A - 0.303 \times B - \\ - 0.093 \times C - 0.045 \times AB - 0.130 \times AC - \\ - 0.020 \times BC - 0.769 \times A^2 - 0.964 \times B^2 - \\ - 0.700 \times C^2) \times 10^{10}$$

The regression equation results (Table VII) revealed that the regression model has relevance ($p < 0.0001$) and that the model has a high goodness of fit value ($R^2 = 0.9348$), indicating that the model is a good reflection of the relationship between the investigated factors and response values. The colony spore yield lack of fit was not significant ($p = 0.6030$), indicating that the model did not lose fit; that is, the modified model could be used for the theoretical prediction of solid spore yield optimization of strain MJ1015. According to the response surface diagrams showing the effects of rice (A), $NaNO_3$ (B), and $K_2HPO_4 \cdot 3H_2O$ (C), on sporulation (Fig. 3), A, B, and C have maximum values.

Table III
Plackett-Burman design and response values.

Order	Variable code								Y Sporulation (conidia/l) 1×10^9
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	
1	1	1	−1	1	1	1	−1	−1	22.370 ± 1.833
2	−1	1	1	−1	1	1	1	−1	2.860 ± 0.282
3	1	−1	1	1	−1	1	1	1	4.267 ± 0.466
4	−1	1	−1	1	1	−1	1	1	0.923 ± 0.117
5	−1	−1	1	−1	1	1	−1	1	9.043 ± 1.283
6	−1	−1	−1	1	−1	1	1	−1	1.487 ± 0.289
7	1	−1	−1	−1	1	−1	1	1	33.453 ± 3.836
8	1	1	−1	−1	−1	1	−1	1	30.840 ± 2.155
9	1	1	1	−1	−1	−1	1	−1	11.113 ± 0.934
10	−1	1	1	1	−1	−1	−1	1	5.773 ± 0.636
11	1	−1	1	1	1	−1	−1	−1	6.950 ± 0.865
12	−1	−1	−1	−1	−1	−1	−1	−1	5.650 ± 1.211
13	0	0	0	0	0	0	0	0	10.628 ± 1.353
14	0	0	0	0	0	0	0	0	13.027 ± 1.477
15	0	0	0	0	0	0	0	0	19.129 ± 1.886

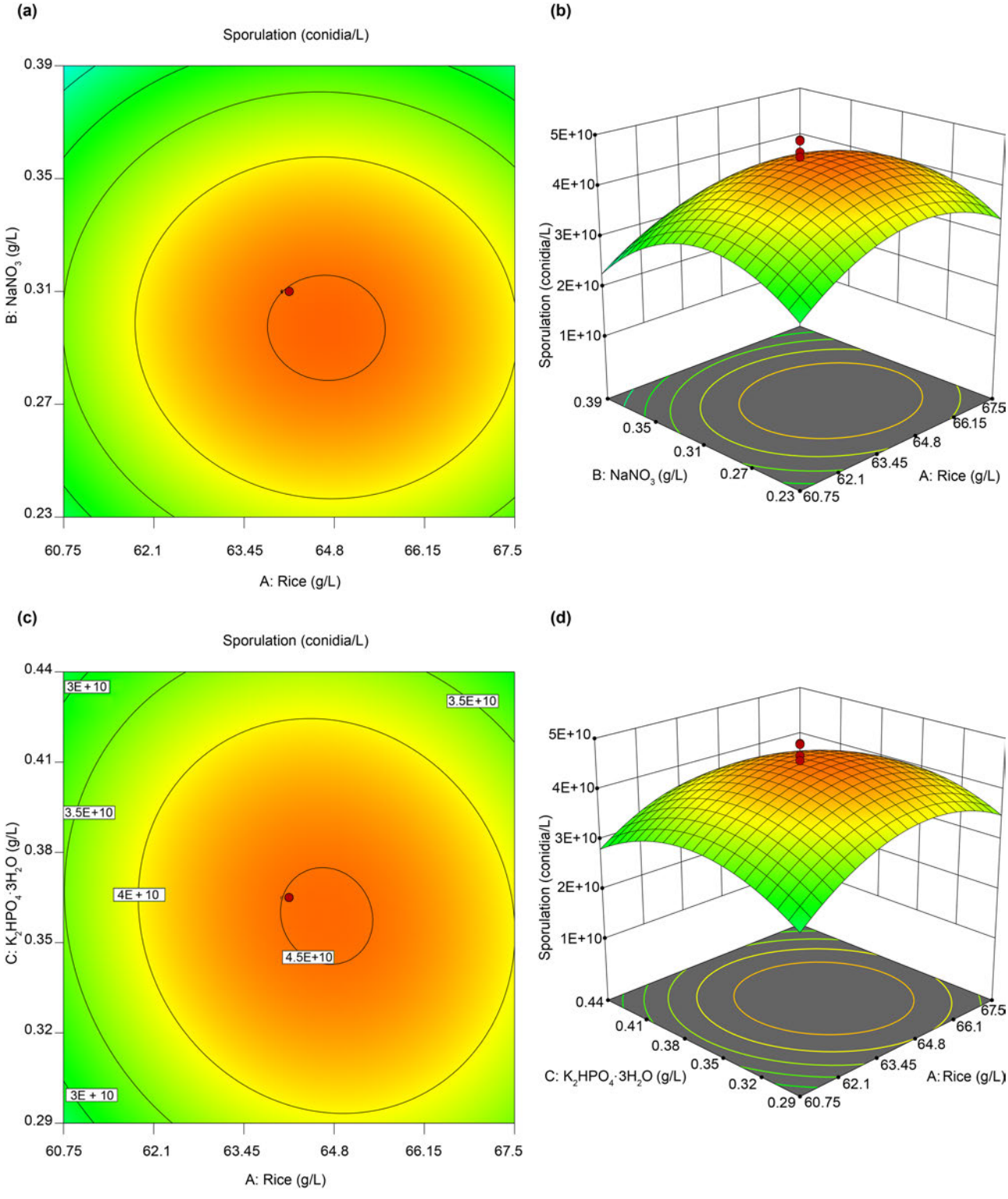

Fig. 3 (a–d). Response surface diagrams showing the effects of rice and $NaNO_3$ (contour (a) and 3D plots (b)), rice and $K_2HPO_4 \cdot 3H_2O$ (contour (c) and 3D plots (d)), $NaNO_3$ and $K_2HPO_4 \cdot 3H_2O$ (contour (e) and 3D plots (f)) on the sporulation of *Beauveria majiangensis* strain MJ1015.

In summary, based on changes in sporulation with nutrient composition, combined with response surface optimization experiments, Design-Expert 12 software was used to analyze spore production as the primary response value and indicated that the optimal medium conditions for MJ1015 sporulation were 64.70 g/l of rice, 0.30 g/l of $NaNO_3$, and 0.36 g/l of $K_2HPO_4 \cdot 3H_2O$. The spore yield of strain MJ1015 when grown on the optimal medium, was predicted to be 4.56×10^{10} conidia/l.

Validation test. The average growth rate of strain MJ1015 on the optimized solid phase medium was (4.46 ± 0.04) mm/d, 85% and 96% faster than that on

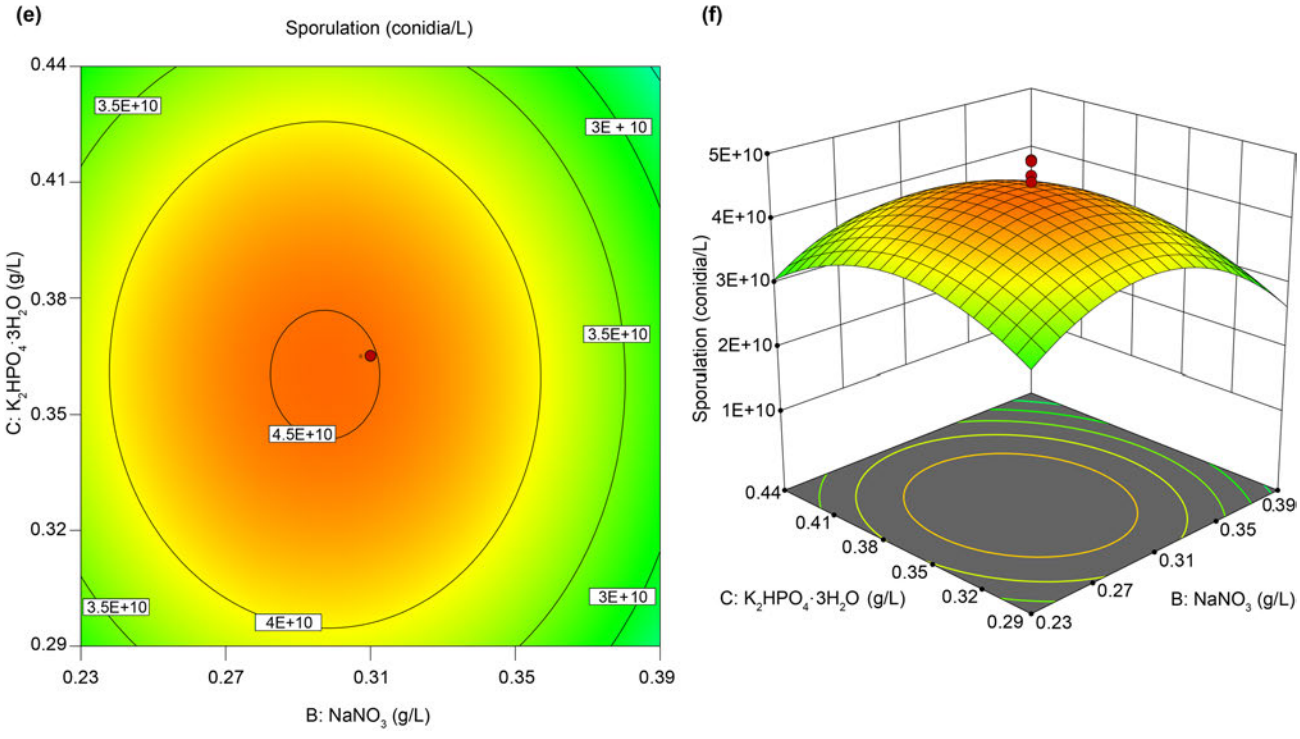

Fig. 3 e–f. Response surface diagrams showing the effects of rice and $NaNO_3$ (contour (a) and 3D plots (b)), rice and $K_2HPO_4 \cdot 3H_2O$ (contour (c) and 3D plots (d)), $NaNO_3$ and $K_2HPO_4 \cdot 3H_2O$ (contour (e) and 3D plots (f)) on the sporulation of *Beauveria majiangensis* strain MJ1015.

SDAY and PDA, respectively. Furthermore, the sporulation of strain MJ1015 ($(4.54 \pm 0.16) \times 10^{10}$ conidia/l) was 43.90 times and 9.65 times that on SDAY and PDA, respectively.

Discussion

Entomopathogenic fungi have been reported to tolerate a wide range of temperatures (i.e., 0 ~ 40°C), with most entomopathogenic fungi showing optimal growth and sporulation at approximately 20 ~ 30°C (Ibrahim and Low 1993; Vega and Kaya 2012; Tumuhaise et al. 2018). This study showed that a temperature of 29°C is most conducive for *B. majiangensis* MJ1015 colony growth and sporulation. Our study supports previous reports that growth rates depend on the fungal isolate (genotype) and temperature. The colony growth rate and the number of spores produced by a fungus are not necessarily related (Safavi et al. 2007; Rangel et al. 2008; Campos-Esquivel et al. 2022), which was also confirmed by our study. Colonies that grew the fastest did not necessarily produce the most spores. The number of conidia produced when grown at 20°C did not differ significantly from that produced at 29°C even though the colony growth rate at 20°C was significantly slower than at 29°C. This may indicate that MJ1015 performed asexual reproduction to produce

Table IV
Results of regression analysis of Plackett-Burman design.

Source	Factor	*df*	Sporulation (*Y*)			
			Sum of squares	Mean square	*F*-value	*p*-value
Model		5	11.69	2.34	7.84	0.0060**
X_1	0.7365	1	6.51	6.51	21.81	0.0016**
X_2	0.0313	1	0.0117	0.0117	0.0393	0.8477
X_4	−0.4789	1	2.75	2.75	9.22	0.0162*
X_5	0.0448	1	0.0241	0.0241	0.0807	0.7836
X_7	−0.4468	1	2.40	2.40	8.03	0.0220*
Residual		8	2.39	0.2985		
Cor total		14	15.30			

* – indicates significant ($p < 0.05$), ** – indicates very significant ($p < 0.001$)

Table V
Experimental design and results of steepest ascent path.

Level of factor	Factor coded value			Factor actual value			Sporulation (conidia/l) 1×10^9
	Rice	$NaNO_3$	$K_2HPO_4 \cdot 3H_2O$	Rice	$NaNO_3$	$K_2HPO_4 \cdot 3H_2O$	
Step lengthΔ	1	0.125	0.125	3.375	0.125	0.125	n.d.
Origin	0	0.000	0.000	30.375	1.125	1.125	10.627 ± 0.342
Origin + 1Δ	1	0.125	0.125	33.750	1.044	1.049	9.043 ± 0.532
Origin + 2Δ	2	0.250	0.250	37.125	0.962	0.973	5.273 ± 0.136
Origin + 3Δ	3	0.375	0.375	40.500	0.881	0.898	4.837 ± 0.229
Origin + 4Δ	4	0.500	0.500	43.875	0.800	0.822	1.880 ± 0.218
Origin + 5Δ	5	0.625	0.625	47.250	0.719	0.746	0.730 ± 0.063
Origin + 6Δ	6	0.750	0.750	50.625	0.637	0.670	0.620 ± 0.008
Origin + 7Δ	7	0.875	0.875	54.000	0.556	0.594	29.487 ± 0.546
Origin + 8Δ	8	1.000	1.000	57.375	0.475	0.518	28.773 ± 0.540
Origin + 9Δ	9	1.125	1.125	60.750	0.393	0.443	35.627 ± 1.806
Origin + 10Δ	10	1.250	1.250	64.125	0.312	0.367	47.587 ± 4.056
Origin + 11Δ	11	1.375	1.375	67.500	0.231	0.291	9.917 ± 0.451
Origin + 12Δ	12	1.500	1.500	70.875	0.150	0.215	2.420 ± 0.079
Origin + 13Δ	13	1.625	1.625	74.250	0.068	0.139	3.967 ± 0.138

offspring when grown under the harsher environment of the 20°C climate chamber. This finding should help to guide the production of MJ1015 and its use in the field. Previous reports have shown that different *Beauveria* species have different pH tolerance ranges, such as 5–6 (Sanzhimitupova 1980), 6–8 (Galani 1988), as well as 10 and above (Shimazu and Sato 1996). An acidic pH (i.e., pH 5) was more suitable for the growth and sporulation of strain MJ1015, unlike *Beauveria bassiana*, which shows optimal growth and spore in media with a pH of 6 to 8 (Karthikeyan et al. 2008). However, the optimum pH for maximal growth of three other *B. bassiana* stains (i.e., BbR1, BbR2, Bbr3) was found to be 6 to 7 (Dhar et al. 2016). A preference for an acidic environment might reflect that *B. majiagensis* strain MJ1015 was isolated from blueberry grubs and that the biological characteristics of *Beauveria* isolated from different geographical sources and hosts are different.

Although entomopathogenic fungi can grow and sporulate on different media, the utilization rate of different media depends on the fungal species (Lin et al. 1988; Aregger 1992). Bhadauria et al. (2012) used the mass ratio method to evaluate 15 different grains as a solid medium for the mass production of *B. bassiana*, with fungal dry weights of 0.38 and 0.56 g/100 g obtained on wheat and rice. Ibrahim et al. (2015) used the same method to show that the dry weight of *B. bassiana* per 100 g of substrate varied greatly, with 20.7, 5.0, and 1.3 g/100 g recorded when grown on burghul, rice, or wheat, respectively. After 14 days of culture, Pham et al. (2010) observed that *B. bassiana* KK5 conidial production was greatest when steamed rice was used as a substrate (2.69 g conidia/100 g substrate), followed by brown rice (1.43 g conidia/100 g substrate).

Table VI
Response surface central composite design and corresponding results.

Run	Coded variable level			*Y* Sporulation (conidia/l) 1×10^9
	A	*B*	*C*	
1	−1	−1	−1	1.95 + 0.108
2	1	−1	−1	2.75 + 0.085
3	−1	1	−1	1.60 + 0.070
4	1	1	−1	2.34 + 0.098
5	−1	−1	1	1.75 + 0.060
6	1	−1	1	2.16 + 0.117
7	−1	1	1	1.45 + 0.062
8	1	1	1	1.55 + 0.076
9	−1.6818	0	0	2.13 + 0.061
10	1.6818	0	0	2.91 + 0.077
11	0	−1.6818	0	2.70 + 0.102
12	0	1.6818	0	1.23 + 0.038
13	0	0	−1.6818	2.58 + 0.076
14	0	0	1.6818	2.85 + 0.059
15	0	0	0	4.90 + 0.077
16	0	0	0	4.88 + 0.088
17	0	0	0	4.67 + 0.119
18	0	0	0	4.25 + 0.110
19	0	0	0	4.57 + 0.085
20	0	0	0	3.70 + 0.082

Table VII
ANOVA for response surface quadratic polynomial model.

Source	*df*	Sporulation (*Y*)				
		Sum of squares	Mean square	*F*-value	*p*-value	
Model	9	2.668×10^{21}	2.965×10^{20}	15.93	<0.0001	significant
A-Rice	1	8.275×10^{19}	8.275×10^{19}	4.450	0.0612	
B-$NaNO_3$	1	1.256×10^{20}	1.256×10^{20}	6.750	0.0266	
C-$K_2HPO_4\cdot3H_2O$	1	1.180×10^{19}	1.180×10^{19}	0.6336	0.4445	
AB	1	1.650×10^{18}	1.650×10^{18}	0.0886	0.7720	
AC	1	1.343×10^{19}	1.343×10^{19}	0.7126	0.4155	
BC	1	3.335×10^{17}	3.335×10^{17}	0.0179	0.8962	
A^2	1	8.529×10^{20}	8.529×10^{20}	45.810	<0.0001	
B^2	1	1.339×10^{21}	1.339×10^{21}	71.900	<0.0001	
C^2	1	7.069×10^{20}	7.069×10^{18}	37.970	0.0001	
Residual	10	1.862×10^{20}	1.862×10^{19}			
Lack of fit	5	8.170×10^{19}	1.634×10^{19}	0.7821	0.6030	not significant
Pure error	5	1.045×10^{20}	2.089×10^{19}			
Cor Total	19	2.855×10^{21}				
R^2		0.9348				
Adjusted R^2		0.8761				

Table VIII
Response surface optimization results validation.

Medium	Colony growth (mm/d)	Sporulation (conidia/l) 1×10^{10}
New medium	4.46 ± 0.04	4.54 ± 0.16
SDAY	2.41 ± 0.03	0.10 ± 0.02
PDA	2.28 ± 0.03	0.47 ± 0.04

SDAY – Sabouraud dextrose agar with yeast extract medium,
PDA – potato dextrose agar medium

However, rice husks were not found to be suitable for producing *B. bassiana* KK5 conidia. Some researchers use a method of spore counting to compare the effect of different media on the growth or sporulation of strains. According to Feng et al. (2000), conidial production of *Verticillium lecanii* on cooked rice and rice bran solid medium was 1.5×10^9 and 1.4×10^9 conidia/g, respectively, which was significantly higher than that on rice husks or a mixture of rice and rice bran under the same culture conditions. In addition, wheat bran and rice bran in the proportion of 3:1 supported maximum conidiospores yields for strain BbR2 (1.9×10^7 conidia/ml) and stain BbR1(1.66×10^7 conidia/ml) and significantly superior over other strains (Dhar et al. 2016). However, there were some differences in our study, the best growth rate and sporulation were achieved when strain MJ1015 was grown on a medium comprising a mixture of rice and wheat, $NaNO_3$, $K_2HPO_4\cdot3H_2O$, and $CaCO_3$. Our results confirm the findings of earlier studies (Ibrahim and Low 1993; Feng et al. 2004; Bhadauria et al. 2012), showing that the fungal species may cause differences in carbon and nitrogen source utilization among strains.

In our study, rice and wheat impart essential factors favoring fungal growth and conidial yields. A possible explanation for higher levels of conidial production on rice and wheat than on other substrates could be the carbon to nitrogen ratio of these substrates. A high carbon-to-nitrogen ratio could promote conidiation under nitrogen starvation (Gao et al. 2007; Safavi et al. 2007; Uzma and Gurvinder 2009; Pham et al. 2010; Goffré et al. 2018; Song et al. 2019; Hassan et al. 2020). However, further investigations are needed to analyze the carbon: nitrogen ratios of the substrates used in this study to verify this idea.

Culture conditions also affect the conidial production of fungi, and, therefore, culture parameters, such as water, temperature, and light, also need to be optimized (Rodríguez-Gómez et al. 2009; Pham et al. 2010; Rizal et al. 2022). In addition, the influence of the medium on the infectivity of the strain should also be considered when selecting a medium (Rodríguez-Gómez et al. 2009; Garza-López et al. 2012; Doolotkeldieva et al. 2019) to ensure that applications of *B. majiangensis* as a pest control agent in the field are practical.

In conclusion, we have taken a first step toward identifying the culture conditions needed for a future commercial-scale and the optimal solid-state fermentation medium operation to produce *B. majiangensis* conidia to control white grubs. It to pave the way towards commercialization of strain MJ1015.

Availability of data and material

The data that support the findings of this study are available on request from the corresponding author.

Acknowledgments

The authors want to thank Chao Kang from the Guizhou Institute of Biology for his suggestions on experimental design.

Author contributions

Conception and design of the study: XW and ML. Material preparation, data collection, and analysis: XW, ZH, CL, GY, LL, YS, YR and JW. The first draft of the manuscript was written by WX and all authors commented on previous versions of the manuscript and approved the submitted version.

Funding

This work was supported by the Natural Science Foundation of Guizhou Province ([2020]1Y110) and (ZK[2022]283).

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Aregger E. Conidia production of the fungus *Beauveria brongniartii* on barley and quality evaluation during storage at 2°C. J Invertebr Pathol. 1992;59(1):2–10. https://doi.org/10.1016/0022-2011(92)90104-c

Bara GT, Laing MD. Entomopathogens: potential to control thrips in avocado, with special reference to *Beauveria bassiana*. In: Warrington I, editor. Horticultural Reviews, vol. 47. Hoboken (USA): John Wiley and Sons, Inc; 2020. p. 325–368. https://doi.org/10.1002/9781119625407.ch7

Bhadauria BP, Puri S, Singh PK. Mass production of entomopathogenic fungi using agricultural products. Bioscan. 2012;7(2):229–232.

Campos-Esquivel L, Hanson PE, Escudero-Leyva E, Chaverri P. Virulence of native isolates of entomopathogenic fungi (Hypocreales) against the "sweetpotato whitefly" *Bemisia tabaci* (Hemiptera: Aleyrodidae), including the effects of temperature and fungicides. J Invertebr Pathol. 2022 Jul;192:107787. https://doi.org/10.1016/j.jip.2022.107787

Castillo Lopez D, Zhu-Salzman K, Ek-Ramos MJ, Sword GA. The entomopathogenic fungal endophytes *Purpureocillium lilacinum* (formerly *Paecilomyces lilacinus*) and *Beauveria bassiana* negatively affect cotton aphid reproduction under both greenhouse and field conditions. PLoS One. 2014 Aug;9(8):e103891. https://doi.org/10.1371/journal.pone.0103891

Chen WH, Liu M, Huang ZX, Yang GM, Han YF, Liang JD, Liang ZQ. *Beauveria majiangensis*, a new entomopathogenic fungus from Guizhou, China. Phytotaxa. 2018;333(2):243–250. https://doi.org/10.11646/phytotaxa.333.2.8

de Farias LV, Ximenes Monteiro K, Rodrigues S, André Narciso Fernandes F, Adolfo Saavedra Pinto G. Comparison of *Aspergillus niger* spore production on Potato Dextrose Agar (PDA) and crushed corncob medium. J Gen Appl Microbiol. 2010 Oct;56(5):399–402. https://doi.org/10.2323/jgam.56.399

Dhar S, Jindal V, Gupta VK. Optimization of growth conditions and medium composition for improved conidiation of newly isolated *Beauveria bassiana* strains. Indian J Exp Biol. 2016 Oct; 54(10):634–643.

Doolotkeldieva T, Bobusheva S, Kulmanbetova A, Zholdoshbekova S, Amanbek Kyzy A. Characterization of *Beauveria bassiana* isolates from Kyrgyzstan. J Invertebr Pathol. 2019 Oct;167:107243. https://doi.org/10.1016/j.jip.2019.107243

Erler F, Ates AO. Potential of two entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* (Coleoptera: Scarabaeidae), as biological control agents against the June beetle. J Insect Sci. 2015 Apr;15(1):44. https://doi.org/10.1093/jisesa/iev029

Feng KC, Liu BL, Tzeng YM. *Verticillium lecanii* spore production in solid-state and liquid-state fermentations. Bioprocess Biosyst Eng. 2000 Jul;23(1):25–29. https://doi.org/10.1007/s004499900115

Feng MG, Pu XY, Ying SH, Wang YG. Field trials of an oil-based emulsifiable formulation of *Beauveria bassiana* conidia and low application rates of imidacloprid for control of false-eye leafhopper *Empoasca vitis* on tea in southern China. Crop Prot. 2004 Jun; 23(6):489–496. https://doi.org/10.1016/j.cropro.2003.10.004

Tamayo-Sánchez F, Tamayo-Mejía F, Marín-Jarrillo A, Santillán-Galicia MT, Pérez-Valdez A, Guzmán-Franco AW. Dynamics of natural infection of white grub larvae by *Beauveria* and *Metarhizium* in maize crops from Mexico. Biocontrol Sci Technol. 2022; 32(10): 1177–1193. https://doi.org/10.1080/09583157.2022.2098925

Fite T, Tefera T, Negeri M, Damte T, Sori W. Evaluation of *Beauveria bassiana*, *Metarhizium anisopliae*, and *Bacillus thuringiensis* for the management of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) under laboratory and field conditions. Biocontrol Sci Technol. 2020:30(3):278–295. https://doi.org/10.1080/09583157.2019.1707481

Galani G. Cultivation of some entomopathogenic fungi in liquid media with various initial pH values. Analel Institutului de Cercetari pentru Protecta Plantelor. 1988;21:54.

Gao L, Sun MH, Liu XZ, Che YS. Effects of carbon concentration and carbon to nitrogen ratio on the growth and sporulation of several biocontrol fungi. Mycol Res. 2007 Jan;111(1):87–92. https://doi.org/10.1016/j.mycres.2006.07.019

Garza-López PM, Konigsberg M, Gómez-Quiroz LE, Loera O. Physiological and antioxidant response by *Beauveria bassiana* Bals (Vuill.) to different oxygen concentrations. World J Microbiol Biotechnol. 2012 Jan;28(1):353–359. https://doi.org/10.1007/s11274-011-0827-y

Goffré D, Cavalloa EC, Cavalitto SF, Folgarait PJ. Selection and yield optimisation of a *Beauveria bassiana* isolate for the biological control of leaf cutter ants. Biocontrol Sci Technol. 2018;28(7):672–687. https://doi.org/10.1080/09583157.2018.1479730

Hassan F, Abdullah S, Assaf L. Molecular identification and biomass production of an endophytic *Beauveria bassiana* isolated from cucumber leaves in Iraq. J Duhok Univ. 2020;22(s2):38–47. https://doi.org/10.26682/cajuod.2020.22.2.5

Ibrahim L, Laham L, Touma A, Ibrahim S. Mass production, yield, quality, formulation and efficacy of entomopathogenic *Metarhizium anisopliae* conidia. Br J Appl Sci Technol. 2015;9(5):427–440. https://doi.org/10.9734/BJAST/2015/17882

Ibrahim YB, Low W. Potential of mass production and field efficacy of isolates of the entomopathogenic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* against *Plutella xylostella*. Int J Pest Manage. 1993;39(3):288–292. https://doi.org/10.1080/09670879309371807

Kankale MD, Kelwatkar NM, Das SB, Sontakke BK. Selection of suitable and economic substrate for mass production of fungus, *Beauveria bassiana*. J Appl Zool Res. 2017;28(2):157–163.

Karthikeyan A, Shanthi V, Nagasathya A. Effect of different media and pH on the growth of *Beauveria bassiana* and its parasitism on leaf eating caterpillars. Res J Agric Biol Sci. 2008;4(2):117–119.

Kim S, Kim JC, Lee SJ, Lee MR, Park SE, Li D, Baek S, Shin TY, Gasmi L, Kim JS. Soil application of *Metarhizium anisopliae*

JEF-314 granules to control, flower chafer beetle, *Protaetia brevitarsis seulensis*. Mycobiology. 2020 Mar 11;48(2):139–147. https://doi.org/10.1080/12298093.2020.1735765

Lin LX, Yu YC, Hu JW. Study on white grub control by *Beauveria brongiartii* in soybean field, in study and application of *Entomogenous Fungi* in China. In: Li YW, Li ZZ, Liang ZQ, Wu JW, Wu ZK, Xu QF, editors. Study and application of entomogenous fungi in China, vol. 1. Beijing (China): Academic Periodical Press; 1988. pp. 135–139.

Liu M, Wang XH, Hauang ZX, Yang GM, Luo LL, Wang H, Zhang SH. [Primary study on the virulence of *Beauveria majiangensis* strain MJ1015 against three species of white grubs] (in Chinese). China Fruits. 2020;2020(3):80–84. https://doi.org/10.16626/j.cnki.issn1000-8047.2020.03.009

Machado ACR, Monteiro AC, de Almeida AMB, Martins MIEG. Production technology for entomopathogenic fungus using a biphasic culture system. Pesq Agropec Bras. 2010;45(10):1157–1163. https://doi.org/10.1590/s0100-204x2010001000015

Mantzoukas S, Tamez-Guerra P, Zavala-Garcia F, Lagogiannis I, Ek-Ramos MJ. Entomopathogenic fungi tested *in planta* on pepper and in field on sorghum, to control commercially important species of aphids. World J Microbiol Biotechnol. 2022 Apr;38(5):84. https://doi.org/10.1007/s11274-022-03268-7

Nong XQ, Liu CQ, Lu X, Wang QL, Wang GJ, Zhang ZH. Laboratory evaluation of entomopathogenic fungi against the white grubs, *Holotrichia oblita* and *Anomala corpulenta* (Coleoptera: Scarabaeidae) from the field of peanut, *Arachis hypogaea*. Biocontrol Sci Technol. 2011;21(5):593–603. https://doi.org/10.1080/09583157.2011.566324

Nozipho K. Development of *Beauveria brongniartii* as a bio-insecticide to control white grub (Coleoptera: Scarabaeidae) species attacking sugarcane in South Africa [Master Thesis]. Pietermaritzburg (South Africa): University of KwaZulu-Natal; 2016.

Ovruski S, Schliserman P, Aluja M. Native and introduced host plants of *Anastrepha fraterculus* and *Ceratitis capitata* (Diptera: Tephritidae) in northwestern Argentina. J Econ Entomol. 2003 Aug; 96(4):1108–1118. https://doi.org/10.1093/jee/96.4.1108

Pandey A. Solid-state fermentation: An overview. In: Pandey A, editor. Solid state fermentation. New Delhi (Indie): Wiley Eastern; 1994. p. 3–10.

Pham TA, Kim JJ, Kim K. Optimization of solid-state fermentation for improved conidia production of *Beauveria bassiana* as a mycoinsecticide. Mycobiology. 2010 Jun;38(2):137–143. https://doi.org/10.4489/myco.2010.38.2.137

Pu ZL, and Li ZZ. Insect mycology. Hefei (China): Anhui Science and Technology Press; 1996. p. 387–446.

Rangel DE, Alston DG, Roberts DW. Effects of physical and nutritional stress conditions during mycelial growth on conidial germination speed, adhesion to host cuticle, and virulence of *Metarhizium anisopliae*, an entomopathogenic fungus. Mycol Res. 2008 Nov; 112(11): 1355–1361. https://doi.org/10.1016/j.mycres.2008.04.011

Rizal LM, Furlong MJ, Walter GH. Responses of diamondback moth to diverse entomopathogenic fungi collected from non-agricultural habitats – effects of dose, temperature and starvation. Fungal Biol. 2022 Oct;126(10):648–657. https://doi.org/10.1016/j.funbio.2022.08.005

Gielen R, Robledo G, Zapata AI, Tammaru T, Põldmaa K. Entomopathogenic fungi infecting lepidopteran larvae: A case from central Argentina. Life. 2022 Jun;12(7):974. https://doi.org/10.3390/life12070974

Rodríguez-Gómez D, Loera O, Saucedo-Castañeda G, Viniegra-González G. Substrate influence on physiology and virulence of *Beauveria bassiana* acting on larvae and adults of *Tenebrio molitor*. World J Microbiol Biotechnol. 2009;25(3):513–518. https://doi.org/10.1007/s11274-008-9917-x

Safavi SA, Shah FA, Pakdel AK, Reza Rasoulian G, Bandani AR, Butt TM. Effect of nutrition on growth and virulence of the entomopathogenic fungus *Beauveria bassiana*. FEMS Microbiol Lett. 2007 May;270(1):116–123. https://doi.org/10.1111/j.1574-6968.2007.00666.x

Sanzhimitupova RD. Effect of the pH of the medium on the growth and development of the causal agent of mycosis of the seabuckthorn moth (*Gelecjia hippophaella* Schrk). Izvestiya Sibirskogo Otdeleniya Akademii Nauk SSSR Biology. 1980;15:39–41.

Shimazu M, Sato H. Media for selective isolation of an entomogenous fungus *Beauveria bassiana* (Deuteromycotina: Hyphomycetes). Appl Entomol Zool. 1996;31(2):291–298. https://doi.org/10.1303/aez.31.291

Sikura AI, Primak TA. Native formulation of Boverin. Moscow (USSR): Microbiological Industry of Soviet Union, Department of Information; 1970. p. 1–44.

Soccol CR, da Costa ESF, Letti LAJ, Karp SC, Woiciechowski AL, de Souza Vandenberghe LP. Recent developments and innovations in solid state fermentation. Biotechnol Res Innovation. 2017; 1(1):52–71. https://doi.org/10.1016/j.biori.2017.01.002

Song MH, Yu JS, Kim S, Lee SJ, Kim JC, Nai YS, Shin TY, Kim JS. Downstream processing of *Beauveria bassiana* and *Metarhizium* anisopliae-based fungal biopesticides against *Riptortus pedestris*: solid culture and delivery of conidia. Biocontrol Sci Technol. 2019; 29(6):514–532. https://doi.org/10.1080/09583157.2019.1566951

Soni S, Mehta PK, Chandel RS. Susceptibility of white grub, *Brahmina coriacea* (Hope) infesting potato to local strains of *Beauveria brongniartii* (Saccardo) in Himachal Pradesh. J Biol Control. 2018; 32(1):41–47. https://doi.org/10.18311/jbc/2018/16288

Tumuhaise V, Ekesi S, Maniania NK, Tonnang HEZ, Tanga CM, Ndegwa PN, Irungu LW, Srinivasan R, Mohamed SA. Temperature-dependent growth and virulence, and mass production potential of two candidate isolates of *Metarhizium anisopliae* (Metschnikoff) Sorokin for managing *Maruca vitrata* Fabricius (Lepidoptera: Crambidae) on cowpea. Afr Entom. 2018;26(1):73–83. https://doi.org/10.4001/003.026.0073

Ugine TA, Wraight SP, Brownbridge M, Sanderson JP. Development of a novel bioassay for estimation of median lethal concentrations (LC_{50}) and doses (LD_{50}) of the entomopathogenic fungus *Beauveria bassiana*, against western flower thrips, *Frankliniella occidentalis*. J Invertebr Pathol. 2005 Jul;89(3):210–218. https://doi.org/10.1016/j.jip.2005.05.010

Uzma M, Gurvinder K. Effects of carbon and nitrogen sources and ratio on the germination, growth and sporulation characteristics of *Metarhizium anisopliae* and *Beauveria bassiana* isolates. Afr J Agric Res. 2009;3(10):922–930. https://doi.org/10.5897/AJAR.9000244

Vega FE, Kaya HK. Insect pathology. Second edition. Waltham (USA): Academic Press; 2012.

Visalakshi M, Bhavani B, Rao SG. Field evaluation of entomopathogenic fungi against white grub, *Holotrichia consanguinea* blanch in sugarcane. J Biol Control. 2015;29(2):103–106. https://doi.org/10.18311/jbc/2015/3228

Wang Y, Fan Q, Wang D, Zou WQ, Tang DX, Hongthong P, Yu H. Species diversity and virulence potential of the *Beauveria bassiana* complex and *Beauveria scarabaeidicola* complex. Front Microbiol. 2022 Mar;13:841604. http://doi.org/10.3389/fmicb.2022.841604

Wraight SP, Ramos ME, Avery PB, Jaronski ST, Vandenberg JD. Comparative virulence of *Beauveria bassiana* isolates against lepidopteran pests of vegetable crops. J Invertebr Pathol. 2010 Mar; 103(3): 186–199. https://doi.org/10.1016/j.jip.2010.01.001

Yu JS, Lee SJ, Shin TY, Kim WJ, Kim JS. Enhanced thermotolerance of entomopathogenic *Beauveria bassiana* and *Metarhizium anisopliae* JEF-isolates by substrate modification. Int J Ind Entomol. 2020;41(2):28–35. https://doi.org/10.7852/ijie.2020.41.2.28