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RESEARCH ARTICLE

# Comparative evaluation of various substrates for cost-effective mass multiplication of *Beauveria bassiana*

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## Abstract

The white muscardine fungus, *Beauveria bassiana* (Bals.-Criv.) Vuill., with wide biocontrol potential, requires cost-effective mass multiplication methods to enhance adoption at the field level. A study was conducted at Biocontrol Laboratory, Department of Entomology and Agricultural Zoology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, for a comparative evaluation of grain-based and artificial substrates for its mass multiplication. Among 6 grains tested, sorghum, chickpea, wheat, rice, maize and green gram; sorghum showed the highest spore yield of 90.96 × $10^7$ spores $g^{-1}$ and a peak growth rate increase of 138.68 % between days 14 and 21. It also exhibited as the most cost effective substrate, producing 2860.35 × $10^7$ spores ₹$^{-1}$, surpassing chickpea (60.12 × $10^7$ spores $g^{-1}$) and wheat (33.08 × $10^7$ spores $g^{-1}$). Maize and green gram performed poorly in biological and economic terms. Among artificial media, PDA (Potato Dextrose Agar) was the most effective, showing the highest mean radial growth of 36.83 mm and sporulation of 52.54 × $10^7$ spores $mL^{-1}$, yielding 24,783.02 × $10^7$ spores ₹$^{-1}$. P1DA (Papaya peels Dextrose Agar) also performed well with radial growth of 31.00 mm, sporulation of 35.41 × $10^7$ spores $mL^{-1}$ and economical yield of 21,862.96 × $10^7$ spores ₹$^{-1}$, nearly twice that of BDA (Banana Dextrose Agar) which produced 18.5 × $10^7$ spores $mL^{-1}$ and 11,419.75 × $10^7$ spores ₹$^{-1}$. Thus, while PDA is biologically superior, P1DA offers a promising, cost-effective alternative for fungal propagation. This evaluation confirms sorghum and PDA as the best substrates for field- and lab-scale production, respectively.

**Keywords:** Artificial media; *Beauveria bassiana*; biocontrol; cost-efficiency; grain media

## Introduction

The increasing global emphasis on sustainable agriculture has intensified the search for eco-friendly alternatives to chemical pesticides. Among biological control agents, *Beauveria bassiana* (Bals.-Criv.) Vuill., a well-known entomopathogenic fungus, has gained recognition for its effectiveness in controlling a wide range of insect pests (1). It has demonstrated biocontrol potential against whiteflies, beetles, termites and even vectors of human diseases such as *Anopheles* mosquitoes (2, 3). Unlike many microbial agents, *B. bassiana* infects insects by directly penetrating the cuticle, eliminating the need for ingestion and enabling broader applicability (4, 5). Despite its efficacy, the large-scale adoption of *B. bassiana* remains limited, particularly in developing countries, due to the high cost and technical requirements of mass production. Conventional submerged fermentation methods rely on expensive liquid media such as corn steep liquor or sugarcane molasses and require sterile conditions and sophisticated infrastructure (6, 7). These factors pose significant barriers for small-scale farmers and decentralized biocontrol production units (8, 9).

To address these challenges, researchers have focused on solid-state fermentation (SSF) using low-cost, locally available agricultural substrates (10). Solid substrates like sorghum, rice, wheat and chickpea have shown promise in supporting conidial production (11–13). SSF offers several advantages: It closely mimics the natural growth environment of entomopathogenic fungi, reduces energy consumption, lowers contamination risks and enhances conidial yield and shelf life (14–16). Studies have shown that optimizing substrate particle size, moisture and nutrient supplementation can further improve mass production efficiency (17, 18).

However, comprehensive comparative evaluations of these substrates under local laboratory conditions remain scarce, particularly considering economic feasibility and spore quality (19, 20). This study was conducted to explore pooled data from 2023 and 2024 to assess the suitability, yield potential and cost-effectiveness of various grain-based and agro-waste substrates for the mass multiplication of *B. bassiana* under laboratory conditions. The goal is to identify economically viable and biologically efficient substrates to support sustainable, farmer-accessible biopesticide production systems in Indian agriculture.

## Materials and Methods

### Fungal culture source

A pure culture of *B. bassiana* was obtained from the Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India. The culture was maintained on Potato Dextrose Agar (PDA) at 4 ± 1 °C for short-term preservation Prior to each trial, sub-culturing was done to ensure the viability and active growth of conidia. The same fungal isolate was used consistently across both the 2023–24 and 2024–25 trials to maintain experimental uniformity.

### Experimental design

The study was conducted over two consecutive year 2023–24 and 2024–25 in the Biocontrol Laboratory, Department of Entomology and Agricultural Zoology, BHU, Varanasi. A Completely Randomized Design (CRD) with three replications per treatment was adopted each year. Observations were taken at 7, 14, 21 and 28 days after inoculation to assess fungal growth and sporulation on different substrates. Data from both years were polled for comparative evaluation and statistical consistency.

### Preparation of substrates

Six grain substrates i.e., Sorghum, Chickpea, Wheat, Rice, Maize and Green Gram were selected for mass multiplication of *B. bassiana* based on their diverse nutritional profiles, structural attributes and documented success in supporting high conidial yields of entomopathogenic fungi. Sorghum offers high starch (70–75 %) and protein (9–13 %) content with a fibrous, porous husk that enhances aeration, moisture retention and mycelial colonization. Chickpea provides elevated protein (20–25 %) and minerals (e.g., phosphorus 312 mg/100 g, iron 5.2 mg 100 $g^{-1}$), while wheat and rice supply readily fermentable carbohydrates (starch 60–80 %) ideal for fungal metabolism, though rice's compact kernel limits harvesting. Maize and green gram, with balanced lipids (4–6 %) and moderate protein (8–24 %), serve as comparative baselines, as their pericarp texture influences penetration and nutrient release (21). Grains were cleaned to remove debris and impurities, soaked in distilled water (1:2 w/v ratio) for 12 hr at room temperature to raise moisture content from an initial 10–12 % (typical for dry grains) to approximately 35–40 %, air-dried under shade for 24–48 hr to achieve a final moisture of 30–35 % optimal for fungal colonization and sterilized in autoclavable polybags (100 gram per packet) at 121 °C and 15 psi for 20 min prior to inoculation. Whole grains were retained to ensure structural integrity, uniform aeration and mycelial penetration, as broken grains reduce yield due to clumping and contamination risks (22, 23).

Four synthetic agar-based media-Potato Dextrose Agar (PDA), $P_1$DA ($P_1$ means Papaya peels extract), Sabouraud Dextrose Agar (SDA) and Banana Dextrose Agar (BDA) were prepared following protocols established in mentioned literature (24, 25). In $P_1$DA and BDA, we used the same composition as PDA but replaced potato with papaya peels and banana peels respectively.

### Sterilization and inoculation

Grain substrates (100 g per packet) were sterilized in autoclavable polypropylene bags at 121 °C for 20 min at 15 psi. Artificial media were poured into sterile Petri plates under aseptic conditions. After cooling, 5 mm agar discs from 7-day-old cultures were transferred to each substrate using sterile techniques in a laminar airflow cabinet (26).

### Incubation conditions

Inoculated samples were incubated at 26 ± 2 °C and 70–80 % relative humidity in a B.O.D. incubator. Solid substrates were manually shaken every 48 hr to ensure uniform aeration and colonization (27). To maintain aseptic conditions, the incubation chamber was periodically fumigated with a $KMnO_4$ formaldehyde mixture (2 g $KMnO_4$ :4 mL formaldehyde $m^{-3}$) and properly aerated before use. Grain-based substrates were supplemented with a broad-spectrum antibiotic (streptomycin sulfate, 50–100 mg $g^{-1}$) after sterilization to prevent bacterial contamination without affecting fungal growth.

### Spore harvesting and quantification

At each observation interval, spores were harvested by adding 100 mL of 0.05 % Tween-80 solution to the substrates. The spore-laden solution was filtered through sterile muslin cloth, serially diluted using Koch's serial dilution method (1883) and the concentration (conidia $mL^{-1}$) was determined using a Neubauer hemocytometer under a compound microscope.

$$\text{Spores/mL} = \text{Average count per square} \times \text{Dilution factor} \times 10^4$$

### Mycelial growth and sporulation measurement

For agar media, radial mycelial growth (in mm) was measured at 7, 14, 21 and 28 days post-inoculation using a digital Vernier caliper. Two perpendicular colony diameters were averaged for accuracy. For sporulation studies, the fungal isolate was grown in broth media and incubated in a shaker incubator (BOD) for 7, 14, 21 and 28 days. Spore quantification was carried out using the serial dilution method. A similar approach, as used for spore harvesting from solid grain substrates, was followed by suspending the spores in 0.05 % Tween-80 solution.

### Growth rate dynamics calculation

Spore production data of fungal isolates grown on different host substrates were recorded at 7, 14, 21 and 28 days intervals. Growth rate dynamics between intervals were calculated as percentage increase in mean spore count relative to the preceding interval using the formula:

$$\text{Growth rate (\%)} = \frac{N_2 - N_1}{N_1} \times 100$$

Where $N_1$ and $N_2$ represent mean spore counts at successive intervals

### Cost efficiency analysis

Cost efficiency was evaluated for both years by calculating the cost per $10^7$ conidia for each substrate. The market price of each medium was divided by the total number of conidia produced per unit, with results expressed as conidia per Indian rupee (₹) (28, 29). Data from both years were averaged and subjected to statistical analysis to determine substrate-specific cost efficiency. These calculations considered only substrate costs, as labor, instrumentation and other operational expenses remained consistent across all media and were therefore excluded. This approach enabled direct comparison of substrate performance.

$$\text{Cost - Efficiency (spores ₹}^{-1}\text{)} = \frac{\text{Cost per kilogram of substrate} \times 1000}{\text{Spore yield per gram}}$$

### Statistical analysis

The experimental data collected during 2023 and 2024 were first analysed separately using one way ANOVA in OPSTAT to evaluate year-wise treatment effects. Mean comparisons were carried out using Tukey's Honestly Significant Difference (HSD) test at a significance level of $P \leq 0.05$. Homogeneity of error variances between 2023 and 2024 datasets was confirmed using Bartlett's test permitting pooled analysis.

For pooled data analysis across both years, the statistical analysis under completely randomized design (CRD) was conducted using the KAU Grapes online platform (developed by Kerala Agricultural University), which provided treatment means, standard error (SEm ±), critical difference (CD) and coefficient of variation (CV) and DMRT. Graphs and trend lines were prepared using Microsoft Excel and KAU Grapes to visually represent treatment performance across different substrates and years.

## Results

### Effect of grain-based substrates on growth and sporulation efficiency of *B. bassiana*

Pooled data from 2023–24 and 2024–25 indicated a highly significant ($p \leq 0.01$) variation among the tested grain substrates with respect to sporulation of *B. bassiana* (Table 1 and Fig. 1). Sorghum supported the maximum mean sporulation ($90.96 \times 10^7$ spores $g^{-1}$), which was statistically superior to all other substrates. Conidial yield on sorghum increased progressively from $27.83 \pm 1.83 \times 10^7$ spores $g^{-1}$ at 7 days to $182.33 \pm 2.58 \times 10^7$ spores $g^{-1}$ at 28 days, indicating rapid fungal colonization and high sporogenic potential. Chickpea ranked second with a mean yield of $60.12 \times 10^7$ spores $g^{-1}$, significantly higher than green gram ($34.71 \times 10^7$) and wheat ($33.08 \times 10^7$ spores $g^{-1}$), which were statistically comparable. Rice ($25.96 \times 10^7$) and maize ($9.96 \times 10^7$ spores $g^{-1}$) recorded the lowest sporulation, being significantly inferior to all other media.

#### Growth rate dynamics between intervals

Temporal analysis revealed clear differences in sporulation increments across successive intervals (Table 2 and Fig. 2). Sorghum showed a consistent and robust increase in spore production between 7–14, 14–21 and 21–28 days, with percentage increments of 64.08 %, 138.68 % and 67.28 % respectively. Chickpea exhibited similar but slightly lower growth dynamics (69.67 %, 110.14 % and 56.32 %), remaining statistically close to sorghum up to the 14–21-day interval. Green gram and wheat showed moderate proportional increases, whereas rice and maize exhibited significantly slower sporulation rates–the latter recording the minimum overall increase (19.04–41.67 %), reflecting poor substrate utilization.

#### Cost-efficiency of different grain media

Cost–benefit analysis based on per-rupee spore yield indicated sorghum as the most cost-efficient substrate, producing $2860.35 \times 10^7$ spores ₹$^{-1}$(spore yield $90.96 \times 10^7$ spores $g^{-1}$; cost ₹31.80 $kg^{-1}$) (Table 3 and Fig. 3). Wheat ($1556.89 \times 10^7$ spores ₹$^{-1}$) and rice ($1189.14 \times 10^7$ spores ₹$^{-1}$) formed a statistically similar group, showing moderate economic efficiency. Despite comparatively high sporulation, chickpea ($1126.97 \times 10^7$ spores ₹$^{-1}$) was less economical due to its higher procurement cost (₹53.35 $kg^{-1}$). Maize and green gram recorded the lowest efficiencies ($476.36 \times 10^7$ and $405.55 \times 10^7$ spores ₹$^{-1}$ respectively), differing significantly from other treatments.

**Table 1.** Effect of different grain media on sporulation of *B. bassiana*

| Grain media | Treatment | No. of spores on different grain media of *B. Bassiana* at different day after inoculation (1 x $10^7$ spore/g) | | | | |
|---|---|---|---|---|---|---|
| | | 7th day | 14th day | 21st day | 28th day | Mean |
| **Green gram** | $T_1$ | $16.16^c \pm 1.94$ | $24^c \pm 1.78$ | $41.16^c \pm 1.47$ | $57.66^c \pm 1.75$ | 34.707 |
| **Chickpea** | $T_2$ | $20.33^b \pm 1.86$ | $34.5^b \pm 1.64$ | $72.5^b \pm 1.76$ | $113.33^b \pm 2.58$ | 60.124 |
| **Wheat** | $T_3$ | $14.83^d \pm 1.16$ | $23.16^c \pm 1.47$ | $38.16^d \pm 1.16$ | $56.33^c \pm 1.75$ | 33.084 |
| **Rice** | $T_4$ | $12.33^e \pm 1.50$ | $18.5^d \pm 1.52$ | $32.0^e \pm 2.09$ | $41.33^d \pm 2.58$ | 25.959 |
| **Maize** | $T_5$ | $7.00^f \pm 1.41$ | $8.33^e \pm 1.63$ | $10.0^f \pm 1.41$ | $14.16^e \pm 1.47$ | 9.956 |
| **Sorghum** | $T_6$ | $27.83^a \pm 1.83$ | $45.66^a \pm 1.63$ | $109.0^a \pm 2.00$ | $182.33^a \pm 2.58$ | 90.959 |
| **C.V.** | | 3.42 | 3.94 | 2.09 | 1.62 | 1.17 |
| **P Value** | | 0.84 | 0.90 | 0.25 | 0.37 | 0.96 |
| **SE(m) ±** | | 0.23 | 0.18 | 0.19 | 0.23 | 0.11 |
| **C.D. at 5 %** | | 0.83 | 1.50 | 1.56 | 1.86 | 0.63 |

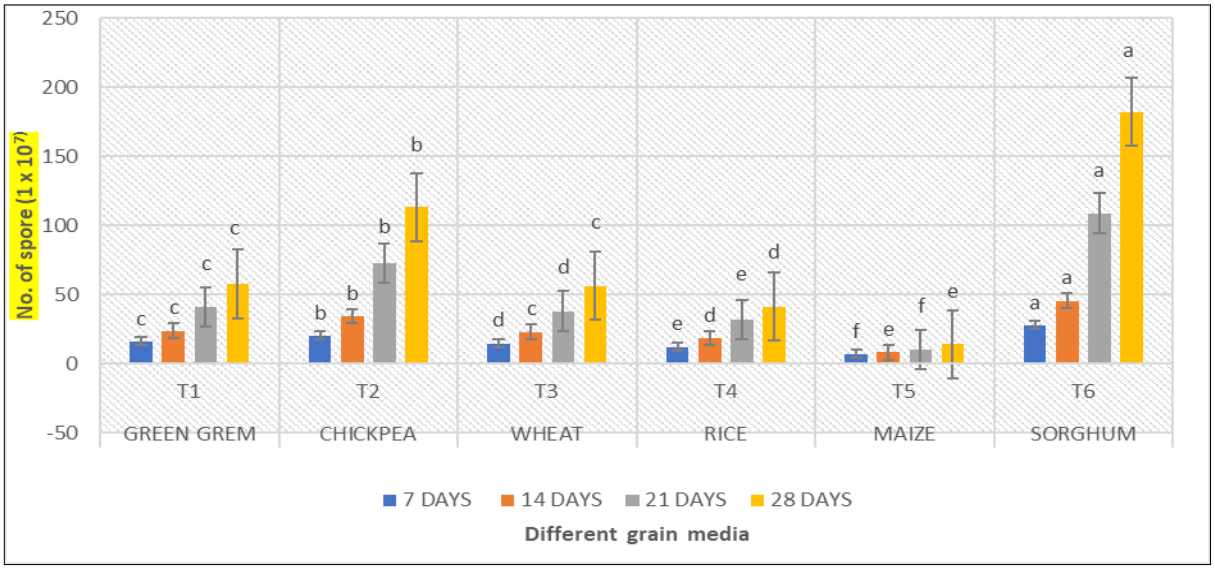


**Fig. 1.** Comparison of spore production of *B. bassiana* on different grain media.

**Table 2.** Percent rate of increase in growth of *B. bassiana* on different grain media

| Grain media | Treatment | Percent rate of increase in growth of *B. bassiana* | | |
|---|---|---|---|---|
| | | 7 to 14 days | 14 to 21 days | 21 to 28 days |
| Green gram | $T_1$ | 48.45 | 71.53 | 40.08 |
| Chickpea | $T_2$ | 69.67 | 110.14 | 56.32 |
| Wheat | $T_3$ | 56.19 | 64.75 | 47.60 |
| Rice | $T_4$ | 50.00 | 72.97 | 29.17 |
| Maize | $T_5$ | 19.04 | 20.00 | 41.67 |
| Sorghum | $T_6$ | 64.08 | 138.68 | 67.28 |

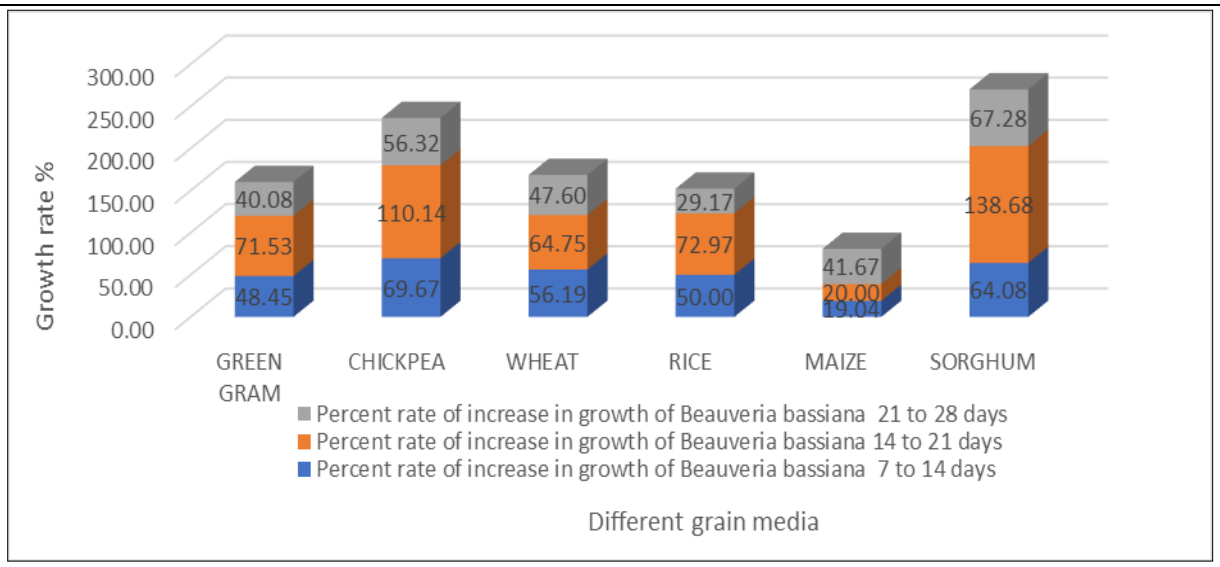


**Fig. 2.** Percent growth rate of *B. bassiana* over time on various grain media.

**Table 3.** Cost efficiency analysis of different grain-based substrates for mass multiplication of *B. bassiana*

| Grain media | Treatment | Mean no. of spore/gram (1 x $10^{7)}$ | Rate of grain/kg | No. of spore per rupee (1 x $10^{7)}$ | Rank |
|---|---|---|---|---|---|
| **Green gram** | **$T_1$** | 34.707 | 85.58 | 405.5504 | 6 |
| **Chickpea** | **$T_2$** | 60.124 | 53.35 | 1126.973 | 4 |
| **Wheat** | **$T_3$** | 33.084 | 21.25 | 1556.894 | 2 |
| **Rice** | **$T_4$** | 25.959 | 21.83 | 1189.143 | 3 |
| **Maize** | **$T_5$** | 9.956 | 20.9 | 476.3636 | 5 |
| **Sorghum** | **$T_6$** | 90.959 | 31.8 | 2860.346 | 1 |

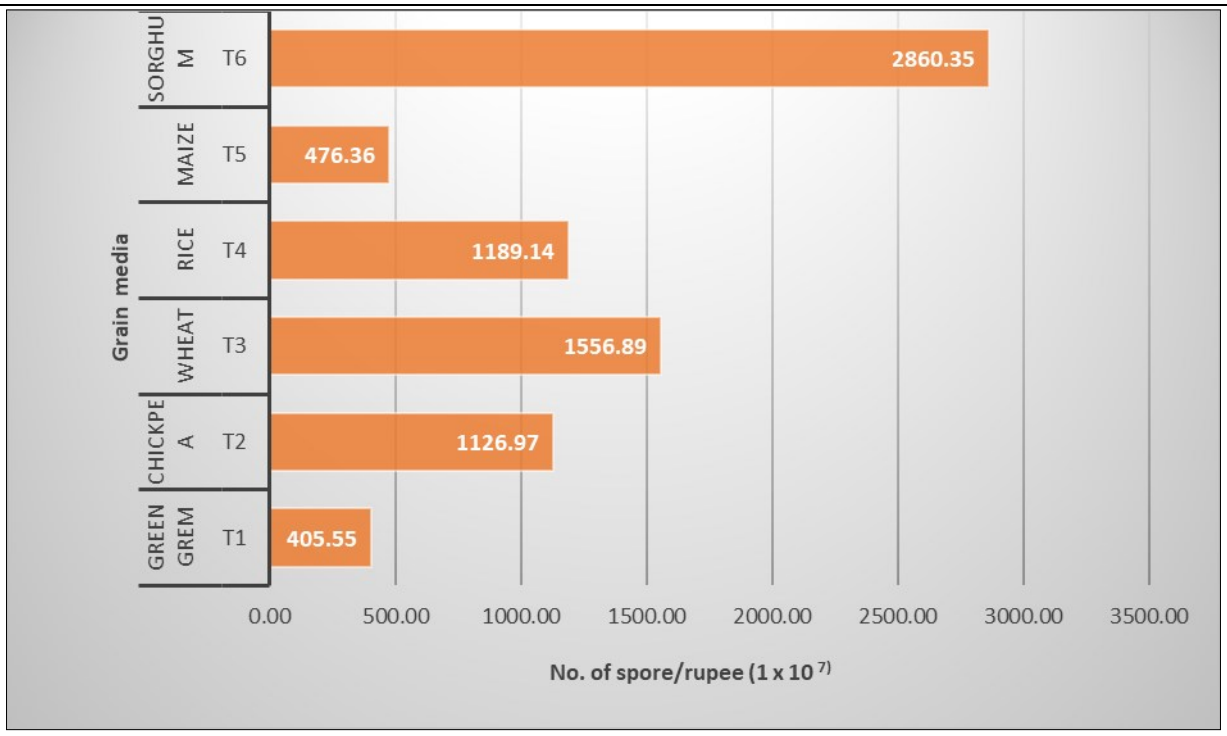


**Fig. 3.** Cost efficiency of grain-based substrates used for *B. bassiana* production.

**Effect of different artificial media on radial growth and sporulation of *B. bassiana***

The four tested artificial media exhibited significant ($p \leq 0.01$) differences in promoting radial growth and sporulation (Table 4, Fig. 4 and 5 respectively). Potato Dextrose Agar (PDA) supported the maximum radial growth (36.83 ± 1.47 mm at 28 days), which was significantly higher than all other media. Papaya Peel Dextrose Agar (P1DA) ranked second (31.00 ± 1.41 mm) and was statistically distinct from PDA but superior to SDA (25.83 ± 1.69 mm) and BDA (17.66 ± 1.21 mm).

Sporulation followed a similar pattern, with PDA yielding the highest conidial density (52.54 × $10^7$ spores $mL^{-1}$), significantly exceeding that of P1DA (35.41 × $10^7$), SDA (30.33 × $10^7$) and BDA (18.50 × $10^7$ spores $mL^{-1}$).

**Cost-efficiency of different artificial media**

Marked variation was observed among artificial media with respect to cost-efficiency (Table 5 and Fig. 6). PDA recorded the highest spore yield per rupee (24783.02 × $10^7$ spores ₹$^{-1}$), being significantly superior to all others. P1DA followed (21862.96 × $10^7$ spores ₹$^{-1}$), showing comparable efficiency to PDA. BDA produced moderate conidial yield per cost unit (11419.75 × $10^7$ spores ₹$^{-1}$), while SDA, due to its relatively higher component cost, exhibited the lowest economic return (4500.30 × $10^7$ spores ₹$^{-1}$).

## Discussion

The superior performance of sorghum for *B. bassiana* production is attributed to its fibrous husk and porous structure, which promote better fungal colonization and moisture retention, facilitating spore development with highest mean sporulation of 90.96 x $10^7$ Spore $g^{-1}$. Starch content (70–75 %) and structural properties as key for fungal growth (30). Chickpea's protein content (20–25 %) and soft texture likely enhanced nutrient availability, supporting high spore yields of 60.12 x $10^7$ Spore $g^{-1}$ (31). Moderate results on wheat (33.08 x $10^7$ spores $g^{-1}$) and green gram (34.71 x $10^7$ spore $g^{-1}$) may stem from their lower nutrient complexity and smoother surfaces, limiting fungal substrate interaction. Rice (25.96 × $10^7$ spores $g^{-1}$) and maize (9.96 × $10^7$spores $g^{-1}$) with compact kernels and hard pericarps, resulted in poor fungal penetration and sporulation (32). The growth rate dynamics further strengthen sorghum's suitability, showing sustained increases of 64.08 %, 138.68 % and 67.28 % across intervals. Suitability of sorghum showing sustained and rapid increases in sporulation across all intervals (33).

Among artificial media, PDA provided the richest environment for *B. bassiana* growth (36.83 mm) and sporulation (52.54 x $10^7$Spore $mL^{-1}$), likely due to its abundant potato starch and dextrose content, facilitating energy metabolism and colony expansion (30). $P_1$DA (31.00 mm growth, 35.41 × $10^7$ spores $mL^{-1}$), made from papaya peel extracts, demonstrated promising results offering a cost-effective and sustainable alternative substrate owing to its nutritious and fermentable sugar content. Lower performance in BDA (17.66 mm) might be attributed to nutrient limitations or inhibitory compounds in banana peel (34).

Cost-efficiency analyses validated these findings; despite PDA exhibiting highest productivity (24783.02 × $10^7$ spores ₹$^{-1}$), P1DA made it economically competitive (21862.96 × $10^7$ spores ₹$^{-1}$) while sorghum excelled among grains (2860.35 × $10^7$ spores ₹$^{-1}$). The high cost of SDA (4500.30 × $10^7$ spores ₹$^{-1}$) and lower sporulation render it less favourable for large-scale biopesticide production.

**Table 4.** Effect of different artificial media on radial growth and sporulation of *B. bassiana*

| Artificial media | Treatment | Effect of media on radial growth and sporulation of *B. Bassiana* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Radial growth (mm) | | | | | Sporulation $10^7$ Spore $mL^{-1}$ | | | | |
| | | 7th | 14th | 21st | 28th | Mean | 7th | 14th | 21st | 28th | Mean |
| **BDA** | $T_1$ | 8.00$^c$ ± 1.27 | 13.00$^d$ ± 1.09 | 15.83$^d$ ± 1.72 | 17.66$^d$ ± 1.21 | 13.709 | 7.00$^d$ ± 2.10 | 14.66$^d$ ± 1.03 | 24.66$^d$ ± 2.34 | 27.66$^c$ ± 2.34 | 18.5 |
| **$P_1$DA** | $T_2$ | 13.0$^{ab}$ ± 1.1 | 22.16$^b$ ± 1.16 | 28.83$^b$ ± 1.6 | 31.00$^b$ ± 1.41 | 23.75 | 19.16$^b$ ± 1.47 | 30.83$^b$ ± 2.93 | 43.83$^b$ ± 2.40 | 47.83$^b$ ± 2.79 | 35.41 |
| **PDA** | $T_3$ | 15.0$^a$ ± 2.0 | 25.66$^a$ ± 1.03 | 33.00$^a$ ± 1.41 | 36.83$^a$ ± 1.47 | 27.375 | 22.16$^a$ ± 2.23 | 40.33$^a$ ± 2.66 | 62.66$^a$ ± 1.63 | 84.83$^a$ ± 2.64 | 52.54 |
| **SDA** | $T_4$ | 11.83$^b$ ± 1.4 | 17.33$^c$ ± 1.21 | 24.33$^c$ ± 1.63 | 25.83$^c$ ± 1.69 | 19.83 | 13.50$^c$ ± 1.64 | 23.16$^c$ ± 1.94 | 34.83$^c$ ± 2.64 | 49.83$^c$ ± 3.06 | 30.33 |
| C.V. | | 9.40 | 4.3 | 5.51 | 3.88 | 2.41 | 7.59 | 4.33 | 5.51 | 3.88 | 4.27 |
| P value | | 0.13 | 0.1 | 0.13 | 0.96 | 0.97 | 0.83 | 0.09 | 0.13 | 0.12 | 0.98 |
| SE(m) ± | | 0.25 | 0.19 | 0.16 | 0.24 | 0.10 | 0.26 | 0.26 | 0.51 | 0.46 | 0.28 |
| C.D.at 5 % | | 2.15 | 1.54 | 4.20 | 1.98 | 0.81 | 2.16 | 2.17 | 4.20 | 3.74 | 2.33 |

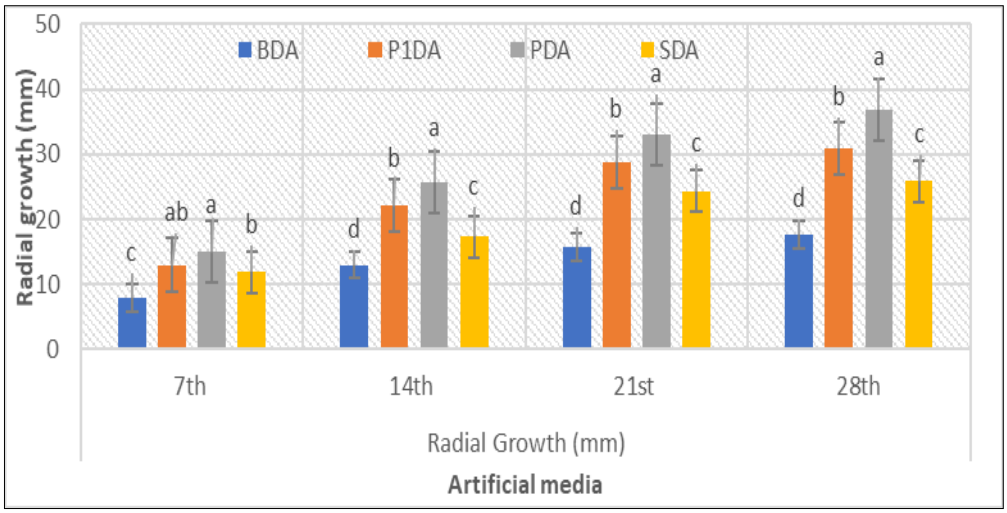


**Fig. 4.** Radial growth of *B. bassiana* on different artificial media.

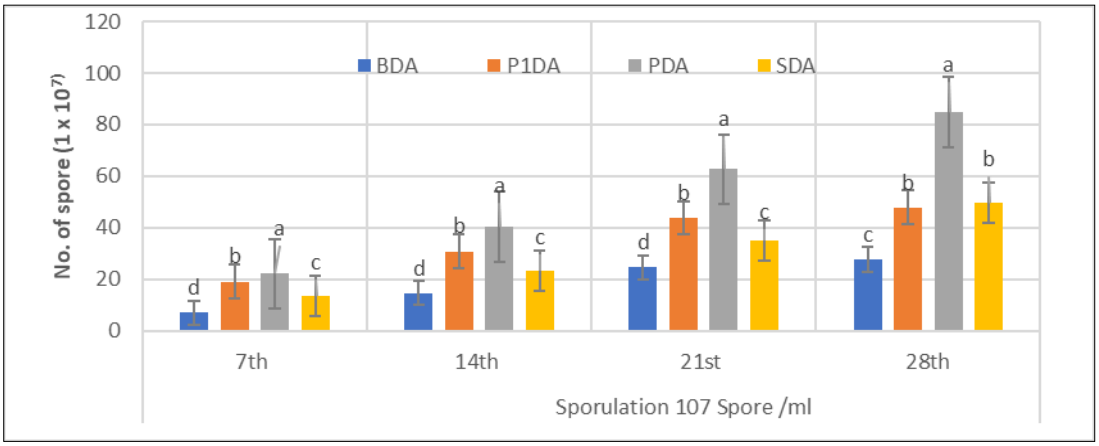


**Fig. 5.** Spore production of *B. bassiana* on artificial media.

**Table 5.** Cost efficiency of different artificial media for sporulation of *B. bassiana*

| Artficial media | Total cost (₹ mL$^{-1}$) | Mean number of spore mL$^{-1}$ | Spore production (per ₹) (1 x 10$^7$) |
|---|---|---|---|
| BDA | 0.0016 | 18.50 | 11419.75 |
| $P_1$DA | 0.0016 | 35.42 | 21862.96 |
| PDA | 0.0021 | 52.54 | 24783.02 |
| SDA | 0.0067 | 30.33 | 4500.29 |

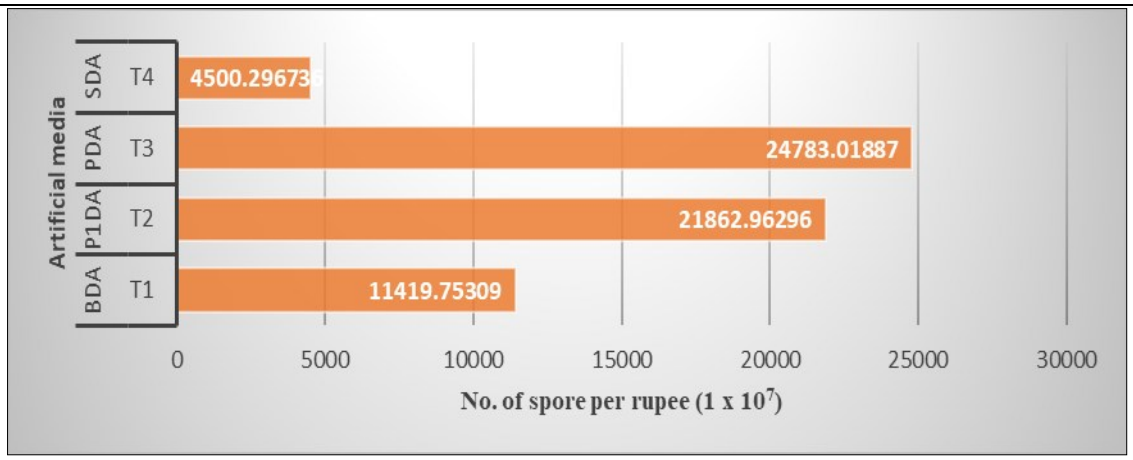


**Fig. 6.** Cost efficiency of artificial media used for *B. bassiana* mass production.

## Conclusion

This investigation confirms that substrate selection is a critical determinant in the efficient mass multiplication of *B. bassiana*. Among grain-based media, sorghum was the most effective, offering the highest spore yield (90.96 × 10$^7$ spores g$^{-1}$), rapid growth rate (138.68 % in mid-development) and superior cost-efficiency (2860.35 × 10$^7$ spores ₹$^{-1}$), owing to its favourable structure and nutrient profile. Chickpea and wheat were also effective alternatives, while maize and rice were suboptimal both biologically and economically. Instead of whole rice, rice husk can be utilized as a substrate to enhance the growth and production of fungal spores (35). Among artificial media, Potato Dextrose Agar (PDA) outperformed others in radial growth (36.83 mm), sporulation (52.54 × 10$^7$ spores mL$^{-1}$) and cost-effectiveness (24783.02 × 10$^7$ spores ₹$^{-1}$), due to its balanced nutrient composition. Papaya Peel Dextrose Agar ($P_1$DA) proved to be a viable, low-cost alternative.

Overall, sorghum and PDA are identified as the most suitable substrates for large-scale, cost-effective and consistent production of *B. bassiana*. In the cost efficacy analysis, Only the cost of substrates was considered; labour and machinery costs were not included, as the comparison focused solely on substrate-based cost differences. These findings provide a practical basis for enhancing the efficiency of biological control programs in sustainable and integrated pest management systems.

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## Authors' contributions

PK and VS contributed to data collection and manuscript preparation. PK, RSM and SPS were involved in the study, while RSM and SVSR developed the concept and design. GSG and VS handled manuscript review and editing. All authors reviewed and approved the final version of the manuscript.

## Compliance with ethical standards

**Conflict of interest:** The Authors do not have any conflicts of interest to declare.

**Ethical issues:** Not applicable

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