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Characterization of Endophytic Entomopathogenic Fungal Isolates: Growth Dynamics, Viability, Density, and Morphological Attributes

S. S. Monica [a*], P. Rajanikanth [a], P. Duraimurugan [b], S. Ameer Basha [a] and D. Srinivasa Chary [a]

[a] Professor Jayashankar Telangana State Agriculture University, Hyderabad, India.
[b] Indian Institute of Oilseeds Research, Hyderabad, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author SSM did the conceptualization, methodology design, fieldwork, data collection and analysis, manuscript drafting. Author PR did the study conceptualization, entomopathogenic fungi expertise, fieldwork assistance, data analysis, manuscript preparation. Author PD did the fungal ecology expertise, methodology design, data interpretation, manuscript writing. Author SAB did the fieldwork participation, data collection and analysis, critical manuscript input. Author DSC did the project supervision, guidance, manuscript review and editing, funding acquisition. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aims to comprehensively characterize endophytic entomopathogenic fungal isolates, focusing on their growth dynamics, viability, density, and morphological attributes, with implications for biocontrol strategies against insect pests.

**Corresponding author: E-mail: monicasubburaj30@gmail.com;*

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Study Design: The research tracked radial growth rates of fungal isolates over 21 days, conducted conidial viability assessments at 24 and 48 hours, measured conidial density, and performed morphological analysis to identify distinct characteristics.
Methodology: Endophytic entomopathogenic fungal isolates, including Beauveria bassiana and Lecanicillium lecanii, were subjected to radial growth rate tracking, viability assessments, density measurements, and morphological analysis. Statistical analysis was employed to identify significant variations among isolates and time intervals.
Results: Significant variations in radial growth rates were observed among isolates, with Beauveria bassiana isolate ChBb exhibiting a rate of 3.66 mm/day, followed by B. bassiana isolate NaBb with 3.92 mm/day, and Lecanicillium lecanii isolate KmLl with 3.06 mm/day. Conidial viability assessments revealed temporal dynamics, with NaBb maintaining the highest viability at both 24 and 48 hours. Additionally, significant differences in conidial density were noted, with NaBb exhibiting the highest density followed by ChBb and KmLl. Morphological analysis unveiled distinct characteristics among isolates, including differences in conidia length, width, and length-to-width ratio.
Conclusion: The findings underscore the potential of Beauveria bassiana and Lecanicillium lecanii isolates in biocontrol strategies against insect pests, highlighting their varying growth dynamics, viability, density, and morphological attributes. These insights contribute to the understanding of entomopathogenic fungi and their applications in sustainable pest management practices.

Keywords: Entomopathogenic fungi; radial growth; conidial density; endophyte; viability; morphology.

1. INTRODUCTION

Endophytes, comprising fungi and bacteria, are microorganisms that establish symbiotic relationships within healthy plants, contributing to plant health and defense mechanisms by producing bioactive compounds such as antibiotics, enzymes, and secondary metabolites [1]. Among these, entomopathogenic fungal endophytes stand out for their ability to infect insects while residing asymptomatically in plant tissues. Extensive research has explored their potential applications in pest management and their capacity to enhance plant growth and resistance to pathogens [2,3]. For instance, treatments utilizing *B. bassiana* have demonstrated efficacy in protecting emerging tomato and cotton seedlings against fungal pathogens [4].

Endophytic entomopathogenic fungi, notably *B. bassiana* and *L. lecanii*, have emerged as focal points of research due to their promising applications in biological control strategies against insect pests. Understanding key characteristics such as growth dynamics, viability, density, and morphological attributes of these fungal isolates is paramount for elucidating their efficacy and optimizing their utilization in biocontrol programs [5,6,7]. Radial growth dynamics serve as critical indicators of fungal colony vigour and proliferation potential [8,9]. Viability assessments, including spore survival over time, play a crucial role in evaluating the effectiveness of fungal treatments [10]. Additionally, conidial density measurements provide insights into the reproductive capacity of fungal isolates, directly influencing their biocontrol effectiveness. Moreover, morphological characterization offers a deeper understanding of fungal behavior and interactions with target pests [11]. By integrating these parameters, researchers can comprehensively evaluate the growth, viability, reproductive potential, and behavior of fungal isolates, essential for developing effective biocontrol strategies against pests in agriculture and other fields. In this study, we undertake a systematic examination of the growth dynamics, viability, density, and morphological attributes of endophytic entomopathogenic fungal isolates, with a specific focus on *B. bassiana* and *L. lecanii*. By elucidating these key characteristics, we aim to provide valuable insights into the potential of these fungal isolates for integrated pest management strategies, ultimately contributing to sustainable and environmentally friendly pest control practices.

2. MATERIALS AND METHODS

2.1 Radial Growth

Spores from cultures aged 14 days were collected from plates and evenly distributed on separate 90 mm Petri dishes filled with fresh SDA and PDA media. These isolates were then allowed to incubate for a period of three days at

a temperature of 27±1°C. After the three-day incubation, a 5 mm diameter section of the mycelium from the cultures was extracted using a cork borer and positioned at the center of individual 90 mm Petri dishes containing SDA and PDA media. The cultures were subsequently incubated at 27±1°C, following the protocol outlined by Membang et al. [12]. The study was carried out using a Completely Randomized Design (CRD) with three different treatments, and each treatment was replicated seven times. Data on radial growth (RG) were recorded in mm/day for every three days interval from the 3rd to the 21st days and calculated by:

RG/day= (Colony diameter at the end of incubation period − Fungal disc diameter/ Total incubation days)

Where, RG/day is radial growth per day.

2.2 Conidial Density / Conidial Concentration

Conidial density calculations were conducted for endophytic fungi aged 7 days. The enumeration of conidial density followed the procedure outlined by Sumikarsih et al. [13]. A fungal suspension measuring 10 x 10 mm was collected from a 7-day-old culture plate, and 10 ml of sterilized distilled water was added to it. Subsequently, the fungal suspension underwent vortexing using a turbo mixer for 20 seconds to achieve a homogeneous suspension. This suspension was then diluted by adding 9 ml of distilled water into 1 ml of the suspension and homogenizing it. The final suspension culture was counted for its conidial density using a hemocytometer under a compound microscope at 400X magnification. The experiment was carried out in a completely randomized design with three treatments and seven replications under laboratory conditions.

Conidial density= (Number of spores counted X Dilution factor/ Volume of sample)

2.3 Spore Germination Test for the Viability of Fungal Isolates

The validation of the spore germination test followed standard procedures, specifically the conidial germination test technique as outlined by Goettel and Inglis [14]. Fungal spores were obtained from a 21-day-old culture through scraping with a sterilized spatula. These harvested spores were then mixed with 10 ml of sterile water containing Tween 80 (0.001% v/v) as a surfactant in a falcon tube, followed by vortexing. The concentration of fungal spores was adjusted to 1×10^8 conidia ml^{-1} using a Neubauer hemocytometer (USA) under a SC50 microscope. A total of 100 µl of the spore suspension was evenly spread on fresh Potato Dextrose Agar (PDA), and two sterilized glass slides were positioned on the media. The setup was incubated at 28°C and observed at 1 x 24 hr and 2 x 24 hr. After this incubation period, spore germination was arrested by applying 70% ethanol. Both germinated and non-germinated cells were quantified using a Neubauer hemocytometer under a SC50 microscope with a magnification of ×40. The experiment adhered to laboratory conditions using a Completely Randomized Design (CRD) with a three number of treatments and seven replications.

Germination was determined by considering a germ tube with more than half the diameter of the spore as germinated, and conversely for non-germinated spores. The percentage of spore germination was calculated following the methodology established by Vega et al. [15].

Percent spore germination= (Number of spores germinated/ Total spore counted) × 100

3. RESULTS AND DISCUSSION

3.1 Radial Growth

The research investigated the radial growth rates of three endophytic fungal isolates (ChBb, NaBb, and KmLl) over a period spanning 3 to 21 days with three-day intervals, with a focus on calculating the radial growth per day. Significant variations were observed among the isolates and over different time intervals regarding radial growth, indicating noteworthy differences in their growth patterns. The radial growth patterns of three endophytic entomopathogenic fungal isolates (ChBb, NaBb, and KmLl) over a 21-day period, measured at intervals of three days. At the initial observation on day 3, ChBb exhibited the largest colony diameter at 18.86 mm, followed by NaBb with 15.29 mm and KmLl with 12.86 mm. As the observation period progressed, all isolates displayed consistent increases in colony diameter. By day 21, ChBb had the largest colony diameter at 81.86 mm, followed by NaBb at 87.29 mm and KmLl at 69.29 mm. Regarding the relative growth per day, NaBb consistently demonstrated the highest

Table 1. Radial growth rate of the endophytic entomopathogenic fungal isolates at different time intervals

Isolate	Colony diameter (mm) ± SE at different time intervals							RG/ day (mm)
	3	6	9	12	15	18	21	
ChBb	18.86±0.57[a]	32.29±0.81[b]	45.14±0.70[b]	63.29±0.57[b]	71.57±1.00[b]	79.71±0.92[b]	81.86±0.70[b]	3.66[b]
NaBb	15.29±0.42[b]	35.57±0.97[a]	47.86±0.40[a]	65.43±0.90[a]	77.14±0.51[a]	82.57±1.43[a]	87.29±0.89[a]	3.92[a]
KmLl	12.86±0.67[c]	24.86±0.67[c]	36.43±1.04[c]	48.00±0.44[c]	59.71±0.47[c]	64.29±0.61[c]	69.29±0.47[c]	3.06[c]
SE(m)	0.07	0.07	0.06	0.04	0.04	0.06	0.04	0.008
CD	0.20	0.22	0.18	0.12	0.12	0.17	0.12	0.02

Data are the mean of seven replications; Means followed by the same letter within a column are not significantly different according to Duncan's Multiple Range Test (DMRT) test, at α ¼ 0.05

Table 2. Spore viability/ germination percentage of endophytic entomopathogenic fungal isolates

Isolate	Conidial viability ± SE	
	24 hrs	48 hrs
ChBb	91.14 ± 0.26[b]	93.14 ± 0.40[a]
NaBb	93.20 ± 0.51[a]	94.14 ± 0.51[a]
KmLl	90.20 ± 0.42[b]	91.43 ± 0.43[b]
SE(m)	0.36	0.52
CD	1.08	1.54

Data are the mean of seven replications; Means followed by the same letter within a column are not significantly different according to Duncan's Multiple Range Test (DMRT) test, at α ¼ 0.05

Table 3. Conidial density of the endophytic entomopathogenic fungal isolates

Isolate	Conidial density ± SE (1 x 10^8 conidia / ml)
ChBb	4.90 ± 0.12[b]
NaBb	5.51 ± 0.13[a]
KmLl	4.72 ± 0.08[b]
SE(m)	0.07
CD	0.02

Table 4. Conidial size of the endophytic entomopathogenic fungal isolates

Isolate	Conidial length (µm ± SE)	Conidial width (µm ± SE)	Length/ width ratio
ChBb	3.62 ± 0.16[a]	2.45 ± 0.06[a]	1.48 ± 0.08[b]
NaBb	3.19 ± 0.11[b]	1.86 ± 0.06[b]	1.72 ± 0.08[a]
KmLl	2.20 ± 0.10[c]	1.81 ± 0.04[c]	1.22± 0.07[c]
SE(m)	0.03	0.02	0.02
CD	0.09	0.05	0.07

rate, averaging 3.92 mm/day, followed by ChBb at 3.66 mm/day and KmLl with the lowest rate of 3.06 mm/day (Table 1). Radial growth dynamics vary among substrates, with Afifah and Saputro [16] showing similar growth among maize, mungbean, and potato dextrose agar (PDA) media. Gebremariam et al. [17] reported radial growth rates of B. bassiana isolates reaching up to 3.43 mm/day for AAUMFB-77 on PDA media at 25°C. Geremew et al. [18] revealed a radial growth rate of 3.24 mm/day for *B. bassiana* from rhizosphere soil. The study emphasizes the significance of conidial density in fungal biocontrol efficacy against insect pests.

3.2 Conidial Viability

This study investigates the conidial viability of fungal spores at 24 and 48 hours. Three endophytic entomopathogenic fungal isolates, namely ChBb, NaBb, and KmLl, are examined. By assessing conidial viability at these two specific time points, the research aims to understand the temporal dynamics of fungal spore survival and to evaluate the effectiveness of the treatments in either promoting or inhibiting conidial viability. At the 24-hour interval, the research data demonstrates the conidial viability rates of three distinct endophytic entomopathogenic fungal strains (ChBb, NaBb, and KmLl). NaBb exhibited the highest viability at 93.20%, closely followed by ChBb at 91.14%. There was a significant difference in viability between NaBb and ChBb, while KmLl displayed the lowest viability at 90.20%, though not significantly different from ChBb. At the 48-hour interval, NaBb maintained its lead with a viability of 94.14%, suggesting either sustained or improved viability over time. ChBb also experienced an increase in viability, reaching 93.14%. However, there was no significant difference in viability between NaBb and ChBb at this time interval. Conversely, KmLl remained significantly different from both NaBb and ChBb, with the lowest viability at 91.43%. This slight enhancement in viability for KmLl compared to the 24-hour interval indicates some improvement over time (Table 2). Germination rates impact virulence, as shown by Gebremariam et al. [17] and Sumikarsih et al. [13], with spore germination percentage varying between 85.43% and 99.67% within 24 hours. Conidial germination of *Beauveria* isolates ranged from 76.33% to 95.75% [19], and 89.30% to 99% of spore viability were recorded by 22 isolates of *B. bassiana* and *M. robertsii* [20]. Afifah and

Saputro [16] documented viability percentages of 68.37% for maize and 72.83% for PDA media. However, direct cross-study comparisons may encounter constraints attributable to variances in experimental protocols and conditions. Geremew et al. [18] underscored dynamic spore germination kinetics, spanning from 79.33% to 99.03% within an 18-hour window.

3.3 Conidial Density

The conidial density of three endophytic entomopathogenic fungal isolates, namely ChBb, NaBb, and KmLl, was assessed and presented in Table 3. Among the isolates, *B. bassiana* isolate NaBb exhibited the highest mean conidial density, recorded at 5.51 x 10^8 conidia ml^{-1}. This density was significantly different from that of the other isolates. Following NaBb, ChBb displayed a mean conidial density of 4.90 x 10^8 conidia ml^{-1}. Conversely, the L. lecanii isolate KmLl demonstrated the lowest conidial density, recorded at 4.72 x 10^8 conidia ml^{-1}. Notably, there was no significant difference observed between the conidial densities of ChBb and KmLl isolates. These findings underscore the variation in conidial density among the examined fungal isolates and highlight the distinct characteristics of each isolate in terms of spore production (Table 3). *B. bassiana* isolate NaBb exhibited the highest mean conidial density at 5.51 x 10^8 conidia/ml, corresponding to CPJW8 and PD1 isolates with spore densities of 4.83 x 10^8 and 5.15 x 10^8 conidia/ml respectively [21]. Sumikarsih et al. [13] observed high conidial densities in Beauveria isolates, with the BTmSo isolate reaching 6.8 x 10^7 conidia/ml. In contrast, KmLl isolate had notably lower density at 4.72 x 10^8 conidia/ml [22]. Afifah and Saputro [16] reported comparable conidia density between maize and PDA media, while rice medium exhibited the lowest density at 4.49 x 10^7 conidia/ml. Membang et al. [12] obtained similar conidial density results at 4.63 x 10^8 conidia/ml.

3.4 Conidial Length and Width

The data presented in Table 4 illustrates the significant morphological variations observed among fungal isolates regarding spore length, width, and length-to-width ratio. Across all examined isolates, there were notable differences in both length and width, indicating distinct morphological characteristics. Specifically, *Beauveria* isolate ChBb exhibited the longest conidia, measuring 3.62 µm, followed by *B. bassiana* isolate NaBb with a mean conidial length of 3.19 µm. Conversely, the *L. lecanii* isolate displayed the shortest conidial length at 2.20 µm. In terms of width, *B. bassiana* isolate ChBb recorded the widest spores at 2.45 µm, followed by NaBb (1.86 µm), while KmLl displayed the narrowest width at 1.81 µm. The calculated length-to-width ratio, a parameter indicative of spore morphology further elucidated the observed morphological variations. Despite *Beauveria* isolate ChBb having the maximum length and width, the highest length-to-width ratio was observed in B. bassiana isolate NaBb (1.72 µm), suggesting a more elongated or slender morphology. Following NaBb, isolate ChBb (1.48 µm) exhibited a substantial length-to-width ratio, indicating a similar elongated morphology albeit to a slightly lesser extent. In contrast, the L. lecanii isolate KmLl displayed the lowest length-to-width ratio of 1.22 µm indicating a comparatively more spherical or compact morphology. Conidial lengths vary among studies, with B. bassiana isolates ranging from 2.08 to 3.24 µm [18]. Doolotkeldieva et al. [23] reported conidial lengths and widths of 2.27 µm and 1.85 µm respectively. Sugimoto et al. [24] observed broader conidial length ranges in *L. lecanii*, from 5.8 to 10.5 µm. Ramarethinam et al. (2005) provided insights into *L. lecanii* spore morphology, revealing aerial spores with relatively uniform sizes at 6.1 ± 0.9 µm in length and 2.2 ± 0.3 µm in width under diverse cultivation conditions.

4. CONCLUSION

In summary, the research findings underscore the intricate dynamics of endophytic entomopathogenic fungal isolates, encompassing radial growth, conidial viability, density, and morphological attributes. Regarding radial growth, significant variations among isolates and over time intervals were observed. NaBb consistently exhibited the highest growth rate, with an average radial growth of 3.92 mm/day, followed by ChBb at 3.66 mm/day and KmLl with the lowest rate of 3.06 mm/day. These differences were evident from the initial observation on day 3, where ChBb displayed the largest colony diameter at 18.86 mm, followed by NaBb with 15.29 mm and KmLl with 12.86 mm. By day 21, ChBb had the largest colony diameter at 81.86 mm, followed by NaBb at 87.29 mm and KmLl at 69.29 mm. Analysis of conidial viability at 24 and 48 hours revealed differences among isolates. At the 24-hour interval, NaBb exhibited the highest viability at 93.20%, closely followed by ChBb at 91.14%, while KmLl displayed the lowest viability at 90.20%. By the 48-hour interval, NaBb maintained its lead with a viability

of 94.14%, followed by ChBb at 93.14%, and KmLl at 91.43%. Conidial density variations among isolates further underscore their distinct characteristics in spore production. NaBb exhibited the highest mean conidial density at 5.51 x 10^8 conidia/ml, followed by ChBb at 4.90 x 10^8 conidia/ml, and KmLl with the lowest density at 4.72 x 10^8 conidia/ml. Morphological analysis revealed significant variations in spore length, width, and length-to-width ratio among isolates. Beauveria isolate ChBb exhibited the longest conidia at 3.62 µm, followed by B. bassiana isolate NaBb with a mean conidial length of 3.19 µm. B. bassiana isolate ChBb recorded the widest spores at 2.45 µm, followed by NaBb at 1.86 µm, while KmLl displayed the narrowest width at 1.81 µm. Despite ChBb having the maximum length and width, the highest length-to-width ratio was observed in NaBb (1.72 µm), followed by ChBb (1.48 µm), and KmLl with the lowest ratio at 1.22 µm. In conclusion, these numerical findings provide a comprehensive understanding of the biology and potential applications of endophytic entomopathogenic fungal isolates in sustainable pest management strategies. Further research in this area could enhance the development of tailored biocontrol measures harnessing the unique attributes of these fungal isolates.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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