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Fungal Biopriming: *Ophiocordyceps neovolkiana* Mycelial Extract Enhances Rice (*Oryza sativa* L) Seed Germination

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Rice (*Oryza sativa* L.) is an important global staple, with promising germination being a pivotal factor for optimum yield and crop establishment. This study investigated the potential of *Ophiocordyceps neovolkiana*, an entomopathogenic fungus native to Kerala, as a novel biopriming agent to increase rice seed germination. Rice seeds of the Jyothi variety were sterilized and bioprimed by soaking in mycelial extract of *O. neovolkiana* for 30 minutes. Per cent germination was observed over one week. Results demonstrated a significant increase in germination percentage for seeds treated with the mycelial extract (94%) compared to both the solvent control (79%) and the absolute control (80%). Furthermore, the mycelial extract treatment induced earlier germination (3.25 days) compared to the solvent control (5 days) and the absolute control (4.75 days). These findings align with existing research on various microbial biopriming agents that enhance seed vigor

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and early seedling development. This study provides convincing evidence that *O. neovolkiana* mycelial extract is a promising biological tool for improving rice seed germination, offering a sustainable method to boost crop establishment and productivity.

Keywords: Rice; biopriming; germination; Ophiocordyceps neovolkiana.

1. INTRODUCTION

Cordyceps, a genus of parasitic fungi, has been used in traditional Chinese medicine for centuries. Well-known species like *Ophiocordyceps sinensis* and *Cordyceps militaris* are recognized for their diverse medicinal properties, including antiviral, antifungal, antibacterial, anticancer, and immunomodulatory actions. Beyond medicinal values, the non-pathogenic efficiency of this entomopathogen is also reported. Several studies have documented the endophytic nature of *Ophiocordyceps* (Evans *et al.*, 2011; Gazis *et al.*, 2014; Wang *et al.*, 2020; Saltamachia and Araujo, 2020). Seed treatment with *Cordyceps fumosorosea* on brinjal seeds significantly improved plant growth and reduced the incidence of the sucking pest *Bemisia tabaci* (Sun *et al.*, 2020). *Ophiocordyceps neovolkiana*, a species endemic to the Kasaragod district of Kerala, India, parasitizes coconut root grubs (*Leucopholis coneophora* Burm.). The comprehensive characterization of *O. neovolkiana*, including its morphology, physiology, and molecular characteristics, has been previously documented (Laya, 2018). This study investigates the impact of *O. neovolkiana* mycelial extract on the germination of 'Jyothi', a prominent rice variety cultivated in Kerala. Rice is a globally significant crop, with approximately 163.1 million hectares cultivated worldwide, yielding 520.5 million metric tons at an average of 4.00 tons per hectare (FAO, 2025). Asia dominates both global rice production and consumption. Achieving rapid and uniform seed germination and seedling emergence is crucial for optimizing rice yield and quality. Poor germination and seedling uniformity can severely reduce production. High-vigor seeds, characterized by uniform and rapid germination, are essential for robust seedling growth, enhanced tolerance to adverse conditions, and ultimately, higher yields. The initial stages of plant development are foundational for the entire crop cycle. Uniform germination promotes more effective competition with weeds and optimizes resource utilization, including light, water, and nutrients (Joshi *et al*., 2013; Dass *et al*., 2017). Furthermore, a strong start during germination minimizes the incidence of pests and diseases, fostering healthier plants and increasing yield potential (Chithrashree *et al.*, 2011; Pal *et al.*, 2021; Xu *et al.*, 2023). Efficient germination is particularly vital in direct-sown rice systems, where planting depth control is limited (Farooq *et al.*, 2009). Studies indicate that rice cultivars with superior germination rates often exhibit higher yields due to the development of robust root systems that support growth and nutrient uptake throughout the crop cycle (Yang *et al.,* 2022; Zhang *et al.,* 2023). Moreover, enhanced germination efficiency reduces seed usage, thereby offering economic advantages to farmers and supporting environmentally sustainable agricultural systems. Thus, achieving a high germination rate is a critical determinant for successful rice growth and productivity.

2. MATERIALS AND METHODS

2.1 Isolation and Culturing of the Fungus

Isolation of the fungus was carried out from the stroma of a fresh fruiting body by following standard tissue culture technique. The sample was washed under running tap water and cut into small pieces, then disinfected with 1% sodium hypochlorite solution for one minute. After three washings using sterilized distilled water, the tissue bits were placed aseptically on solidified potato dextrose agar (PDA) medium in sterile Petri dishes under a laminar airflow chamber. All dishes were incubated at room temperature (28 ± 2ºC) and observed from the next day onwards. The fungal growth obtained was subsequently sub cultured to Yeast Extract Potato Fructose broth (Laya, 2018).

2.2 Mycelial Extract Preparation

After complete growth, the mycelium was harvested from the growing medium and freeze-dried. The dried sample was then extracted by stirring with methanol (40 ml methanol/1g sample) for 1 hour in a magnetic stirrer and subsequently filtered through Whatman No. 1 filter paper. The residue was then extracted again with methanol (20 ml methanol/1g sample) for 1 hour by following the same procedure. The combined methanolic extract was evaporated to dryness at 40°C in a rotary evaporator and re-suspended in sterile distilled water (Reis *et al.*, 2013).

2.3 Biopriming

Rice seeds (100 grains per treatment) were surface sterilized with 1% sodium hypochlorite solution for two minutes, followed by three rinses with sterile distilled water. Subsequently, 25 sterile seeds were placed into each of four replicate Petri plates and soaked in 20 mL of the mycelial extract for 30 minutes (Purwanto *et al.*, 2020). These treated seeds were then transferred to Petri plates lined with double-layered moist cotton, which were then covered with an upper lid containing moist blotter paper. For comparative analysis, a solvent control (treated with methanol) and an absolute control (treated with sterile distilled water) were maintained (Fig. 1). All setups were incubated for one week in a plant growth chamber under optimum temperature and relative humidity. Seed germination was then assessed across all treatments.

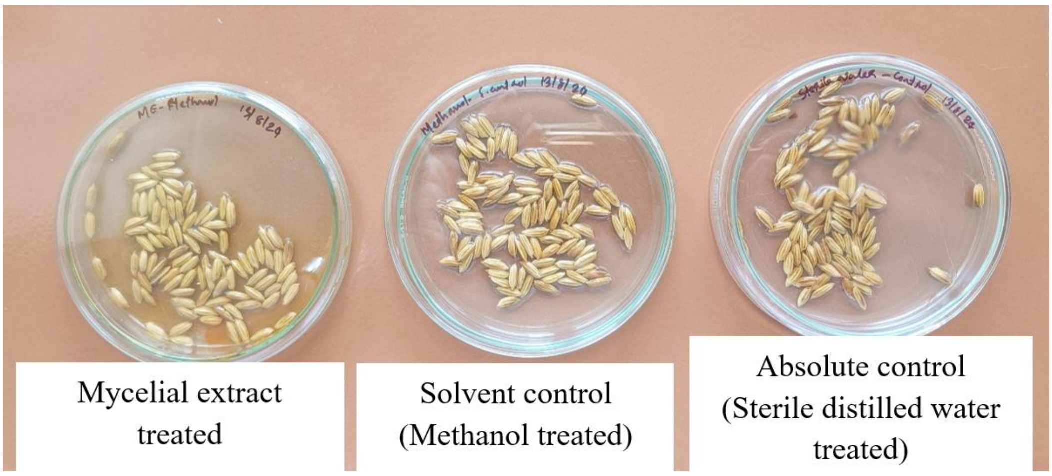


Fig. 1. Rice seeds treated with mycelial extract, methanol and distilled water

3. RESULTS AND DISCUSSION

There was a significant increase in percent germination in the mycelial extract treatment (94%) compared to the solvent control (79%) and absolute control (80%) (Fig. 2, Table 1, Graph 1). It was also observed that there was an induction of early germination in the mycelial extract treatment (3.25 days) compared to the solvent control (5 days) and absolute control (4.75 days) (Table 2, Graph 2). Data were subjected to analysis of variance (ANOVA) using the statistical package GRAPES (General R-based Analysis Platform for Experimental Statistics) developed by the Department of Agricultural Statistics, Kerala Agricultural University (Gopinath *et al*., 2020).

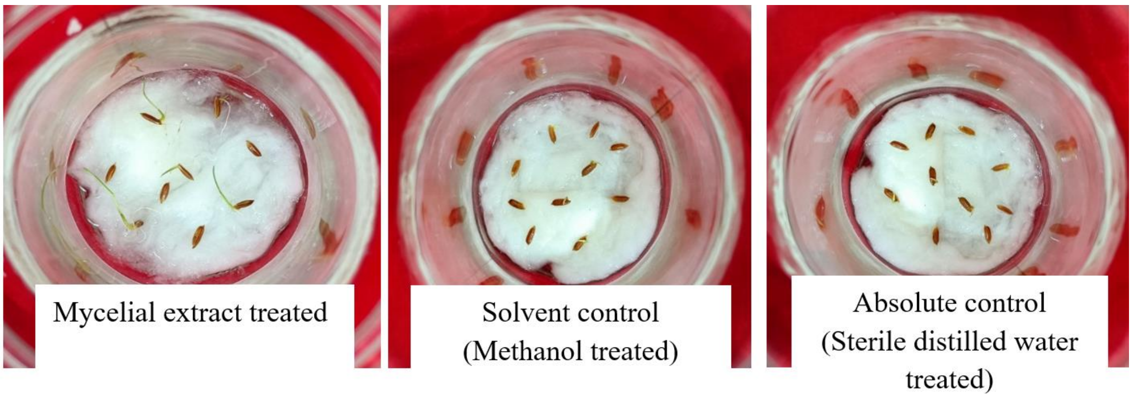
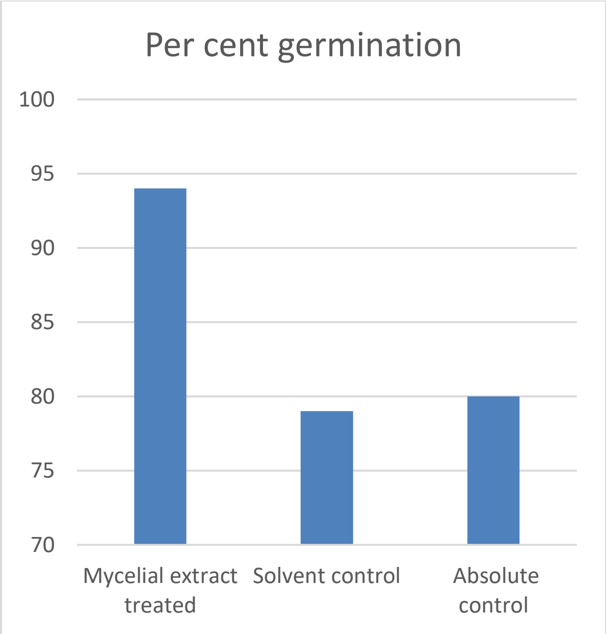


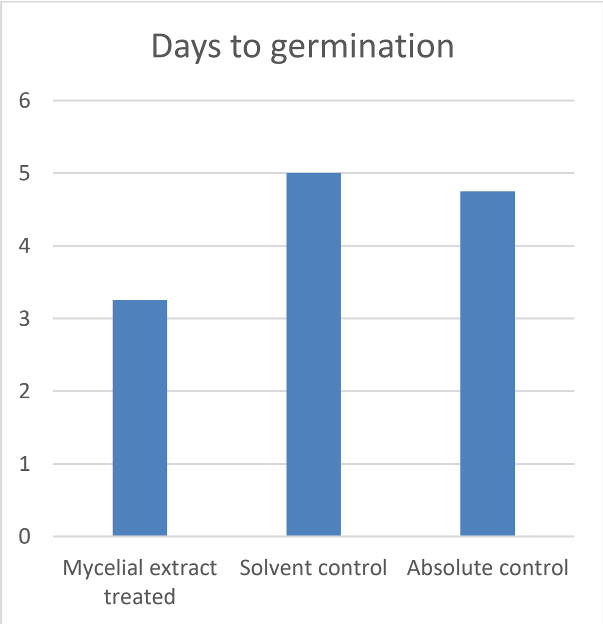
Fig. 2. Germinated rice seeds in different treatments

Table 1. Per cent germination in different treatments (mean of four replications, figures followed by same letter do not differ significantly according to LSD test, p-value is 0.04, since this is less than 0.05, there is a significant difference between at least one pair of treatments.)

Treatments	Per cent germination±SD
Mycelial extract treated	94±2.31[a]
Solvent control	79±8.64[b]
Absolute control	80±10.00[b]
CD	12.39
SE(m)	3.87



Graph 1. Per cent germination in different treatments



Graph 2. Days to germination in different treatments

Table 2. Days to germination in different treatments (mean of four replications, figures followed by same letter do not differ significantly according to LSD test, p-value is 0.007, since this is less than 0.05, there is a significant difference between at least one pair of treatments)

Treatments	Days to germination±SD
Mycelial extract treated	3.25±0.50[b]
Solvent control	5±0.50[a]
Absolute control	4.75±0.82[a]
CD	1.00
SE(m)	0.31

The positive outcomes observed in the present study regarding the effect of *O. neovolkiana* mycelial extract on rice germination are well-supported by and align with a growing body of research demonstrating the efficacy of biological priming agents in enhancing seed performance. Similar to the significant improvements in seed germination rates, growth parameters, and root traits reported with *Bacillus megaterium* 3456789 by Vaishnavi et al. (2024), Li et al. (2025) reported that multiple plant growth-promoting fungi, such as *Aspergillus tubingensis*, *A. brunneoviolaceus*, several *Penicillium* species, and *Talaromyces purpureogenus* isolated from converted non-grain land, substantially enhanced rice seedling height, root development, and fresh/dry biomass. These enhancements were achieved through mechanisms including phosphate solubilization, siderophore production, and IAA synthesis. The *O. neovolkiana* extract likely promotes initial seed development and overall seedling vigor through similar mechanisms. This aligns with findings on liquid microbial cultures in paddy by Raja *et al*. (2017), which also showed enhanced and more uniform germination rates, even under suboptimal conditions, suggesting a broad resilience-improving effect that the *O. neovolkiana* extract might also confer. The effectiveness of specific microbial isolates, such as the superior performance of *Trichoderma harzianum* WAI-D in improving germination and vigor in rice, provides a precedent for the study on targeted benefits of particular fungal extracts by Devi *et al*. (2019), indicating that *O. neovolkiana* could similarly possess unique beneficial properties. Furthermore, the enhanced photosynthetic efficiency and increased antioxidant activity seen with yeast extract priming in rice done by Johnson and Puthur (2021), which led to reduced oxidative stress and improved stress adaptation, suggest that the *O. neovolkiana* extract might also induce beneficial physiological and biochemical changes within the germinating seeds. This concept is further reinforced by the general understanding that beneficial microorganisms, as discussed in a review on various crops by Cardarelli (2022), improve nutrient uptake and plant resilience to abiotic stress, contributing to overall better plant growth, a benefit likely shared by the *O. neovolkiana* treatment. The remarkable increases in dry weight, fresh weight, and root length observed with plant endophyte extracts on rice seedlings in the study by Wang (2014) underscore the potential for substantial growth promotion beyond just germination, a broader impact that future studies on *O. neovolkiana* could investigate. Moreover, just as in the study conducted by Makhaye (2021) on biopriming with seaweed extract and microbial-based biostimulants influenced various germination parameters in *Abelmoschus esculentus*, the *O. neovolkiana* extract likely optimizes the timing and success rate of germination. The study on mycorrhizal fungi in curly chili carried out by Meo (2024), which improved germination quality and vigor, further highlights the diverse fungal agents capable of enhancing early plant establishment. Finally the improved seedling establishment and yield from seed priming combined with *Trichoderma* application in Boro rice by Rahman (2015), and significant enhancements in germination speed, root/shoot growth, dry matter production, and vigor index achieved by biopriming rice seeds with phosphobacteria by Sivakumar (2017), and, noticeable enhancements in rice growth metrics on biopriming using rhizobacteria (e.g., *Bacillus altitudinis, Herbaspirillum huttiense, Pseudomonas mohnii*) by Ishaq *et al.* (2025) collectively provide strong evidence that the positive results from the *O. neovolkiana* mycelial extract represent a promising avenue for sustainable agricultural practices aimed at improving rice crop performance.

4. CONCLUSION

The application of *O. neovolkiana* mycelial extract significantly enhances rice seed germination, leading to both a higher percentage of germination and an induction of early sprouting compared to control groups. These positive results align comprehensively with a growing body of scientific literature that demonstrates the efficacy of various biological priming agents in improving seed vigor and seedling establishment across different crops. The observed improvements in germination, root and shoot development, and overall plant health in these comparative studies suggest that *O. neovolkiana* mycelial extract holds considerable promise as a novel biostimulant for rice. This research underscores the potential for sustainable agricultural practices, offering an eco-friendly alternative to conventional methods for improving rice productivity and ensuring robust crop establishment, which is crucial for global food security. Further investigation into the specific mechanisms of action and optimal application strategies for *O. neovolkiana* mycelial extract could pave the way for its broader adoption in rice cultivation.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

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

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDICES

Appendix 1. CRD analysis of per cent germination

Treatment mean and other statistics:

	Treatment_means	std	LCL	UCL	Min	Max
T1	94	2.309	85.239	102.761	92	96
T2	80	8.641	71.239	88.761	72	92
T3	79	10.000	70.239	87.761	68	92

ANOVA table:

SoV	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	2	562.667	281.333	4.689	0.04
Error	9	540.000	60.000	NA	NA

Other Important Statistics:

MSE	SE(d)	SE(m)	CV(%)
60	5.477	3.873	9.185

Since the P-value in ANOVA table is < 0.05, There is a significant difference between at least a pair of treatments, so multiple comparison is required to identify best treatment(s)

LSD test:

MSerror	Df	Mean	CV	t.value	LSD
60	9	84.333	9.185	2.262	12.39

Treatment Grouping:

	trt_mean	Grouping
T1	94	a
T2	80	b
T3	79	b

Treatments with same letters are not significantly different

Appendix 2. CRD analysis of days to germination

Treatment mean and other statistics:

	Treatment_means	std	LCL	UCL	Min	Max
T1	3.25	0.500	2.545	3.955	3	4
T2	4.75	0.500	4.045	5.455	4	5
T3	5.00	0.816	4.295	5.705	4	6

ANOVA Table:

SoV	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	2	7.167	3.583	9.214	0.007
Error	9	3.500	0.389	NA	NA

Other important Statistics:

MSE	SE(d)	SE(m)	CV(%)
0.389	0.441	0.312	14.391

Since the P-value in ANOVA table is < 0.05, there is a significant difference between atleast a pair of treatments, so multiple comparison is required to identify best treatment(s)

LSD test:

MSerror	Df	Mean	CV	t.value	LSD
0.389	9	4.333	14.391	2.262	0.998

Treatment Grouping:

	trt_mean	Grouping
T3	5.00	a
T2	4.75	a
T1	3.25	b

Treatments with same letters are not significantly different

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