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ORIGINAL ARTICLE

Bioprospecting of Endophytic Mycobiota Associated with Traditional Rice Varieties for Functional Traits

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ABSTRACT
Plant-associated endophytic mycobiota constitute a rich microbial niche with immense potential for bioactive metabolite production and adaptive traits relevant to sustainable agriculture. The present study was aimed to isolate and evaluate fungal endophytes from seeds of traditional rice varieties of Tamil Nadu for their bioactive potential and functional traits. Four endophytic fungi isolated from Vaalan and Seeraga Samba seeds namely Ovatospora brasiliensis (V1), Aspergillus quadrilineatus (V2), Aspergillus tamarii (SS2), and Aspergillus flavus (SS6) were screened against fungal pathogens (Macrophomina phaseolina, Fusarium oxysporum, and Colletotrichum sp.) and bacterial pathogens (Xanthomonas oryzae pv. oryzae, Bacillus subtilis, and Pseudomonas aeruginosa). Among the isolates, SS6 and SS2 showed maximum inhibition against Colletotrichum sp. and Macrophomina phaseolina. All the isolates exhibited cellulase, amylase, lipase, protease, and pectinase activities, while SS2 and SS6 showed chitinase, and V1 and V2 exhibited peroxidase activity. Laccase and ligninase production were observed only in Ovatospora brasiliensis. The endophytic strains also showed a significant level of salt tolerance up to 100 mM concentration. The isolates were found to possess varied sensitivity and resistance towards commercial fungicides such as bavistin, tricyclazole and sixer. The antimicrobial activity, enzymatic production, tolerance to salt and fungicide resistance of these endophytes indicate their role in strengthening plant defense, stress conditions and microbiome interactions. The study highlights the potential of seed-borne fungal endophytes as sustainable and eco-friendly biological agents for growth and development of crop plants.
Keywords: *Traditional rice, fungal endophyte, antibacterial, antifungal, extracellular enzymes, salt tolerance, fungicide.*

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INTRODUCTION

Traditional rice varieties represent an important component of cultural heritage and nutritional security owing to their richness in essential micronutrients retained in the bran layer, unlike many modern polished rice cultivars [1]. These indigenous rice varieties are increasingly valued for their health benefits and their ability to tolerate diverse environmental stresses developed through adaptation to varied agroclimatic conditions [2]. Such resilient plant systems also harbor diverse communities of endophytic microorganisms that inhabit internal plant tissues without causing immediate harm to the host. Fungal endophytes are known to enhance plant growth and provide protection against biotic and abiotic stresses, including pathogen and pest attacks [3,4]. The composition and functional properties of endophytic fungi differ among plant species and even among varieties of the same crop, making each endophytic association unique. Certain endophytes possess plant growth-promoting and antagonistic traits that can be effectively exploited in integrated crop management programmes as sustainable biocontrol agents [5]. The extensive use of chemical fertilizers and pesticides has adversely affected soil fertility, nutrient balance, and agricultural sustainability, ultimately reducing cultivable land quality and productivity [6,7]. Therefore, the exploration of novel endophytic fungi from traditional rice varieties has gained significant importance for developing eco-friendly alternatives in agriculture. Endophytic fungi are capable of suppressing phytopathogens through the production of antimicrobial metabolites and signaling compounds during host–pathogen interactions [8]. In addition, the secretion of extracellular enzymes such as cellulase, chitinase, protease, and pectinase enables these microorganisms to degrade pathogen cell walls and restrict their growth [9]. Characterization of endophytic fungi for antibacterial, antifungal, fungicidal tolerance, and extracellular enzymatic activities may therefore facilitate the identification of potent strains with applications in sustainable agriculture, biocontrol strategies, and industrial biotechnology. In view of these prospects, the present study focuses on the isolation and characterization of seed-associated fungal endophytes from the traditional rice varieties Vaalan and Seeraga Samba of Tamil Nadu, India, with special emphasis on their antimicrobial potential, extracellular enzyme production, salt tolerance, resistance towards commonly used fungicides, and for their possible utilization as efficient biological agents in sustainable crop management.

MATERIAL AND METHODS

ISOLATION OF ENDOPHYTIC FUNGUS FROM THE SEEDS OF TRADITIONAL RICE VARIETIES

In the current study, two traditional rice varieties, namely Vaalan and Seeraga Samba, were obtained from Swami Dyananda Educational Trust Farm, Manjakudi, Tamil Nadu. These seeds were surface sterilized and then placed over the potato dextrose agar medium [10].

AUTHENTICATION OF ENDOPHYTIC FUNGAL STRAINS WITH ACCESSION NUMBERS

The DNA of the four endophytic fungal strains obtained from the seeds of traditional rice varieties Vaalan and Seeraga Samba were isolated using the method given by White *et al.,* 1990 [11]. PCR amplification of the fungal endophytes was done using the 18S rRNA with universal primers ITS 1 and ITS 4 and the PCR products were sequenced at Eurofins Genomics India Pvt. Ltd., Karnataka, India. Sequences submitted to NCBI GenBank, to procure accession numbers and used for further studies.

ANTAGONISTIC ACTIVITY OF ENDOPHYTIC FUNGAL STRAINS

The antibacterial and antifungal activity of the fungal strains were determined using the agar plug method [12] and dual culture method respectively [13,14]. Each of the fungal isolates were tested against three bacterial (*Xanthomonas oryzae* pv. *oryzae*, *Bacillus subtilis*, *Pseudomonas aeruginosa*) and fungal pathogens (*Macrophomina phaseolina*, *Fusarium oxysporum* and *Colletotrichum* sp.).

EXTRACELLULAR ENZYME ACTIVITY

The extracellular enzyme activities of cellulase, amylase, laccase, lipase, pectinase, and protease produced by the endophytic fungal strains were evaluated using substrate-amended media following the method described by Sunitha *et al.,* 2013 [15]. Chitinase activity was assessed according to the method of Mendrofa *et al.,* 2021 with slight modifications [16]. Ligninase activity was assessed by inoculating Guaiacol amended agar media with endophytic fungal strains and incubated in dark for seven days at 28 C. Development of brown colouration around the mycelial discs, indicated the oxidation of guaiacol and the intensity of the colour developed,was observed and measured [17-19]. Endophytic fungal strains were tested for the production of peroxidases, by treating the mycelia with hydrogen peroxide solution and observed for the effervescence [20,21].

SCREENING FOR SALT TOLERANCE

Endophytic fungal strains were screened for salt tolerance using NaCl at 4 varying concentrations (0 mM, 50 mM, 100 mM, 200 mM), where the 0 mM serves as a control to calculate the salt tolerance index [22].

EFFECT OF FUNGICIDES ON FUNGAL ENDOPHYTES

Invitro resistance of the endophytic fungal strains against commercially available fungicides, namely Sixer (Mancozeb 63% WP + Carbendazim 12% WP), Beam Tricyclazole 75% WP) and Bavistin (Carbendazim 50% WP), at three different concentrations 0.010 g/L, 0.020 g/L and 0.030 g/L was determined using the food poison technique [23] and their percentage of inhibition was recorded [24].

STATISTICAL ANALYSIS

All the results analysed were represented as mean ± standard error. Analysis of Variance (ANOVA) was done using Microsoft Soft Excel, 2021 to further validate the significance of the study.

RESULT AND DISCUSSION

The endophytic fungal isolates were identified as *Ovatospora brasiliensis* (V1), *Aspergillus quadrilineatus* (V2), *Aspergillus tamarii* (SS2) and *Aspergillus flavus* (SS6) and deposited to get their accession numbers from PP795172- PP795175 through NCBI GenBank.

ANTAGONISTIC ACTIVITY OF ENDOPHYTES

Antagonistic activity of the endophytes against the fungal phytopathogens were determined with the percentage of inhibition (Table 1). Findings revealed that all the fungal phytopathogens (*Macrophomina phaseolina*, *Fusarium oxysporum* and *Colletotrichum* sp.) were inhibited by the endophytes. *Macrophomina phaseolina* was inhibited maximum by SS2 (62.5 ± 4.17) followed by V2 (50.99 ± 5.37) and V1 (43.03 ± 2.42). *Fusarium oxysporum* was inhibited strongly by SS2 (42.11 ± 2.26) followed by SS6 (36.09 ± 0.75) and V2 (27.82 ± 3.76). Similarly, for *Colletotrichum* sp. inhibitory percentage was found higher by the strain SS6 (51.57 ± 10.94) followed by SS2 (37.51 ± 9.38) and the least was recorded with V1 (6.25 ±0). Antagonism score card varied based on the growth of the endophytic fungus and the pathogen over the plates as described by Bell *et al.,* 1982 is shown in Table 2 [14]. The antagonistic potential exhibited by the fungal endophytes in the present study is consistent with earlier reports on endophytic microorganisms against phytopathogens. Previous studies demonstrated that Trichoderma sp. showed varying antagonistic score values against *Macrophomina phaseolina* and Rhizoctonia solani, which closely corresponds with the observations of the current investigation [25]. Similarly, Streptomyces sp. GL4, QS4 and 3L3 exhibited different percentages of antagonistic activity against *Macrophomina phaseolina* and *Fusarium oxysporum* f. sp. *lycopersici* [26]. Furthermore, endophytic isolates EFTL-03, EFTL-04, EFTL-10, EFTL-12, and EFTL-13 obtained from Tahiti lime were reported to strongly inhibit *Colletotrichum acutatum* [27]. These findings collectively support the potential of fungal endophytes as effective bioresources for integrated crop management and sustainable disease control strategies [13]. Antagonistic effects of the endophytic fungal strains against the bacterial pathogens (*Xanthomonas oryzae* pv. *oryzae*, *Bacillus subtilis*, *Pseudomonas aeruginosa)* were evident with the zone of inhibition measured in mm (Fig 1). Endophytic fungal isolates did not show any observable zone against *Pseudomonas aeruginosa*. The endophytic strain SS6 showed a maximum zone of inhibition of 21 ± 2 against *Xanthomonas oryzae* pv. *Oryzae* followed by SS2 (20.5 ± 0.5). Strains V1 and V2 had no zone of inhibition. The highest degree of antagonism was found in SS2 (36 ± 3) followed by SS6 (30 ± 2) and V1 (25 ± 0) against *Bacillus subtilis*. *Dioscorea zingiberensis* rhizome had Dzf2 as fungal endophyte with the closest similarity to *Fusarium rodolens* with highest antibacterial activity against *Bacillus subtilis* [28]. Different strains of *Aspergillus* had better inhibitory potential with detectable zone of inhibition against *Xanthomonas oryzae* pv. *oryzae* as per the study conducted by Jiang *et al.,* 2019 [29]. Antagonistic activity of the fungal endophytes against bacterial and fungal pathogens was found with high statistical significance ($P < 0.05$).

EXTRACELLULAR ENZYME PRODUCTION BY ENDOPHYTES

The tested endophytic fungal strains (V1 and V2) were positive for peroxidase activity. Peroxidase-producing endophytic fungi play an important role in crop protection by enhancing host defense mechanisms against pathogens and environmental stresses. These enzymes detoxify reactive oxygen species, strengthen plant cell walls through lignification, and promote induced systemic resistance, thereby improving tolerance to biotic and abiotic stresses [21]. Similar findings were reported by Rodriguez *et al.,* 2009, Kusari *et al.,* 2012, and Lata *et al.,* 2018, who highlighted the role of endophytic fungi in enhancing plant defense responses and stress tolerance [30-32]. Similarly, peroxidase production was documented in other endophytic fungus namely *Setosphaeria rostrata KaL-1, Diaporthe sp. KaL-2, Corynespora cassiicola KaL-3, Setosphaeria rostrata KaL-4, Diaporthe* sp. KaL-5[20]. Extracellular enzymes such as cellulase, amylase, lipase, laccase, protease, pectinase, ligninase, and chitinase were evaluated in the four endophytic fungal strains (Fig 2). All the tested strains exhibited cellulase, amylase, lipase, protease, and pectinase activities, whereas chitinase activity was observed only in strains SS2 and SS6. Laccase and ligninase production were detected exclusively in strain V1. Similar findings have been reported in previous studies, where endophytic fungi isolated from different host plants exhibited diverse extracellular enzymatic activities associated with plant protection and nutrient acquisition. Lignin degradation had brown colouration around the colony, which is observed over the bottom of the plates with the score value of 2 indicating light brown, as per oxidation scale provided by Irawan *et al.,* 2014 [19]. Likewise, manganese peroxidase production was observed in fungal endophytic strains *Chaetomium cervicicola, Penicillium rubens, Trichoderma harzianum* from different parts of potato plant helps in degradation of lignin mainly due to oxidation of guaiacol [12]. Endophytic fungi from *Tamarix nilotica* showed cellulase, amylase, lipase, and protease activities that contributed to host defense and nutrient utilization [33]. Likewise, *Fusarium* sp. and *Trichoderma* sp. isolated from *Coleus aromaticus* exhibited amylase activity [34], while *Aspergillus flavus* complex, *Pestalotiopsis microspora*, *Simplicillium obclavatum*, and *Penicillium cataractarum* from *Ocimum* species demonstrated higher amylase, cellulase and protease activities respectively [35]. Chitinase, amylase, cellulase, protease and lipase activities were also reported in endophytic fungi isolated from *Cupressus torulosa*. These extracellular enzymes play a significant role in crop protection by facilitating endophytic colonization, nutrient mobilization, and degradation of phytopathogen cell wall components [36].

SALT TOLERANCE IN FUNGAL ENDOPHYTES

The NaCl salt tolerance index of all the fungal endophytic isolates was evaluated at 4-day intervals up to 12 days of incubation to assess their adaptability under saline conditions. The isolates exhibited varying degrees of tolerance at different NaCl concentrations (50, 100, and 200 mM) when maintained at 28 ± 2 C.

Among the isolates, *Ovatospora brasiliensis* showed a gradual increase in salt tolerance index up to the 8th day of incubation at concentrations up to 100 mM NaCl; however, at 200 mM NaCl, a steady decline in fungal growth and salt tolerance index was observed, indicating the inhibitory effect of elevated salinity on fungal growth and metabolic activity. Similarly, *Aspergillus quadrilineatus* exhibited increased salt tolerance during the initial 4 days of incubation at 50 and 100 mM NaCl concentrations, followed by a decline at 200 mM NaCl. During the 8th and 12th days of incubation, no significant variation in salt tolerance index was observed beyond 50 mM NaCl, where the value remained at 1.16 ± 0.10 and subsequently stabilized at 1 ± 0 across all concentrations with prolonged incubation. In contrast, *Aspergillus flavus* and *A. tamarii* remained largely unaffected and exhibited tolerance indices comparable to the control under all tested NaCl concentrations. Similar findings have been reported in previous studies, where fungal endophytes demonstrated enhanced growth under moderate salinity but reduced growth at higher salt concentrations. Our results were in accordance with the findings of Hammami *et al.,* 2016 that strains like *Xylaria sp., A. tenuissima, Fusarium* sp.1 *and Fusarium* sp.2 had higher salt tolerance with higher concentrations and several endophytic strains had increased NaCl tolerance till 100 mM and then declined gradually.[22] Studies by Hosseyni Moghaddam *et al.,* 2022 and Mutungi *et al.,* 2024 reported that halotolerant fungal endophytes improve plant growth and stress tolerance under saline conditions through osmotic regulation, nutrient acquisition, and antioxidant defense mechanisms[37,38]. Furthermore, Manjunatha *et al.,* 2022 and Gupta *et al.,* 2023 emphasized the potential application of salt-tolerant fungal endophytes as eco-friendly bioinoculants in sustainable agriculture and integrated crop management under salinity stress conditions [39,40]. Therefore, the moderate salt tolerance exhibited by *Ovatospora brasiliensis* and *Aspergillus quadrilineatus* in the present study highlights their potential role in enhancing crop resilience in salt-affected soils (Fig 3 and 4).

EFFECT OF FUNGICIDES ON ENDOPHYTES

The effect of commercial fungicides, namely Bavistin, Sixer, and Beam, on the endophytic fungal strains was evaluated at concentrations of 0.010, 0.020, and 0.030 g/L by measuring radial growth inhibition (Fig 5). The results revealed differential sensitivity among the isolates toward the tested fungicides. Bavistin exhibited complete (100%) inhibition of strains SS2 and SS6, whereas strains V1 and V2 showed progressive inhibition with increasing fungicide concentration. In the case of Beam (tricyclazole 75% WP), only strain V2 exhibited inhibition, while the remaining isolates demonstrated resistance. Sixer showed comparatively higher inhibitory effects against all the tested endophytic fungal strains at all concentrations. The observed variations in fungicide sensitivity among the endophytic isolates were statistically significant ($P < 0.05$). This study aligns with the uniqueness of *Fusarium solani,* an endophyte (non-pathogenic) showed resistance against mancozeb [41]. Notably, the resistance exhibited by certain endophytic strains against commonly used fungicides in the study suggests their adaptability and potential compatibility with chemical disease management practices. Such fungicide-tolerant endophytes could therefore be exploited as promising biocontrol agents in integrated disease management strategies, where they may function synergistically with reduced fungicide application for sustainable crop protection.

Figure 1: Antibacterial activity of endophytic fungal strains against (a) *Bacillus subtilis* (b) *Xanthomonas oryzae* pv. *oryzae*

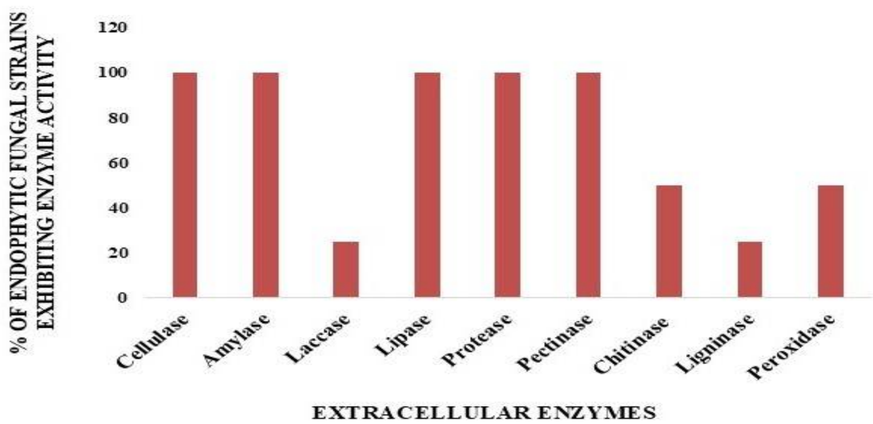

Figure 2: Percentage of endophytic fungal strains with extracellular enzyme activity

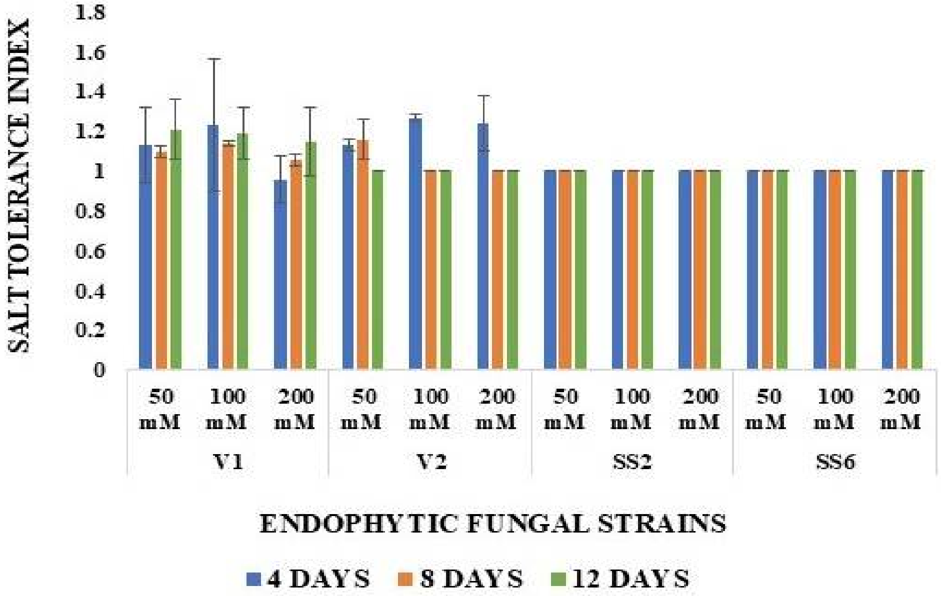

Figure 3: Salt tolerance index of the endophytic fungal strains

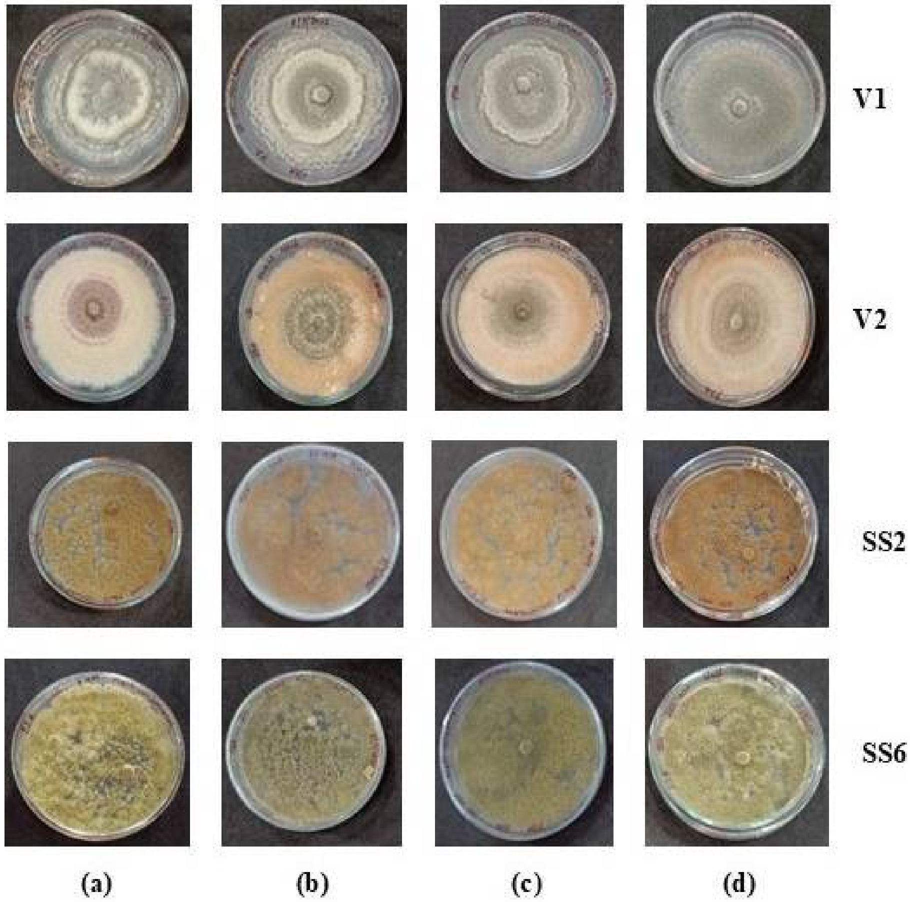

Figure 4: NaCl tolerance of the endophytic fungal strains - V1, V2, SS2 and SS6 (a) 0 mM (b) 50 mM (c) 100 mM (d) 200 mM at the end of 12 days

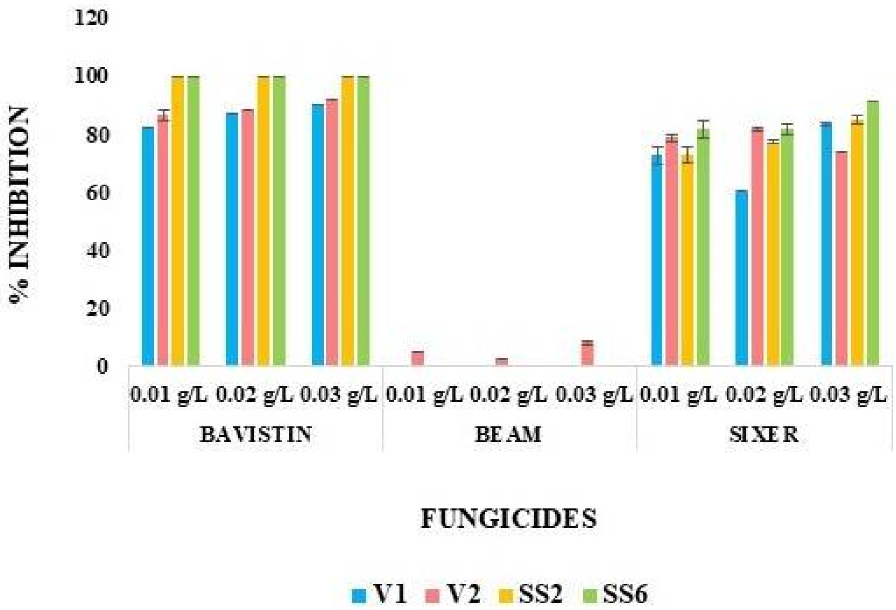

Figure 5: Effect of different fungicides on the growth of endophytic fungal strains

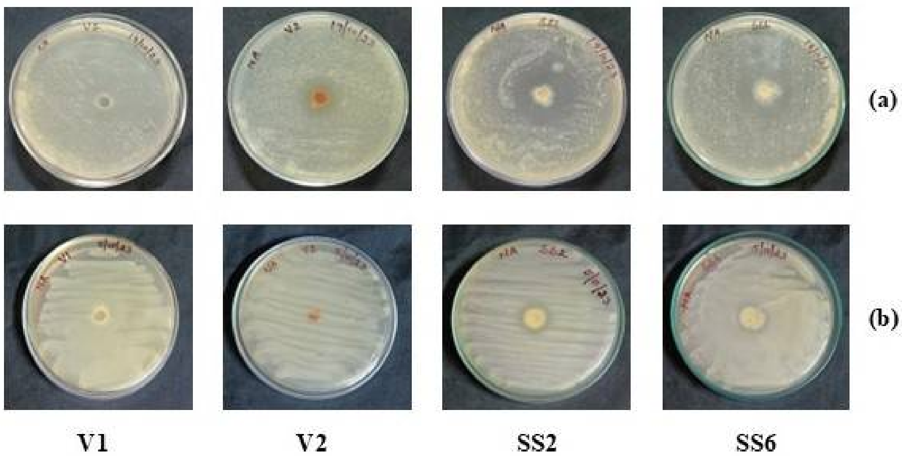

Table 1: Antagonistic activity of endophytic fungal strains against fungal phytopathogens after 4 days of incubation

S. No	Endophytic Fungal Strains	Fungal Phytopathogens		
		Fusarium oxysporum (OFO)	*Macrophomina phaseolina* (MP)	*Colletotrichum* sp. (CO)
1.	V1	6.36 ± 0.91	33.09 ±5.15	0
2.	V2	14.55 ± 1.82	41.18 ± 5.89	0
3.	SS2	32.73 ± 3.64	55 ± 5	36 ± 4
4.	SS6	25.45 ± 0	23 ± 3	46 ± 2

Table 2: Antagonistic activity of endophytic fungal strains against fungal phytopathogens after 7 days of incubation

S. No	Endophytic Fungal Strains	Fungal Phytopathogens			Antagonism Reaction		
		Fusarium oxysporum (OFO)	*Macrophomina phaseolina* (MP)	*Colletotrichum* sp. (CO)	OFO	MP	CO
1.	V1	18.05 ± 3.76	43.03 ± 2.42	6.25 ± 0	4	4	2
2.	V2	27.82 ± 1.5	50.99 ± 5.37	0	2	3	2
3.	SS2	42.11 ± 2.26	62.5 ± 4.17	37.51 ± 9.38	2	1	1
4.	SS6	36.09 ± 0.75	35 ± 1.67	51.57 ± 10.94	2	3	1

CONCLUSION

The present study demonstrated that the endophytic fungal isolates obtained from traditional rice varieties of Tamil Nadu- Valan and Seeraga Samba possess significant bioactive potential and stress adaptability. The isolates exhibited diverse extracellular enzymatic activities associated with pathogen suppression, nutrient mobilization, and successful host colonization, along with notable antagonistic activity against phytopathogens, moderate salt tolerance, and selective fungicide resistance. These characteristics highlight their possible role in enhancing plant health and resilience under stress conditions. Overall, the findings emphasize the agricultural significance of fungal endophytes from traditional rice varieties as promising eco-friendly bioresources for integrated disease management and sustainable crop protection.

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COMPETING INTEREST

The authors declare that no competing interest exists.

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