

**Natural defense: The inhibitory effects of endophytic *Bacillus* and *Xylaria* sp. against rice pathogens**

**ABSTRACT**

**Aim:** Endophytes living inside the plant tissues are known to play a crucial role in the physiology and developmental functioning of host plants. Rice is the major food crop that feeds half of the world population. But pest and diseases lead to drastic yield reduction in rice. Sustainable and eco-friendly disease management strategies are urgently needed to reduce the reliance on chemical pesticides and mitigate the environmental impact of intensive agriculture. The aim of the study was the selection of natural suppressors of the brown spot and sheath blight diseases in rice. In this study, three bacterial endophytes viz., *Bacillus subtilis* isolate PAL 11, *B. altitudinis* isolate TSL-4 and *B. altitudinis* isolate KAM-11 and one endophytic fungal isolate *Xylaria* sp. isolate KTD-2 were tested for their potential to inhibit *Bipolaris oryzae* and *Rhizoctonia solani* under *in vitro* conditions.

**Methodology:** *In vitro* evaluation of endophytes against two major rice pathogens viz., *Rhizoctonia solani* and *Bipolaris oryzae* causing sheath blight and brown leaf spot respectively were tested by dual culture technique.

**Results:** All the tested bacterial and fungal endophytes significantly inhibited both the pathogens under *in vitro* conditions. Among the bacterial endophytes, *B. subtilis* isolate PAL-11 exhibited highest inhibition of both the pathogens. The mycelial inhibition of *B. oryzae* was 56.04 and 74.48 per cent respectively at 5 and 8 days after dual culturing whereas the inhibition of *R. solani* was 80.31 and 80.22 per cent respectively at 3 and 5 days after dual culturing. The fungal endophyte *Xylaria* sp. isolate KTD-2 exhibited 63.2 and 67.95 per cent inhibition of mycelial growth of *B. oryzae* at 5 and 8 days respectively after the pathogen inoculation. The mycelial growth of *R. solani* was also significantly inhibited (54.95 %) by the fungal endophyte at 5 days after pathogen inoculation.

**Conclusion:** This research highlights the potential role of endophytes in sustainable agriculture and suggests that integrating beneficial microbes into rice disease management strategies could help enhance plant health, increase crop yields, and contribute to global food security.

*Key words: Endophytes, Bacillus sp., Xylaria sp., Brown spot, Sheath blight*

## INTRODUCTION

Rice is the major food crop that feeds half of the world's population [1]. More than 3.5 billion of the world population depends on rice for 20 per cent or more of the daily calorie intake [2]. In view of the alarmingly growing human population, the demand for rice is predicted to rise further. However, rice crop is highly susceptible to biotic and abiotic stresses, leading to significant losses in grain yield and quality [3]. Pest and disease incidences cause severe primary and secondary yield loss ranging from 26 to 38 per cent. Among these, rice diseases alone account for 16 per cent yield loss under various systems of rice cultivation [4]. Rice is infected by more than 70 diseases which are caused by fungi, bacteria, viruses and nematodes [5] during the whole growth period leading to wide spread use of chemical pesticides for disease control. The major diseases causing severe yield loss in rice are blast, sheath blight, brown spot and bacterial leaf blight.

Sheath blight caused by the necrotrophic fungus *Rhizoctoniasolani* Kuhn is one of the most serious diseases of rice worldwide, causing considerable yield loss [6]. Among the fungal diseases causing significant yield loss in rice, sheath blight is ranked as the second most important disease after rice blast. Use of high yielding semi dwarf cultivars with dense planting and high dose of nitrogenous fertilizers accelerate the incidence of sheath blight in rice [7]. *R. solani* can infect the plant at any growth stage. The yield loss ranging from 4 to 50 per cent have been reported depending on the crop stage at the time of infection, severity of the disease and environmental conditions [8]. Its diverse host range and ability to remain dormant under unfavourable conditions make the pathogen more difficult to manage

Brown spot of rice caused by *Bipolarisoryzae* is one of the disastrous fungal pathogens causing huge production losses in rice crop [9]. The pathogen attacks the crop from seedling to milk stage and infects leaves, leaf sheath, coleoptile, panicle branches, glumes, and spikelets [10]. *B. oryzae* infects the coleoptiles, leaves

forming oval, dark brown lesions, which ultimately destroy the leaves [11]. Apart from reducing plant vigour, number of grains per panicle and kernel weight, it also causes grain discoloration at maturity, thus reducing market value [12]. The infection especially occurs in environments where water supply is scarce and it is often combined with imbalances in plant mineral nutrition, especially the lack of nitrogen and results in yield losses in the range of 4 - 56 per cent [13]. But the epidemics caused by *B. oryzae* led to Bengal famine during 1942- 43, where 50 to 90 per cent rice grain was lost and about two million people starved to death [14].

Lack of adequate level of resistance, pathogenic variability, diverse host range and ability to survive under unfavourable conditions make these pathogens difficult to manage. Even though many fungicides can manage brown spot and sheath blight to an extent, searching for beneficial microorganisms for biocontrol will improve sustainable integrated pest management. In addition, the use of chemicals is raising several public concerns such as the emergence of fungicide resistant strains, environmental pollution, residual toxicity, soil quality reduction, natural ecosystem damage, and human health issues as well demanding alternate options to chemical management. In the present day scenario, where everyone is shifting towards organic cultivation due to the harmful side effects of the pesticides, use of endophytes plays an important role in the management of different diseases of rice crop

A diverse range of microorganisms dwell in various parts of a plant. In plant-microbe interactions, some microbes exert deleterious effects but most turn out to be highly beneficial to the hosts. Among these, endophytes are diverse plant symbionts that dwell within the host plant tissues without causing any disease symptoms. One of the most important contributions of endophytic mycobiota in agriculture is their antagonistic potential against a broad spectrum of pathogens, insects, and nematodes by different mechanisms including competition, production of antimicrobial compounds and inducing systemic resistance in host plants [15]. Plant benefits extensively by harboring these microorganisms as they promote plant growth as well. These microbes can be exploited for managing pathogens in a sustainable way. The development of biocontrol strategies using endophytes is an emerging area in crop protection to reduce the damage caused by plant pathogens in economically important crops. Therefore, the present investigation was made to

study the biocontrol potential of bacterial and fungal endophytes against *R. solani* and *B. oryzae* in *in vitro* conditions.

## **MATERIALS AND METHODS**

### **Isolation, purification and maintenance of *B. oryzae***

Diseased plants showing typical brown spot symptoms were collected from Integrated Farming System Research Station (IFSRS), Karamana, Thiruvananthapuram of Kerala Agricultural University. The samples were collected in clean polythene bag and brought to the laboratory for isolation of the pathogen. Samples were thoroughly washed with tap water and then the infected plant parts were cut in such a way that the leaf bits contain 50 per cent area of healthy part and 50 per cent from the lesion. The leaf bits were then surface sterilized with sodium hypochlorite (1%) for 1 minute followed by rinsing with sterile water with three changes of water. The leaf bits were blot dried on sterile blotting paper and placed on PDA medium aseptically. The inoculated plates were incubated at 25-28°C for 4-5 days. The fungal mycelia emerging from the leaf bits were sub cultured on PDA medium. The pure culture of *B. oryzae* was obtained by single spore isolation technique [16] and pathogenicity was confirmed by following Koch's postulates. The purified culture was preserved at 4°C for further use.

### **Isolation, purification and maintenance of *R. solani***

Diseased plants having typical symptoms of sheath blight were collected from the fields of IFSRS Karamana and the pathogen was isolated following the surface sterilization procedure as mentioned above. The fungal mycelia emerging from the incubated leaf bits were sub cultured on PDA medium. The culture was further purified by hyphal tip method [17]. The pathogenicity was confirmed by following Koch's postulates. The purified culture was preserved at 4°C for further use.

### **Morphological and cultural characterisation of *B. oryzae* and *R. solani***

Cultural and morphological characterization *B. oryzae* and *R. solani* were carried out in PDA medium. Fungal discs were inoculated on a fresh PDA medium and incubated at room temperature for 7 days. Mycelial growth, texture, colour and sporulation were observed. For sporulation of *B. oryzae*, alternate light

and dark periods and ultraviolet radiations were provided. The sclerotial emergence, size, shape, colour and distribution pattern were also observed in the case of *R. solani*. Mycelia and asexual spores were examined under the microscope for identification of the pathogens [18].

### ***In vitro* evaluation of the endophytes against *B. oryzae* and *R. solani***

Endophytic bacterial cultures of *Bacillus subtilis* isolate PAL 11 (Acc. No. OR81210), *B. altitudinis* isolate TSL-4 (Acc. No. OR81212) and *B. altitudinis* isolate KAM-11 (Acc. No. OR81213) and endophytic fungal isolate *Xylaria* sp. isolate KTD-2 (Acc. No. OR787561) maintained at Plant Pathology laboratory at IFSRS Karamana, Kerala Agricultural University were used for the study. The bacterial cultures preserved in glycerol stock at -20°C were sub-cultured on Tryptic soy agar (TSA) and the fungal isolate stored in PDA slants was sub-cultured on fresh PDA medium for the study.

### **Evaluation of the endophytic bacteria against *B. oryzae* and *R. solani***

*In vitro* evaluation of the bacterial endophytes against *B. oryzae* and *R. solani* was checked by dual culture technique [19]. TSA and PDA media mixed in 1:1 proportion was used for the study. Culture disc (5 mm) from an actively growing culture of the pathogen was placed at the centre of the mixed medium in a Petri plate. Each bacterial antagonist was streaked at two sides of the pathogen disc 2.5 cm away from the centre. Culture disc without antagonistic bacteria served as the control. Five replications were maintained for each pathogen and the plates were incubated at 28±2°C. The growth of the pathogen was recorded both in the treatment plate and control plate. In the case of dual culture with *R. solani*, observations were recorded at 3 and 5 days after incubation; whereas in case of *B. oryzae*, observations were recorded 5 and 8 days after incubation.

The results were expressed as percent inhibition of the mycelial growth of the pathogen over control [20].

$$\text{Inhibition percentage (\%)} = \frac{c - t}{c} \times 100$$

Where,

c = Growth of the pathogen in the control plate

**t** = Growth of the pathogen in the treatment plate

### **Evaluation of the endophytic fungus against *B. oryzae* and *R. solani***

The antagonism of the endophytic fungus *Xylaria* sp. isolate KTD-2 against the pathogens *B. oryzae* and *R. solani* was also checked by dual culture technique [19]. A 5 mm diameter culture disc of the endophyte was placed at one end of the Petri plate, 1 cm away from the edge. After 3 days of incubation, a culture disc (5 mm) of the pathogen was placed at the opposite side, 1 cm away from the edge. Pathogen without antagonistic fungi served as the control. Five replications were maintained for each pathogen and the plates were incubated at  $28 \pm 2$  °C. Growth of the pathogen was recorded both in the treatment plate and control plate. In the case of dual culture with *R. solani*, observations were recorded 3 and 5 days after incubation, whereas in the case of *B. oryzae*, observations were recorded 5 and 8 days after incubation. The inhibition of pathogen growth by the fungal endophyte was calculated as above.

## **RESULTS AND DISCUSSION**

### **Morphological and cultural characterisation of *B. oryzae* and *R. solani***

*B. oryzae* initially produced greyish white mycelium turning to dark grey when fully grown (Fig. 1). The mycelia are fluffy on the PDA medium and took 8-9 days for attaining full plate growth. Conidiophores and conidia were septate. Conidia are brown in colour with 5-10 septations and its shape varied from oval to cylindrical, slightly curved with a bulge in the middle and tapering towards the ends (Fig 2). Many previous research findings explained that *B. oryzae* isolates generally show different morphological characters in culture. In a study conducted to isolate and characterize *B. oryzae* from nine states across India, 17 isolates were formed into four groups such as black with fluffy growth, grey with fluffy growth and white spots, grey with fluffy growth and grey with suppressed growth based on colony morphology and growth pattern [21]. Similarly, *B. oryzae* isolated from Fogera area of Ethiopia was grouped into nine categories based on its colony morphology [22]. The conidia of *B. oryzae* are generally 5-10 septate with oldest conidia at the base [23]. The conidia are brownish in colour, slightly curved towards the end and broadest in the middle [24].

In the present study *R. solani* presented fast growth on the PDA medium and reached 9 cm growth within 3-4 days. The mycelia were white to cream in colour. The growth pattern of the mycelia on the medium were flat and also produced moderate quantity of aerial mycelium on the colony surface as well as on the lid and produced some sclerotia on the lid. The sclerotial emergence started after 96 h and majority of sclerotia were irregular in shape and some were spherical. The sclerotial colour was white at first and became light to dark brown after maturation and exuded droplet on the sclerotial surface. The sclerotia were firmly attached to the hyphae and produced superficially at the periphery of the Petri plate (Fig. 3). Many of the previous research findings stated that *R. solani* exhibited a range of morphological variations in culture. The mycelia colour varied from white to cream to brown and dark brown in many isolates of *R. solani* [25]. Mycelial growth nature of *R. solani* isolates varied between areal and flat growth patterns [26]. The mycelial growth is comparatively faster in *R. solani*. The growth was rapid if mycelia have grown to Petri dish diameter (90 mm) on the third day, slow if mycelial growth is less than 90 mm on the third day [27]. *R. solani*, isolated from infected rice plants from Bangladesh, exhibited significant variation in sclerotial size, shape and distribution. The brown to black sclerotia having exuded droplet were attached superficially on the mycelium. The distribution of sclerotia within the colonies was highly variable. Sclerotia were produced either near the inoculum or peripheral region or near the margin or scattered and so on. Some isolates produced a moderate quantity of aerial mycelium on the colony surface as well as on the lid and produced at least some sclerotia on the lid [28]. In a study on the diversity of field isolates of *R. solani* from sheath blight-infected rice fields of south India, most of the isolates (75.4%) were fast growers and reached 90mm radial growth on PDA within 48h. The sclerotial emergence was started after 72h in majority of the isolates and the sclerotia matured from fourth day onwards. Two types of sclerotia i.e. micro and macro sclerotia with light and dark brown colour, were observed. Two types of sclerotial attachments, i.e. firm and loose, were observed in the study. Sclerotia shape also varied from irregular to spherical or globose [29].

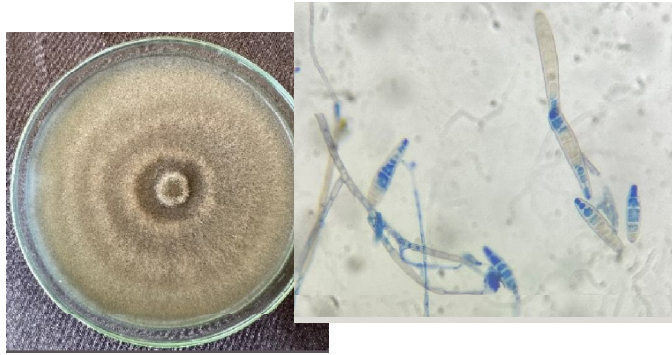


Fig. 1. Pure culture of *B. oryzae*

Fig. 2. Conidia and Conidiophores of *B. oryzae*



Fig. 3. Pure culture of *R. solani*

#### **Evaluation of endophytic bacteria against *B. oryzae* and *R. solani***

All the tested bacterial endophytes significantly inhibited the mycelial growth of both the pathogens in dual culture. The highest inhibition of mycelial growth of *B. oryzae* was exhibited by the bacterial endophyte *B. subtilis* isolate PAL-11 and it was 56.04 per cent and 74.48 per cent respectively at 5 and 8 days after incubation followed by *B. altitudinis* isolate TSL-4 with 47.80 and 71.15 per cent inhibition at 5 and 8 days after incubation respectively (Table 1) (Fig. 4). In the case of dual culture of endophytes with *R. solani* also, the maximum inhibition of 80.31 and 80.22 per cent respectively at 3 and 5 days after inoculation was obtained when the bacterial endophyte *B. subtilis* isolate PAL-11 was used. It was followed by *B. altitudinis* isolate TSL-4 with 57.31 and 67.69 per cent inhibition respectively at 3 and 5 days after inoculation (Table 2) (Fig. 5).

Plants are constantly involved in interactions with a wide range of endophytic microorganism living inside the plant system. These endophytes are sheltered from environmental stresses and act as plant growth promoters and biocontrol agents

[30]. *Bacillus* species are among the most common species of bacteria that are found to colonize plants endophytically [31]. Several investigators have successfully applied *Bacillus* spp. to control diseases in many crops including rice. The antagonistic potential of endophytic *Bacillus* spp. against important rice diseases have already been investigated including *B. subtilis* against *Pyricularia oryzae* [32], *B. amyloliquefaciens*, *B. methylotrophicus* and *B. subtilis* against *Xanthomonas oryzae* pv. *oryzae*, *B. subtilis* against *R. solani* [33], *B. velezensis* against *B. oryzae* [34] and so on. *B. velezensis* LS123N isolated from rice exhibited control against multiple rice pathogens in planta assays including bacterial blight, rice blast, bakanae disease, *Pythium* disease, and brown spot disease. Field trials further demonstrated that LS123N significantly reduced naturally occurring brown spot disease in both seedlings and mature rice plants, and increased rice yields [34]. Similarly, endophytic *B. subtilis* and *B. amyloliquefaciens* isolated from rice plants significantly inhibited *B. oryzae* in *in vitro* studies and controlled the brown spot incidence in the field conditions. Further consortia developed from the two endophytes were also found to be effective in managing the disease [35]. In a similar experiment conducted to evaluate the efficacy of endophytes to control seed borne pathogens of rice, *Bacillus* spp. were more effective in managing the diseases including brown spot and bacterial leaf blight [36]. Adding to these findings, in the present investigation also, *B. oryzae* was significantly inhibited by *Bacillus* spp.

*R. solani* inciting sheath blight of rice has emerged as an economically important rice pathogen causing considerable yield loss. Unfortunately, at present, there is no known rice variety which is either immune or having high degree of resistance to sheath blight disease [37]. Therefore, biological control can be a potent option. In the present study all the bacterial endophytes tested were found to inhibit the *R. solani* in dual culture. Many previous investigations support these findings. For example, in a research study, out of all the isolated endophytes from rice, *B. subtilis* var. *amyloliquefaciens* (FZB24) resulted in maximum inhibition (36 %) of *R. solani* in *in vitro* study and rice plants treated with this endophyte exhibited 55 per cent reduction of disease over control under glasshouse conditions along with increase in secretion of defense related enzymes [38]. Similarly, plant growth promoting *B. subtilis* isolated from soil showed 80.73 per cent inhibition of mycelial growth of *R. solani* in dual culture and lowest disease severity when paddy seeds

were treated in pot culture experiment [39]. *B. altitudinis* isolated from *Ocimum tenuiflorum* when challenged inoculated with *R. solani* in rice had significantly reduced sheath blight disease incidence, infected tillers, recorded maximum induction of defense-related enzymes (phenyl ammonia lyase, peroxidase, and polyphenol oxidase), and enhanced dry matter accumulation [40]. The antagonistic potential of *B. altitudinis* against rice blast disease was also already reported [41]. Some other endophytic *Bacillus* spp. such as *B. methylotrophicus* [42] and *Lysinibacillus sphaericus* [43] were also found to inhibit *R. solani*.

**Table 1. Inhibition of mycelial growth of *B. oryzae* by endophytic bacteria, *Bacillus* spp.**

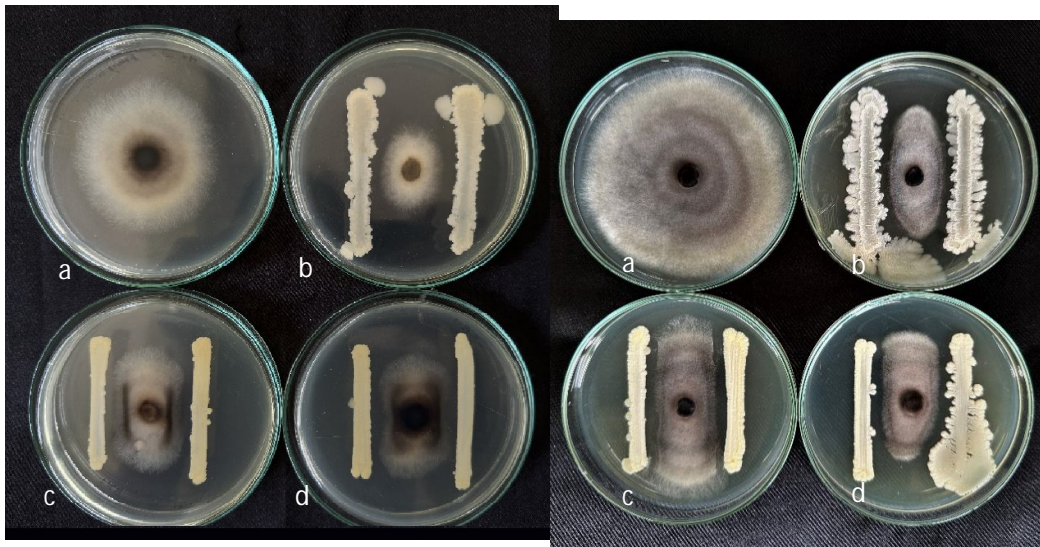
Sl. No.	Treatments	Mycelial growth of <i>B. oryzae</i> (cm)			
		5 days after inoculation		8 days after inoculation	
		<i>B. oryzae</i> diameter (cm)	Inhibition percentage (%)	<i>B. oryzae</i> diameter (cm)	Inhibition percentage (%)
1	<i>B. subtilis</i> isolate PAL-11	2.00 <sup>a</sup>	56.04	2.28 <sup>a</sup>	74.48
2	<i>B. altitudinis</i> isolate TSL-4	2.38 <sup>b</sup>	47.80	2.58 <sup>b</sup>	71.15
3	<i>B. altitudinis</i> KAM-11	2.45 <sup>b</sup>	46.15	2.70 <sup>b</sup>	69.75
4	Control	4.55 <sup>c</sup>	0.00	8.93 <sup>c</sup>	0.00
	S.Ed	0.08		0.08	
	CD (0.05%)	0.17		0.18	

Values are the means of four replications, values followed by similar superscripts are not significantly different at 5% level

**Table 2. Inhibition of mycelial growth of *R. solani* by endophytic bacteria, *Bacillus* spp.**

Sl. No	Treatments	Mycelial growth of <i>R. solani</i> (cm)			
		3 days		5 days	
		<i>R. solani</i> diameter (cm)	Inhibition percentage (%)	<i>R. solani</i> diameter (cm)	Inhibition percentage (%)
1	<i>B. subtilis</i> isolate PAL-11	0.95 <sup>a</sup>	80.31	1.78 <sup>a</sup>	80.22
2	<i>B. altitudinis</i> isolate TSL-4	2.05 <sup>b</sup>	57.51	2.90 <sup>b</sup>	67.69
3	<i>B. altitudinis</i> KAM-11	2.15 <sup>b</sup>	55.44	3.05 <sup>c</sup>	66.02
4	Control	4.83 <sup>c</sup>		8.98 <sup>d</sup>	
	S.Ed	0.08		0.05	
	CD (0.05%)	0.18		0.11	

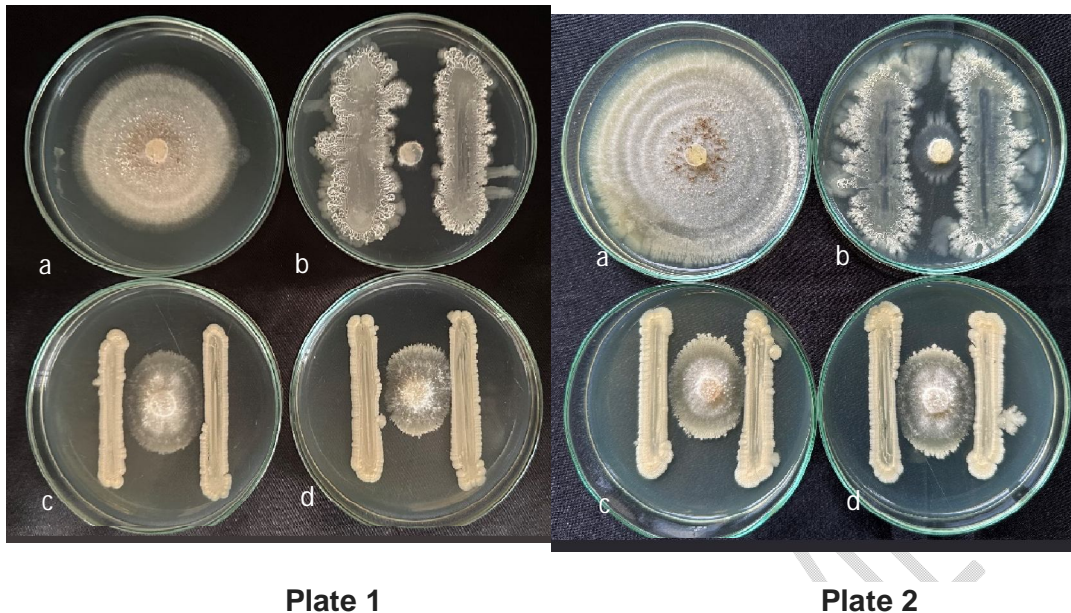
Values are the means of four replications, values followed by similar superscripts are not significantly different at 5% level



**Plate 1**

**Plate 2**

**Fig 4: Inhibition of mycelial growth of *B. oryzae* by endophytic bacteria at 5 days (plate 1) and 8 days (plate 2) after incubation; a. control, b. *B. subtilis* isolate PAL-11, c. *B. altitudinis* isolate TSL-4, d. *B. altitudinis* KAM-11**



**Fig. 5 Inhibition of mycelial growth of *R. solani* by endophytic bacteria at 3 days (plate 1) and 5 days (plate 2) after incubation; a. control, b. *B. subtilis* isolate PAL-11, c. *B. altitudinis* isolate TSL-4, d. *B. altitudinis* KAM-11**

#### **Evaluation of endophytic fungi against *B. oryzae* and *R. solani***

Mycelial growth of *B. oryzae* was significantly inhibited by the fungal endophyte *Xylaria* sp. isolate KTD-2 and the inhibition was 63.2 and 67.95 per cent respectively at 5 and 8 days after pathogen inoculation (Table 3) (Fig. 6). The mycelial growth of *R. solani* was also significantly reduced by the endophyte and the inhibition was 54.95 per cent at 5 days after pathogen inoculation (Table 4) (Fig. 7). Different genera of fungal endophytes have been isolated from rice plants and many were identified to inhibit pathogens [44]. Xylariaceous endophytes have been reported in all major groups of plants including conifers, monocots, dicots, ferns, and liverworts [45]. In the present investigation the endophytic *Xylaria* sp. isolate KTD-2 was found to inhibit rice pathogens *B. oryzae* and *R. solani*. The antagonistic potential of endophytic *Xylaria* against many plant pathogens have been already reported. For example, *X. regalis* isolated from *Tuja plicata* exhibited antagonistic activity against *F. oxysporum* and *Aspergillus niger* [46]. The antagonistic potential of *Xylaria* sp. F0010 against many diseases such as rice blast (*Magnaporthe grisea*), rice sheath blight (*Corticium saski*), wheat leaf rust (*Puccinia recondita*) and barley powdery mildew (*Blumeriagraminis* f. sp. *hordei*) was also reported [47]. Similarly,

reduction in severity of early blight in tomato caused by *Alternariasolani* by the application of endophytic *X. feejeensis* SRNE2BP[48] and inhibition of *Collectotrichumgloeosporioides* by endophytic *Xylaria* spp. [49] was also studied previously. These research works confirm the potential of *Xylaria* sp. to be used as a potent biocontrol agent in managing brown spot and sheath blight diseases of rice.

**Table 3. Inhibition of mycelial growth of *B. oryzae* by fungal endophyte *Xylaria* sp. isolate KTD-2**

Sl. No	Treatments	Mycelial growth of <i>B. oryzae</i> (cm)			
		5 days		8 days	
		<i>B. oryzae</i> diameter (cm)	Inhibition percentage (%)	<i>B. oryzae</i> diameter (cm)	Inhibition percentage (%)
1	Dual culture	2.58	63.2	2.88	67.95
2	Control	7.02		9	
	S.Ed	0.06		0.03	
	CD (0.05%)	0.13		0.06	

Values are the means of thirteen replications

**Table 4. Inhibition of mycelial growth of *R. solani* by fungal endophyte *Xylaria* sp. isolate KTD-2**

Sl. No	Treatments	Mycelial growth of <i>R. solani</i> (cm)			
		3 days		5 days	
		<i>R. solani</i> diameter (cm)	Inhibition percentage (%)	<i>R. solani</i> diameter (cm)	Inhibition percentage (%)
1	Dual culture	4.02	10.77	4.05	54.95
2	Control	4.50		9	
	S.Ed	0.03		0.04	
	CD (0.05%)	0.07		0.08	

Values are the means of thirteen replications

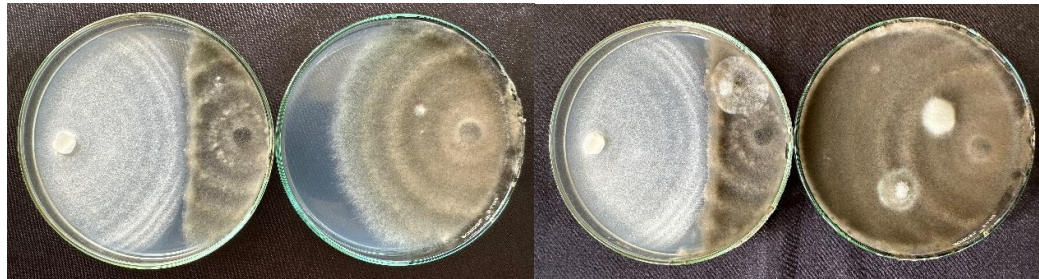


Plate 1

Plate 2

**Fig 6: Inhibition of mycelial growth of *B. oryzae* by endophytic fungi *Xylariasp* isolate KTD-2 at 5 days (plate 1) and 8 days (plate 2) after incubation**

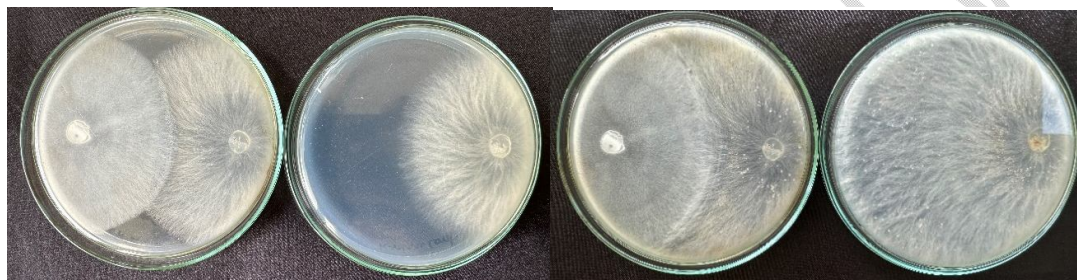


Plate 1

Plate 2

**Fig 7: Inhibition of mycelial growth of *R. solani* by endophytic fungi *Xylariasp* isolate KTD-2 at 3 days (plate 1) and 5 days (plate 2) after incubation**

## CONCLUSION

Brown spot caused by *B. oryzae* and sheath blight caused by *R. solani* are the two major diseases of rice causing severe threat to global rice production. Lack of adequate resistance, variability of the pathogen and ability of these pathogens to survive under extreme conditions make these pathogens difficult to manage. Despite the fact that pesticides are recommended for disease control, they are not considered to be long-term remedies because of concerns about fungicide residues, exposure risks, toxicity to non-target organisms, and other health and environmental issues. Therefore, endophytic microorganisms having biocontrol potential as well as plant growth promotion represent an alternative viable option to chemical management. In the present investigation, endophytic *Bacillus* sp. as well as *Xylaria* sp. were found to significantly inhibit *B. oryzae* and *R. solani* under *in vitro* conditions. The inhibition potential of these endophytes under *in vitro* conditions indicates that these endophytes can be used for field applications as a replacement

for chemicals after proper field trials. Findings of the study suggests that formulations can be developed from these endophytes for large scale application after *in vitro* and field experiments for holistic management of rice diseases.

Disclaimer (Artificial intelligence)

Option 1:

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 the writing or editing of this manuscript.

## Reference

1. Molina J, Sikora M, GarudN, Flowers JM, Rubinstein S, Reynolds A, et al. Molecular evidence for a single evolutionary origin of domesticated rice. Proc Natl Acad Sci. 2011;108:8351-8356.
2. Sanchez RT, Klu KAY, Genkinger MJ, Lacey VJ, Chung T N and Acien NA. Association between rice consumption and risk of cancer incidence in the California teachers study. Cancer Causes Control. 2020;31:1129-40.
3. Cohen SP, Leach JE. Abiotic and biotic stresses induce a core transcriptome response in rice. Sci Rep. 2019;9(1):6273
4. Schulz B, Boyle C, Draeger S, Römmert AK, Krohn K. Endophytic fungi: a source of novel biologically active secondary metabolites. Mycol Res. 2002;106(9):996-1004.
5. Ou SH. Rice diseases. Commonwealth Mycological Institute; 1985.
6. Prasad BN, Reddi MK. Effect of Non-Volatile Compounds Produced by *Trichoderma* spp. on Growth and Sclerotial Viability of *RhizoctoniaSolani*, incitant of Sheath Blight of rice. Indian J FundamAppl Life Sci. 2011;2:2231–6345.
7. Senapati M, Tiwari A, Sharma N, Chandra P, Bashyal BM, Ellur RK, Bhowmick PK, Bollinedi H, Vinod KK, Singh AK, Krishnan SG. *Rhizoctoniasolan*Kühn pathophysiology: status and prospects of sheath blight disease management in rice. Front Plant Sci. 2022;13:881116.
8. Singh A, Rashmi R, Savary S, Willocquet L, Singh US. Infection process in sheath blight of rice caused by *Rhizoctoniasolani*. Indian Phytopathol. 2003;56(4):434-438.

9. Shabana, YM, Abdel-Fattah GM, Ismail AE, Rashad YM. Control of brown spot pathogen of rice (*Bipolarisoryzae*) using some phenolic antioxidants. Braz J Microbiol. 2008;39:438-444.
10. Arshad HM, Hussain N, Ali S, Khan JA, Saleem K, Babar MM. Behavior of *Bipolarisoryzae* at different temperatures, culture media, fungicides and rice germplasm for resistance. Pak J Phytopathol. 2013;25(1):84–90.
11. Aryal L, Bhattarai G, Subedi A, Subedi M, Subedi B, Sah GK. Response of rice varieties to brown spot disease of rice at Paklihawa, Rupandehi. Glob J BiolAgric Health Sci. 2016;5(2):50-54.
12. Mew TW, Gonzales P. A handbook of rice seedborne fungi. Int. Rice Res. Inst; 2002.
13. Chakrabarti NK. Epidemiology and disease management of brown Spot of rice In India. In: Major Fungal Disease of Rice: Recent Advances. Kluwer Academic Publishers; 2001.
14. Bisen K, Biswas SK, Kumar V, Lal K, Kumar R, Kumar N. Biochemical changes in relation to brown leaf spot (*Drechsleraoryzae*) resistance in different rice genotypes. J Plant Stud. 2015; 4(2):23
15. Nalini MS, Prakash HS. Diversity and bioprospecting of actinomycete endophytes from the medicinal plants. Lett App Microbiol. 2017;64(4):261-70.
16. Ricker AJ, Ricker RS. Introduction to research on plant diseases. New York: John Swift Co; 1936
17. Onions AH, Allsopp D, Eggins HO. Smith's introduction to industrial mycology. 7<sup>th</sup> edn. New York :Halsted Press; 1981.
18. Rangaswami G, Mahadevan A. Diseases of crop plants in India. 4th ed. New Delhi: Prentice Hall of India Pvt. Ltd; 1999.
19. Dennis, C. and Webster, J. Antagonistic properties of species-groups of Trichoderma: I. Production of non- volatile antibiotics. Trans Br Mycol Soc. 1971;57(1):25-IN.
20. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature. 1927; 159:850.
21. Valarmathi P, Ladhakshmi D. Morphological characterization of *Bipolarisoryzae* causing brown spot disease of rice. Int J CurrMicrobiolAppl Sci. 2018;7(02):161-170.

22. Ejigu MB, Adimasu MA, Feyissa BB. Phenotypic characterization of rice *Bipolaris oryzae* in Fogera plains. *Cogent Food Agric.* 2024;10(1):2359941.
23. Sunder S, Singh RA, Agarwal R. Brown spot of rice: an overview. *Indian Phytopathol.* 2014;67(3):201-215.
24. Unnikrishna KP. Studies on the effect of plant extracts and antagonistic bacterium against rice brown spot pathogen *Drechslera oryzae* (Breda de Haan) Subram and Jain. M.Sc. (Agri.) Thesis, Tamil Nadu Agricultural University, Coimbatore; 1988.
25. San Aye S, Myint YY, Lwin T, Matsumoto M. Isolation, identification and preservation of *Rhizoctonia* spp. from sheath spot diseases of rice in Myanmar. *Bull. Inst. Trop. Agr., Kyushu Univ.* 2008;31(1):31-8.
26. El-Shafey RA, Elamawi RM, Saleh MM, Tahoon AM, Emeran AA. Morphological, pathological and molecular characterisation of rice sheath blight disease causal organism *Rhizoctoniasolani* AG-1 IA in Egypt. *Arch Phytopathol Plant Prot.* 2019;52(5-6):507-29.
27. Desvani SD, Lestari IB, Wibowo HR, Supyani S, Poromarto SH, Hadiwiyono H. Morphological characteristics and virulence of *Rhizoctoniasolani* isolates collected from some rice production areas in some districts of Central Java. In *AIP Conference Proceedings 2018 Sep 21 (Vol. 2014, No. 1)*. AIP Publishing.
28. Moni ZR, Ali MA, Alam MS, Rahman MA, Bhuiyan MR, Mian MS, Iftikharuddaula KM, Latif MA, Khan MA. Morphological and genetical variability among *Rhizoctoniasolani* isolates causing sheath blight disease of rice. *Rice Sci.* 2016;23(1):42-50.
29. Jayaprakashvel M, Mathivanan N. Morphological and pathological variations of rice sheath blight inciting south Indian *Rhizoctoniasolani* isolates. *Arch Phytopathol Plant Prot.* 2012;45(4):455-67.
30. Nagendran K, Karthikeyan G, Peeran MF, Raveendran M, Prabakar K, Raguchander T. Management of bacterial leaf blight disease in rice with endophytic bacteria. *World Appl Sci J.* 2013;28(12):2229-2241.
31. Mahaffee WF, Kloepper JW. Temporal changes in the bacterial communities of soil, rhizosphere, and endorhiza associated with field-grown cucumber (*Cucumis sativus* L.). *Microb Ecol.* 1997;34:210-23.

32. Saleh MM. Evaluation of endophytes isolated from rice leaves for their antifungal activities against *Pyriculariaoryzae* causative blast disease. Egypt J Phytopathol. 2018;46(1):193-214.
33. Durgadevi D, Harish S, Manikandan R, Prabhukarthikeyan SR, Alice D, Raguchander T. Proteomic profiling of defense/resistant genes induced during the tripartite interaction of *Oryzasativa*, *Rhizoctoniasolani*AG1-1A, and *Bacillus subtilis* against rice sheath blight. PhysiolMol Plant Pathol. 2021;115:101669.
34. Tu CK, Huang WD, Wang PH, Lin WL, Chen HY, Rau ST, Chang TC, Young LS, Wang CL, Lee MH. The rice endophytic bacterium *Bacillus velezensis* LS123N provides protection against multiple pathogens and enhances rice resistance to wind with increase in yield. Biocontrol. 2024;192:105507.
35. Khemmuk W, Boonchuay D, Jungkhun N, Klinmanee C, Kantachan A. Biological Control of Rice Brown Spot by *Bacillus* Spp. in Thailand. In Proceedings 2020 Apr 10 (Vol. 36, No. 1, p. 217). MDPI.
36. Das J, Devi RK. In vitro evaluation of bacterial endophytes against major seedborne pathogens of rice grains. Agric. Res. J. 2022;59(6):1086.
37. Adhipathi P, Singh V, Meena SC. Virulence diversity of *Rhizoctoniasolani* causing sheath blight disease in rice and its host pathogen interaction. Bioscan. 2013;8(3):949-52.
38. Nagendran K, Karthikeyan G, Mohammed Faisal P, Kalaiselvi P, Raveendran M, Prabakar K, Raguchander T. Exploiting endophytic bacteria for the management of sheath blight disease in rice. BiolAgricHortic. 2014;30(1):8-23.
39. Thakur P, Tiwari RK, Nirmalkar VK, Verma SK, Chaure NK. Antagonistic activity and Bioefficacy of *Bacillus subtilis* against Sheath Blight of Rice (*Oryza sativa* L.) caused by *Rhizoctoniasolani*. Biol Forum. 2022;14(3):1305-1311.
40. Sahu PK, Singh S, Gupta AR, Gupta A, Singh UB, Manzar N, Bhowmik A, Singh HV, Saxena AK. Endophytic bacilli from medicinal-aromatic perennial Holy basil (*Ocimumtenuiflorum* L.) modulate plant growth promotion and induced systemic resistance against *Rhizoctoniasolani* in rice (*Oryza sativa* L.). Biocontrol. 2020;150:104353.

41. Lam VB, Meyer T, Arias AA, Ongena M, Oni FE, Höfte M. Bacillus cyclic lipopeptides iturin and fengycin control rice blast caused by *Pyricularia oryzae* in potting and acid sulfate soils by direct antagonism and induced systemic resistance. *Microorganisms*. 2021;9(7):1441.
42. Shrestha BK, Karki HS, Groth DE, Jungkhun N, Ham JH. Biological control activities of rice-associated Bacillus sp. strains against sheath blight and bacterial panicle blight of rice. *PloS one*. 2016;11(1):e0146764.
43. Shabanamol S, Sreekumar J, Jisha MS. Bioprospecting endophytic diazotrophic *Lysinibacillus sphaericus* as biocontrol agents of rice sheath blight disease. *3 Biotech*. 2017;7(5):337.
44. Tian XL, Cao LX, Tan HM, Zeng QG, Jia YY, Han WQ, Zhou SN. Study on the communities of endophytic fungi and endophytic actinomycetes from rice and their antipathogenic activities in vitro. *World J Microbiol Biotechnol*. 2004;20:303-309.
45. Davis EC, Franklin JB, Shaw AJ, Vilgalys R. Endophytic *Xylaria* (Xylariaceae) among liverworts and angiosperms: phylogenetics, distribution, and symbiosis. *Am J Bot*. 2003;90:1661–1667.
46. Hend AA, Perveen K, Tahmaz R, Alhaqbani S. Evaluation of biological control potential of locally isolated antagonist fungi against *Fusarium oxysporum* under in vitro and pot conditions. *Am J Microbiol Res*. 2012;6(2):312–319.
47. Park JH, Choi GJ, Lee SW, Lee HB, Kim KM, Jung HS, Jang KS, Cho KY, Kim JC. Griseofulvin from *Xylaria* sp. strain F0010, an endophytic fungus of *Abies holophylla* and its antifungal activity against plant pathogenic fungi. *J Microbiol Biotechnol*. 2005;15(1):112-117.
48. Brooks S, Klomchit A, Chimthai S, Jaidee W, Bastian. *Xylaria feejeensis*, SRNE2BP a fungal endophyte with biocontrol properties to control early blight and *Fusarium* wilt disease in tomato and plant growth promotion activity. *Curr Microbiol*. 2022;79(4):108.
49. Elias LM, Fortkamp D, Sartori SB, Ferreira MC, Gomes LH, Azevedo JL, Montoya QV, Rodrigues A, Ferreira AG, Lira SP. The potential of compounds isolated from *Xylaria* spp. as antifungal agents against anthracnose. *Braz J of Microbiol*. 2018;49:840-847.

UNDER PEER REVIEW