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Fungal Endophytes from Chilli and their Antagonism against *Colletotrichum capsici*

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ABSTRACT

The *Colletotrichum capsici* incited anthracnose/fruit rot posing a serious threat to commercial chilli production in India. In the present study, the fungal endophytes from chilli were explored in effort to combat the pathogen. 19 endophytic fungal isolates isolated from leaf, stem and root of healthy chilli plant exhibited antagonistic activity against *C. capsici* in dual culture assay under *in vitro*. Among them endophyte, UHSFS5 demonstrated the highest pathogen mycelial inhibition (74.68 %). Based on the cultural and morphological characteristics UHSFS5 was identified to be belonged to *Aspergillus* genus. Upon its accurate molecular identification, pathogenicity and enumerating its efficacy in pot and field and accurate molecular identification it may be employed to combat the chilli anthracnose disease.

Keywords: Anthracnose, *Aspergillus,* Chilli,Fungal endophyte.

INTRODUCTION

India is larger consumer and exporter of chilli (*Capsicum annum* L.) globally, in addition to being its greatest producer with 1.98 Mt and contributes 43 per cent of world chilli production, followed by China, Ethiopia, Thailand, Pakistan and Bangladesh (Kiruthika *et al*, 2024). The commercial production of chilli is potentially hindered by numerous biotic constraints of which the anthracnose disease incited by *Colletotrichum capsici, C. gleosporoides, C. acutatum and C. scoville* poses a significant threat to chilli production, resulting in substantial yield loss. The symptoms of the disease appear on foliage as tiny circular dots that eventually merge into larger elliptical patches, potentially resulting in defoliation under severe circumstances (Jojy *et al*, 2024). Primarily, the symptoms are seen in severe form on ripe fruits in the field and leads to the fruit quality deterioration. The chemical pesticides are indiscriminately applied to chilli in an effort to overcome these biotic constraints. Further, these strategies have detrimental effect on human health, environment and most important one is development of resistance in target populations (Martin and Loper, 1999).

Since not many alternatives to pesticides are available, the biological control by utilizing beneficial microorganisms has been extensively researched (Duffy *et al*, 1995). Early workers found difficulties in incorporating non-native microbial into established and adapted microbial communities, as well as the absence of specificity between microbes and their host plants. However, one potential strategy to address this limitation is to utilize selected communities of native endophytic microorganisms (Amaresan *et al*, 2012). Endophytic microorganisms are a unique group of microbes that inhabit the tissues and internal organs of terrestrial and some aquatic plants (Gao *et al*, 2010). Endophytes are found in all plant species and exhibit varying capacities to benefit their hosts (Sarmah *et al*, 2023). Endophytes are typically defined as microbes residing within the internal tissues of plants and are commonly isolated from these tissues following surface sterilization (Fadiji *et al*, 2020). The studies on endophytic fungi in chilli were attempted by few early researchers (Amaresan *et al*, 2014; Pavithra *et al,* 2021) to combat anthracnose disease but achieved limited success. Hence with this background information the present study was conducted to explore the fungal endophytes in chilli against anthracnose/Fruit rot pathogen *C. capsici*.

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Table 1: List of fungal endophytes isolated from chilli.

Sl. No.	Isolates	Plant Part	Place	GPS coordinates
1	UHSFL1	Leaf	Bagalkot (Karnataka)	16.1691° N 75.6615° E
2	UHSFL2	Leaf		
3	UHSFL3	Leaf		
4	UHSFL4	Leaf	Devihosur (Haveri, Karnataka)	14.7951° N 75.3310° E
5	UHSFL5	Leaf		
6	UHSFS1	Stem		
7	UHSFS2	Stem		
8	UHSFS3	Stem	Bagalkot (Karnataka)	16.1691° N 75.6615° E
9	UHSFS4	Stem		
10	UHSFS5	Stem		
11	UHSFR1	Root		
12	UHSFR2	Root		
13	UHSFR3	Root		
14	UHSFR4	Root		
15	UHSFR5	Root		
16	UHSFR6	Root	Devihosur (Haveri, Karnataka)	14.7951° N 75.3310° E
17	UHSFR7	Root		
18	UHSFR8	Root		
19	UHSFR9	Root		

Table 2: *In vitro* evaluation of fungal endophytic isolates against *C. capsici*

Sl. No.	Isolates	Mycelial growth of pathogen (cm)	Mycelial growth inhibition (%)
1	UHSFL1	4.20	47.50 (43.59)*
2	UHSFL2	5.37	32.81 (34.96)
3	UHSFL3	6.12	23.43 (28.96)
4	UHSFL4	5.57	30.31 (33.42)
5	UHSFL5	5.72	28.43 (32.24)
6	UHSFS1	5.57	30.31 (33.42)
7	UHSFS2	2.60	67.50 (55.27)
8	UHSFS3	5.77	27.81 (31.84)
9	UHSFS4	5.75	28.12 (32.04)
10	UHSFS5	2.02	74.68 (59.82)
11	UHSFR1	6.15	23.12 (28.75)
12	UHSFR2	6.00	25.31 (30.02)
13	UHSFR3	5.75	28.12 (32.04)
14	UHSFR4	6.15	23.12 (28.75)
15	UHSFR5	5.77	27.81 (31.84)
16	UHSFR6	5.77	27.81 (31.84)
17	UHSFR7	4.85	39.37 (38.88)
18	UHSFR8	2.65	66.87 (54.89)
19	UHSFR9	2.47	69.06 (56.23)
S. Em ±		0.42	1.78
CD (0.01)		1.36	6.03

Mycelial growth of pathogen in control (without endophyte) is 9 cm
*Arcsine transformed values

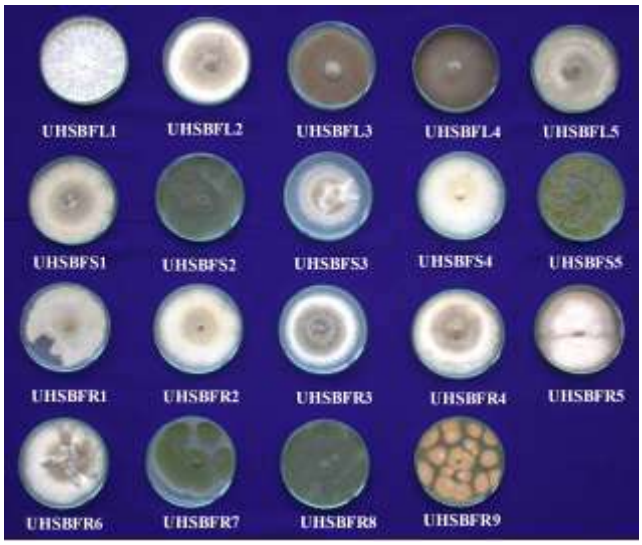

Fig.1: Fungal endophytes isolated from different parts of chilli

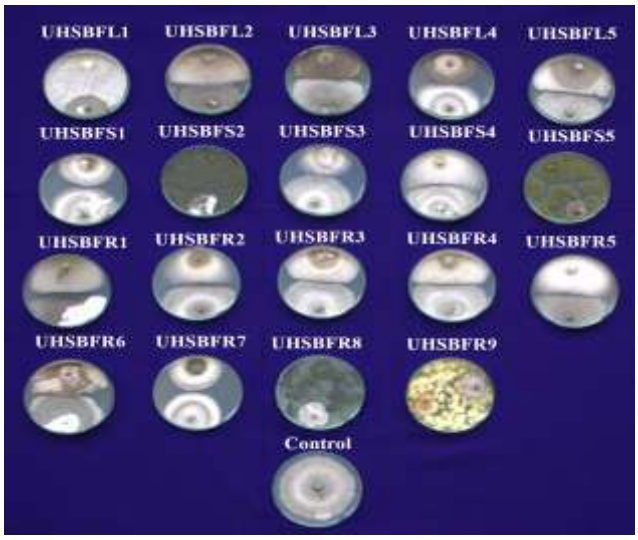

Fig.2: Antagonistic activity of endophytic fungal isolates against *C. capsici*

MATERIALS AND METHODS

Sample collection

The healthy chilli plants were collected in sterile polythene bags from fields in the Bagalkot and Haveri districts of Karnataka and brought to the laboratory for isolation of endophytes during 2023. The samples were used for the isolation of endophytes within 48 hours of sampling to ensure freshness and minimize the contamination.

Media preparation

Potato dextrose agar (PDA) medium [potato extract (200 g/L), dextrose (20 g/L), agar (20 g/L), pH 7] was utilized for the isolation and purification of endophytic fungi. To prevent bacterial contamination, streptomycin was incorporated into the medium at a concentration of 250 mg/L.

Endophytic fungi isolation

Endophytic fungi from the collected samples were isolated using a sterile leaf technique (Schult and Boyle, 2005). Healthy leaves, stems and roots were washed in running tap water to remove soil and dirt, then cut into one cm bits. These bits were surface sterilized in 70 per cent ethanol for one minute, followed by 2 per cent sodium hypochlorite for two minutes. Afterward, the sections were rinsed three times with sterile distilled water and placed on nine cm Petri plates containing Potato Dextrose Agar (PDA) medium supplemented with streptomycin (250 mg/l) to inhibit bacterial growth. To verify the effectiveness of the surface sterilization and ensure that isolations were from internal plant tissues, a 100 µl aliquot from the final rinse and a whole surface-sterilized leaf were separately inoculated on PDA plates. The absence of fungal growth on these plates confirmed the success of the surface sterilization. All plates were incubated at 27±1ºC and observed for fungal growth every day up to 10 days. The total of 19 fungal isolates were recovered from the collected samples (Table 2).

Effect of fungal endophytes against *C. capsici* under *in vitro*

Fungal endophytic fungal isolates were screened for their antagonistic effects using the dual culture method. Each agar plate was inoculated with a five mm diameter agar disk of endophytic fungi on one side and a five mm diameter agar disk of *C. capsici* on the opposite side. Control plates with only the pathogen or only the endophytes were also maintained. Petri plates then incubated at 27±1 ºC for 7 days durations and diameter of the pathogen colony growth was measured in both the control plates and the paired cultures. The percentage inhibition of the pathogen was calculated using the formula; I=(C-T/C) * 100, Where I = per cent inhibition of pathogen, C = Growth of pathogen in absence of Endophyte, T = Growth of pathogen in presence of endophyte.

Cultural and morphological characterization of fungal endophytes

The isolates were cultured for seven days on Potato Dextrose Agar (PDA) medium and incubated at 25°C. Microscopic observations were conducted by preparing slides stained with lactophenol cotton blue.

A small tuft of the fungus, typically containing spores and spore-bearing structures was transferred onto a drop of stain using a flamed, cooled needle. The fungal material was carefully teased apart with two mounted needles and gently mixed with the stain to ensure even distribution. A cover slip was then placed over the sample, avoiding air bubbles in the preparation. Finally, the edges of the cover slip were sealed with fresh nail polish to secure the lactophenol mount for observation under the trinocular microscope.

RESULTS AND DISCUSSION

The endophytic microorganisms reside within the living plants tissues are promising, yet underexplored, sources of novel natural products for agricultural use. The presence of endophytes in plant parts, including leaves, stems, roots, flowers, seeds, and fruits of various plant species has been reported (Anjum and Chandra, 2015). In our study, healthy chilli samples (leaf, stem, flower bud and root) collected were subjected for isolation of endophytes. A total of 19 fungal endophytes were isolated from healthy chilli tissues: 5 from leaves, 5 from stems and 9 from roots. The isolates were designated as UHSFL1, UHSFL2, UHSFL3, UHSFL4, UHSFL5, UHSFS1, UHSFS2, UHSFS3, UHSFS4, UHSFS5, UHSFR1, UHSFR2, UHSFR3, UHSFR4, UHSFR5, UHSFR6, UHSFR7, UHSFR8 and UHSFR9 (Table 1; Fig. 1). All 19 isolates were tested against *C. capsici* to know the antagonistic activity through dual culture technique under *in vitro* condition. Among all tested isolates, UHSFS5 showed highest inhibition of 74.68 per cent (Table 2). It was identified based on macro and microscopic characters. The isolates exhibited a green colony colour with a colourless reverse, and its conidial head was greyish-green. The conidia were globose, smooth to finely rough, and displayed a yellow-green hue, as shown in Fig. 2. These distinctive morphological characteristics led to the identification of the isolate may be belonging to the genus *Aspergillus*.

The present results were in line with Pavithra *et al* (2021) who isolated various fungal endophytes from chilli and recorded highest mycelial growth inhibition of *Colletotrichum capsici* by *Aspergillus niger* to the tune of 90.74% under *in vitro* conditions. Further, Chowdary and Kumar (2022) found that of the 122 endophytic fungal isolates obtained from healthy chilli plants, the isolate ENRF 7 (*Trichoderma asperellum*) was found to be more promising against *Colletotrichum acutatum* with 61.45 % inhibition under *in vitro*. Endophytes reside in protected environments within plant tissues, providing them with a competitive advantage over organisms in the rhizosphere and phyllosphere. They benefit from consistent nutrient flow, stable pH levels, and adequate moisture, while being shielded from high densities of competitors (Backman and Sikora, 2008). Additionally, they benefit the plant by suppressing pathogen access to the host system through the synthesis of bioactive compounds that act against phytopathogens (Singh *et al*, 2020).

CONCLUSION

The present study concluded that based on distinctive morphological characteristics the UHSFS5 isolate was identified belonging to genus *Aspergillus*. After confirming its identity at molecular level and testing its efficacy in field conditions, the fungal endophyte may be exploited as potent bio-control agent in managing the fruit rot disease of chilli.

REFERENCES

Amaresan N, Jayakumar V and Thajuddin N (2014). Isolation and characterization of endophytic bacteria associated with chilli (*Capsicum annuum*) grown in coastal agricultural ecosystem. *IJBT* **13**: 247-255.

Amaresan N, Jayakumar V, Kumar K and Thajuddin N (2012). Isolation and characterization of plant growth promoting endophytic bacteria and their effect on tomato (*Lycopersicon esculentum*) and chilli (*Capsicum annuum*) seedling growth. *Ann Microbiol* **62**(2): 805-810.

Anjum N and Chandra R (2015). Endophytic bacteria: optimization of isolation procedure from various medicinal plants and their preliminary characterization. *Asian J Pharm Clin Res* **8**(4): 233-238.

Asghari S, Harighi B, Ashengroph M, Clement C, Aziz A, Esmaeel Q and Ait Barka E (2020). Induction of systemic resistance to *Agrobacterium tumefaciens* by endophytic bacteria in grapevine. *Pl Patho* **69**(5): 827-837.

Backman P A and Sikora R A (2008). Endophytes: an emerging tool for biological control. *Bio cont* **46**(1): 1-3.

Duffy B K, Simon A and Weller D M (1995). Combination of *Trichoderma coningii* with *Psuedomonas florescence* for control of take-all on wheat. *Phytopath* **86**(2):188-194.

Dutta D, Puzari K C, Gogoi R and Dutta P (2014). Endophytes: exploitation as a tool in plant protection. *Braz Arch Biol Technol* **57**(5): 621-629.

Fadiji A E and Babalola O O (2020). Elucidating mechanisms of endophytes used in plant protection and other bioactivities with multifunctional prospects. *Front Bio Biotech* **8**: 467.

Gao F K, Dai C C and Liu X Z (2010). Mechanisms of fungal endophytes in plant protection against pathogens. *Afr J Microbio Res* **4**(13): 1346-1351.

Jojy E T, Amrutha P, Johnson J M, Sajeena A, Rafeekher M, Radhakrishnan N V and Paul A (2024). *Piriformospora indica* incumbers the incidence and growth of *Colletotrichum capsici* in colonized chilli plants by direct antagonism and activation of phenylpropanoid pathway. *Plant Protect Sci* **60**(4): 354-370.

Kiruthika N (2024). Economics of Mundu Chilli Cultivation in Ramanathapuram District of Tamil Nadu, India. *Asian J Agri Ext Econ Socio* **42**(1): 44-48.

Maheshwari D K and Annapurna K (2017). Endophytes as Contender of Plant Productivity and Protection: An Introduction. *Endophytes: crop productivity and protection*. *Springer* **2**:1-10

Manikantha Chowdary G B S and Kumar S R (2022). *In-vitro* antagonistic potential of fungal endophytes against chilli anthracnose pathogen *Colletotrichum acutatum*. *Int J Botany Stud* **7**(1): 188-193.

Martin F N and Loper J E (1999). Soil borne plant disease caused by *Pythium* spp: Ecology, epidemiology and prospects for biological control. *Crit Rev Pl Sci* **18**(2): 111-181.

Pavithra G, Raghavendra Reddy M, Venkata Avinash A and Seweta S (2021). Evaluation of Isolated Endophytes, Bio-agents and Fungicides against Anthracnose of Chilli. *Biopestic Int* **17**(2):143-149.

Pavithra G, Manda R R, Addanki V A and Srivastava S (2021). Evaluation of Isolated Endophytes, Bio-agents and Fungicides against Anthracnose of Chilli. *Biopestic Int* **17**(2): 143-149.

Sarmah P P, Nath H K D and Nath T (2023). Endophytic *Bacillus* spp from native chilli cultivars and their effect against fruit rot pathogen of Bhut Jolokia (*Capsicum chinense* Jacq.). *Egyptian J of Bio Pest Ctrl* **33**(1): 119.

Saxena A, Raghuwanshi R, Gupta V K and Singh H B (2016). Chilli anthracnose: the epidemiology and management. *Front Microbio* **7**:1527.

Schult B and Boyle C (2005). The endophytic continuum. *Mycol Res* **109**(6): 661-686.

Singh M, Srivastava M, Kumar A, Singh A K and Pandey K D (2020). Endophytic bacteria in plant disease management. *Microb Endo Wood Publ* **1**: 61-89.

Wilson D (1995). Endophyte: the evolution of a term, and clarification of its use and definition. *Oikos* **73**(2): 274–276.

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