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SHORT COMMUNICATION

In vitro antagonistic activity of endophytic fungi associated with medicinal plants of Lamiaceae towards phytopathogenic fungi

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Biocontrol approaches against diseases are widely acknowledged as a step toward organic farming. In the current study, 24 species of endophytic fungi from three Lamiaceae medicinal plants, *Ocimum basilicum*, *Leucas aspera*, and *Elsholtzia stachyodes*, were tested against four common plant pathogenic fungi: *Aspergillus flavus*, *Aspergillus niger*, *Curvularia lunata*, and *Fusarium oxysporum* under *in vitro* dual culture method. Of the 24 endophytic fungi, 19 were inhibited by at least one of the four plant pathogenic fungal species, with antagonistic activity ranging from class 2 to class 4. *Colletotrichum gleosporioides* (ECS2) had the most inhibitory effect on all four plant pathogenic fungi (Class 2), followed by *Nigrospora oryzae* (LAS6) class 2 and *Aspergillus niger* (OBS2) class 3, respectively. In contrast, the endophytic fungi *Alternaria raphani* (OBS1), *Cladosporium cladosporioides* (LAS4, ECS11), *Alternaria citri* (ECS1), and *Cladosporium* sp.3 (ECS4), had no antagonistic effect (class 5) on the pathogenic fungi under investigation. The study demonstrates that endophytic fungal strains hold promise for use in regional biocontrol strategies for plant diseases.

Keywords: Antagonistic activity, biological control, dual culture, fungal endophytes

INTRODUCTION

Plant infections pose a global hazard to agricultural security, causing pre- and post-harvest losses in various crops, particularly in impoverished nations. Economic crop diseases generate an estimated $220 billion in global losses each year. Plant disease management frequently includes resistance cultivars, cultural measures, and chemical control methods. The misuse of synthetic chemicals poses a risk to humans and has a negative impact on the environment by diminishing agricultural sustainability. Excessive usage of these chemicals can lead to pathogen resistance, hindering disease management efforts (Alori and Babalola, 2018). There is a growing interest in non-chemical disease control approaches that are safe for both human health and the environment.

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Using bio-control agents instead of fungicides can effectively manage plant infections, create safe food, and reduce environmental pollution. Biocontrol agents are typically isolated by screening rhizosphere or endophyte populations for their ability to suppress pathogens *in vitro* (Gao *et al*. 2010).

Endophytic fungi are microorganisms that invade plant tissues without causing illness (Devi *et al*. 2022) and resulting in permanent relationships with their host. Endophytic microbes hold significant biotechnological interest. These qualities have numerous applications, including boosting plant development, controlling biological pests, and improving plant resistance to stress (Komeil and Saad, 2021). Several studies have found that endophytes minimize insect and pathogenic fungus attacks on the host plant (Talapatra *et al*. 2017). Endophytes and plants produce secondary metabolites and bioactive substances, which have industrial applications for pathogen control and diverse biological activities (Polonio *et al*. 2015; Devi *et al*. 2022). Endophytic microorganisms are limited in their use in biological control due to external factors such as climatic conditions, interactions with other plants and microbial species, number of endophytes, and phytosanitary safety requirements (Polonio *et al*. 2015).

The selected medical plants, *Ocimum basilicum* L., *Leucas aspera* L., and *Elsholtzia stachyodes* (Link) Raizada & H.O.Saxena (Family: Lamiaceae), were economically exploited in the pharmaceutical, fragrance, and medicinal sectors (Gangadevi and Muthumary, 2007; Banerjee *et al*. 2009; Chowdhary and Kaushik, 2015). *O. basilicum* (sweet basil) is a common medicinal herb that is widely used in cuisine and as a spice. Ethnic medicine uses the leaves and essential oil as a carminative, antispasmodic, and stomachic (Shahrajabian *et al*. 2020). Like, *L. aspera* (Thumbai) is extensively used as a diaphoretic, antipyretic, insecticidal, and stimulant, as well as for migraine and snake bite treatment (Vasudha *et al*. 2019). Similarly, *E. stachyodes* produces essential oil that has anti-inflammatory, antibacterial, antitumor, and anticancer properties. Moreover, Banerjee (2011), Gautam (2014), and Chowdhary and Kaushik (2015) investigated the occurrence of endophytic fungi from different tissue parts of Indian medicinal plant species. However, Banerjee *et al*. (2009) isolated number of endophytic fungi species especially from medicinal plants of Lamiaceae family which were recorded as significant diversity of endophytic fungi. Previous results suggesting that these endophytic fungi could play an important role in the plant development by its potential antifungal activity against phytopathogenic fungi (Paul *et al*. 2012). In this work, 24 endophytic fungi from three medicinal plants *O. basilicum*, *L. aspera*, and *E. stachyodes* were evaluated for their potential antagonistic activity against plant pathogenic fungi *in vitro* found in various crops.

MATERIALS AND METHODS

Microbial strains

Strains of endophytic fungi, used in the current assay (Table 1), were isolated from *Ocimum basilicum* L., *Leucas aspera* L., *Elsholtzia stachyodes* (Link) Raizada & H.O.Saxena (Lamiaceae), by Dr. W. Sanahal Devi and retrieved from Plant Pathology and Microbiology Laboratory, Department of Life Sciences (Botany), Manipur University, Imphal, Manipur, India. The pathogenic fungus strains used were *Aspergillus flavus*, *Aspergillus niger*, *Curvularia lunata* and *Fusarium oxysporum* (Department of Plant Pathology, Central Agricultural University (CAU), Imphal).

In vitro antagonist activity of endophytic fungi

The dual-culture approach described by Bell *et al*. (1982) and modifed by Devi *et al*. (2022) was used to assess antagonist effect of isolated different endophytic fungal strains against selected plant pathogenic fungi species viz., *A. flavus*, *A. niger*, *C. lunata* and *F. oxysporum*. In this method, 5 mm dia. mycelia discs (5 days old culture) were removed from the borders of actively growing colonies of the both test pathogens and endophytic fungus and placed 5 cm apart on opposite ends of a 90 mm dia. Petri plate containing about 20 mL of PDA medium. The paired cultures were cultured at 25±1°C for 5-7 days and rated for degree of antagonism on a scale of class 1 to class 5 (Devi *et al*. 2022), as described as follows, class 1: Endophytic fungi totally outcompete the pathogen and cover the entire medium surface (very hostile or highly antagonistic), class 2: Endophytic fungi colonize at least two-thirds of the medium surface (antagonistic), class 3: Endophytic fungus and the pathogen colonized almost half of the medium surface, and neither appeared to dominate the other (moderately antagonist), class 4: The pathogen colonized at least two-thirds of the medium surface and appeared to tolerate invasion by Endophyte fungi (poor antagonist), and class 5: The pathogen entirely outgrows the endophytic fungus and takes up the entire medium surface (non antagonist) (Devi *et al*. 2022).

RESULTS AND DISCUSSION

A total of 24 species of endophytic fungi such as *Alternaria citri*, *Alternaria raphani*, *Aspergillus flavus*, *Aspergillus niger*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Cladosporium*

Table 1: Antifungal activity of endophytic fungi from *O. basilicum*, *L. aspera,* and *E. stachyodes* against plant pathogenic fungi

Fungal endophytestested (Strain ID)	Plant pathogenic fungi			
	A. flavus	*A. niger*	*C.lunata*	*F. oxysporium*
Ocimum basilicum				
Alternaria raphani J.W.Groves & Skolko (OBS1)	5	5	5	5
Aspergillus niger gr. (OBS2)	3	3	3	2
Chaetomium globosum Kunze (OBS3)	4	4	3	4
Cladosporium sp.1 (OBS4)	5	5	4	4
Epicoccum nigrum Link (OBS5)	4	4	3	4
Nigrospora spherica (Sacc.) E. W.Mason (OBS9)	3	4	4	4
Penicillium echinatum E. Dale (OBS10)	3	4	3	4
Trichoderma longibrachitum Rifai (OBS12)	3	3	4	3
Leucas aspera				
Aspergillus flavus gr. (LAS1)	3	3	3	3
Cladosporium sp.2 (LAS3)	5	4	4	5
Cladosporium cladosporioides (Fresen.) G.A. de Vries (LAS4)	5	5	5	5
Nigrospora sp.1 (LAS5)	4	4	3	3
Nigrospora oryzae (Berke. & Broome) Petch (LAS6)	3	2	2	3
Nigrospora sphaerica (Sacc.) E.W. Mason (LAS7)	3	4	4	3
Elsholtzia stachyodes				
Alternaria citri Ellis & N.Pierce (ECS1)	5	5	5	5
Aspergillus flavus gr. (ECS3)	3	3	4	4
Cladosporium sp.3 (ECS4)	5	5	5	5
Cladosporium cladosporioides (Fresen.) G.A.de Vries (ECS11)	5	5	5	5
Colletotrichum sp.4. (ECS5)	4	5	3	3
Colletotrichum gleosporioides (ECS2)	2	2	2	3
Nigrospora sacchari (Speg.) E.W. Mason (ECS7)	5	5	3	4
Nigrospora sphaerica (Sacc.) E.W. Mason (ECS12)	4	4	3	4
Penicillium verrucosum Dierckx (ECS8)	3	3	3	3
Trichoderma sp. (ECS10)	3	2	3	3

Numbers denote classes.Class1: Highly antagonistic, Class 2: Antagonistic, Class 3: Moderately antagonistic, Class 4: Poor antagonistic, Class 5: No antagonism

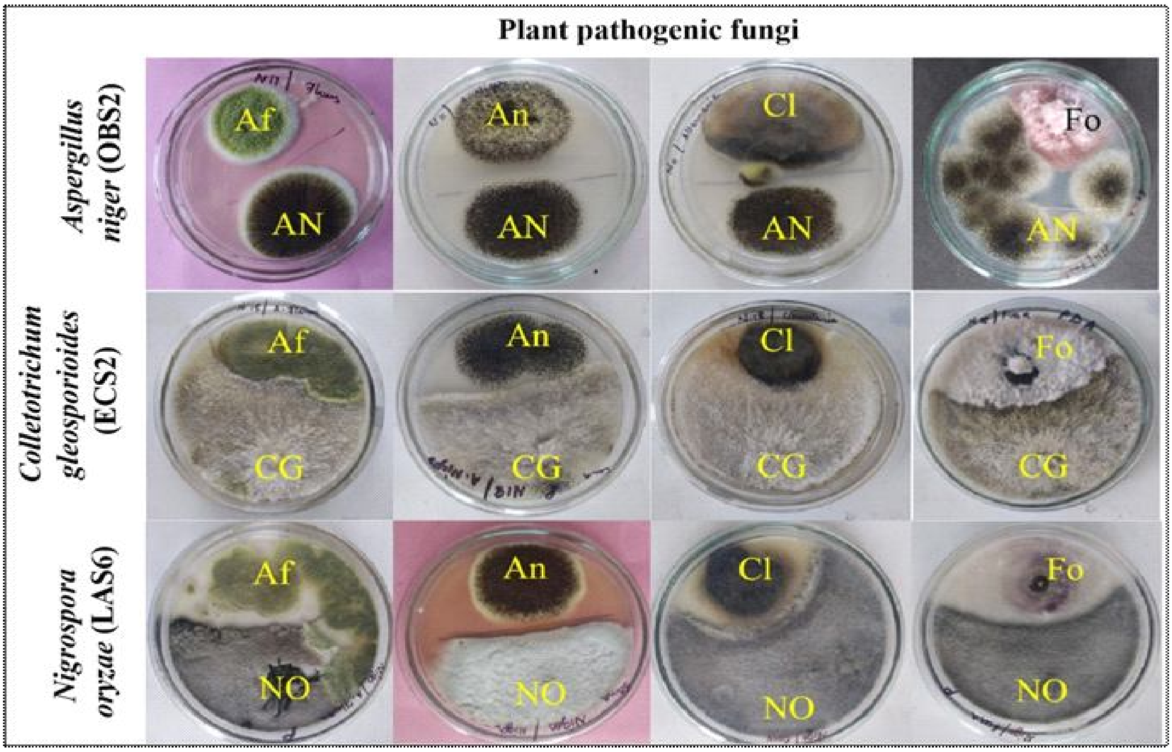

Fig. 1: *In vitro* antogonistic activity between endophytic fungi of *O. basilicum*, *L. aspera*, and *E. stachyodes* and the selected phytopathogens in in dual culture assays. Pathogens: Af=*Aspergillus flavus*; An=*Aspergillus niger*; Cl=*Curvularialunata*; Fo=*Fusarium oxysporum* Endophytes: AN=*Aspergillus niger*(OBS2); NO=*Nigrospora oryzae* (LAS6); CG=*Colletotrichum gleosporioides* (ECS2)

sp.1, *Cladosporium* sp. 2, *Cladosporium* sp. 3, *Colletotrichum gleosporioides*, *Colletotrichum* sp. 4, *Epicoccum nigrum*, *Nigrospora oryzae*, *Nigrospora sacchari*, *Nigrospora* sp.1, *Nigrospora sphaerica*, *Penicillium echinatum*, *Penicillium verrucosum*, *Trichoderma longibrachitum*, and *Trichoderma* sp. etc. from three medicinal plants, *O. basilicum*(8 species), *L. aspera* (6 species), and *E. stachyodes*(10 species), were tested (Table 1) against four common plant pathogenic fungi *Aspergillus flavus*, *Aspergillus niger*, *Curvularia lunata*, and *Fusarium oxysporum*. Of the 24 endophytic fungi, 19 were inhibited the mycelia growth activity by at least one of the four plant pathogenic fungal species with antagonistic activity ranging from class 2 to class 4. *Colletotrichum gleosporioides* (ECS2) had the most inhibitory effect on all four plant pathogenic fungi (Class 2), followed *Nigrospora oryzae* (LAS6) class 2 and *Aspergillus niger* (OBS2) class 3, respectively(Fig.1).

Endophytic microbes can improve plant fitness by increasing tolerance to heavy metals, and dryness can enhance plant growth while decreasing herbivory or phytopathogen settlement (Paul *et al*. 2012). Previous research on endophytic fungal communities connected with diverse types of plants from tropical, subtropical, and temperate habitats revealed a number of fungi (Banerjee, 2011; Gautam 2014; Chowdhary and Kaushik, 2015) and imply that these endophytic fungi may play a function in plant development. Endophytic nonpathogenic *Phomopsis*, *Cladosporium*, and *Diaporthe* from Korean medicinal plants stimulated the development (plant biomass) of pepper (Paul *et al*. 2012). Similarly, *Fusarium oxysporum*, a nonpathogenic endophyte isolate, generated resistance in cucumber to *Pythium ultimum* by combining antibiosis and mycoparasitism, as well as inducing plant defense mechanisms (Benhamou *et al*. 2002). Similar findings as of present studies were also reported by Devi *et al*. (2022), Chowdhary and Kaushik (2015), and Paul *et al*. (2012) and they discovered that endophytic *Phoma* sp., *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., *Diaporthe* sp., can suppress the growth of different harmful fungus. According to our findings, endophytes are a potential source of secondary metabolites and biological control agents.

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DECLARATIONS

Conflict of Interest: Authors declare no conflict of interest.

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