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In vitro screening of fungal endophytes from sandalwood (*Santalum album*) as antagonists to phytopathogens

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Abstract. *Simamora AV, Hahuly MV, Nenotek PS, Kana YR, Kasim M, Pollo R, Ola AR, Pramatana F. 2024. In vitro screening of fungal endophytes from sandalwood (*Santalum album*) as antagonists to phytopathogens. Biodiversitas 25: 361-371.* Endophytic fungi possess bioactive compounds and generate secondary metabolites like their host plants. The phytochemical compounds found in sandalwood plants (*Santalum album* L.) are recognized for their anti-pathogenic properties, suggesting the potential of sandalwood endophytic fungi as effective anti-phytopathogens. *Alternaria solani* and *Fusarium oxysporum* threaten tomato plants, whereas *Phytophthora palmivora* is particularly detrimental to cocoa plants. The aims of this study were: (i) to obtain isolates of endophytic fungi from roots, stems, and leaves of sandalwood plants and (ii) to assess the ability of endophytic fungi as anti-phytopathogens in vitro. Endophytic fungi were isolated through a direct plating method and next by purification and identification. All obtained endophytic fungi underwent testing for their antagonistic potential against *A. solani*, *F. oxysporum*, and *P. palmivora* in vitro through the dual culture method. The inhibition percentage was analyzed using variance analysis and further examined with the 5% HSD test. The study successfully isolated 104 endophytic fungal isolates from the sandalwood; 29 isolates were from the roots, 33 from the stems, and 42 from the leaves. All endophytic isolates demonstrated the ability to inhibit the growth of *A. solani*, *F. oxysporum*, and *P. palmivora* in vitro, with various inhibition percentages ranging from 26.7 to 83.3%. Further research will concentrate on secondary metabolites from selected isolates showing inhibition levels exceeding 70%.

Keywords: *Alternaria solani*, biocontrol agents, *Fusarium oxysporum*, *Phytophthora palmivora*, *Santalum album*

INTRODUCTION

Fungi make up 1,500,000 species with a 33:1 ratio of fungi to plant organisms, and among them, fungal endophytes are a highly prevalent and widespread group (Sumanth et al. 2021). Endophytic fungi are widely distributed in plants and contribute significantly to the equilibrium of plant microecosystems. Extensive research has demonstrated that the abundance of endophytic fungi is notably affected by the specific plant species and the surrounding environment. Furthermore, these fungi often provide various fitness benefits to their host plants (Zhao et al. 2020).

Plants are a source of medicinal raw materials and antimicrobial compounds. Most chemical components derived from plants used as therapeutic and antimicrobial ingredients are secondary metabolites. Sandalwood is a type of plant native to Indonesia that grows endemic to the East Nusa Tenggara (ENT) region, which is often found on the islands of Timor, Sumba, Alor, Solor, Pantar, Flores, Roti, and other islands. It is a hemiparasitic tree native to semiarid areas and is essential because it has high economic value. East Nusa Tenggara sandalwood has distinctive features, including having high oil content and heartwood production. Sandalwood is known for yielding essential oils with a delightful scent, making it highly valued in the market (Ariyanti and Asbur 2018; Fatima et al. 2019). According to Sun et al. (2014), *S. album* contains more than 200 chemical compounds, one of which is a high-value essential oil. Essential oils extracted from roots and sandalwood are reported to have antiviral and antifungal activities and potential as anticancer therapy and skin fungal infections (Kim et al. 2017).

One of the newest ways to obtain secondary metabolites without exploiting the plant of origin is using endophytic microbes in plant tissues. Endophytic fungi play an essential role in symbiosis with plants physiologically and ecologically, protecting plants from pathogens and unfavorable environments by producing secondary metabolites (Jain and Pundir 2017; Kim et al. 2017; Fadiji and Babalola 2020).

Each higher plant may contain several endophytic microbes that produce secondary metabolites because of coevolution or genetic transfer from the host plant to the endophytic microbes. The ability of endophytic microbes to produce certain phytochemical compounds produced by their host plants may be related to the genetic recombination of endophytic microbes with their hosts during their evolution. This ability is a great opportunity and can be relied upon to produce secondary metabolites

through endophytic microbes isolated from the host plant. Endophytic microbes can produce rare and important bioactive compounds like their host plants. In that case, endophytes can reduce their dependence on raw material sources from their host plants, thereby preserving the existing biodiversity (Alam et al. 2021; Rutkowska et al. 2023). In addition, using microbes as a source of efficacious secondary metabolite products can be carried out in an easier and more economical process to produce products at more competitive prices (Chitnis et al. 2020). In addition to delivering bioactive compounds, endophytes can serve as biocontrol agents by releasing antibiotic substances to manage plant diseases. These endophytes protect the plant from insect pests and diseases and can produce biotechnologically useful substances (Giehl et al. 2023).

Research on sandalwood in Indonesia, particularly in the ENT Province, has predominantly focused on its growth and conservation (Seran et al. 2018; Seran et al. 2020). However, more scholarly attention needs to be devoted to exploring sandalwood endophytic fungi and their potential inhibitory effects on phytopathogens, as well as their role as plant growth promoters. Understanding the endophytic fungi associated with sandalwood can provide insight into their contribution to plant health. Therefore, undertaking research in this domain holds significant merit, as it can contribute to the advancement of sandalwood cultivation and its role in ENT and Indonesia. Hence, the primary objectives of this research encompass (i) isolating endophytic fungi from the roots, stems, and leaves of sandalwood and (ii) assessing their in vitro inhibitory potential against three phytopathogens.

MATERIALS AND METHODS

Isolation of endophytic fungi from root, stem, and leaves of sandalwood

Endophytic fungi were sampled from roots, stems, and leaves of sandalwood in Ajaobaki Village, North Mollo Sub-District, and Kuanoel Village, Fatumnasi Sub-District located in South Central Timor District, East Nusa Tenggara Province (Figure 1) and carried out by direct plating (Ramanan et al. 2020; Simamora et al. 2021). Each growing endophytic fungi was purified on fresh Potato Dextrose Agar (PDA) medium supplemented with chloramphenicol at 100 mg/L. Subsequently, the cultures were incubated at room temperature for 7-14 days. Following the incubation period, comprehensive macroscopic and microscopic observations were made, and the identification process was carried out according to the criteria outlined by Watanabe (2010).

Characterization of endophytic fungi from root, stem, and leaves of sandalwood

All fungal endophyte isolates underwent standard characterization procedures, which involved the examination of their macroscopic and microscopic attributes and their reproductive structures under the microscope at both 10X and 40X magnifications. Macroscopic observations were focused on the fungal colonies' growth characteristics in agar medium, including colony diameter, color, texture, reverse side appearance, margins, and pigment production (Olokkaran et al. 2019).

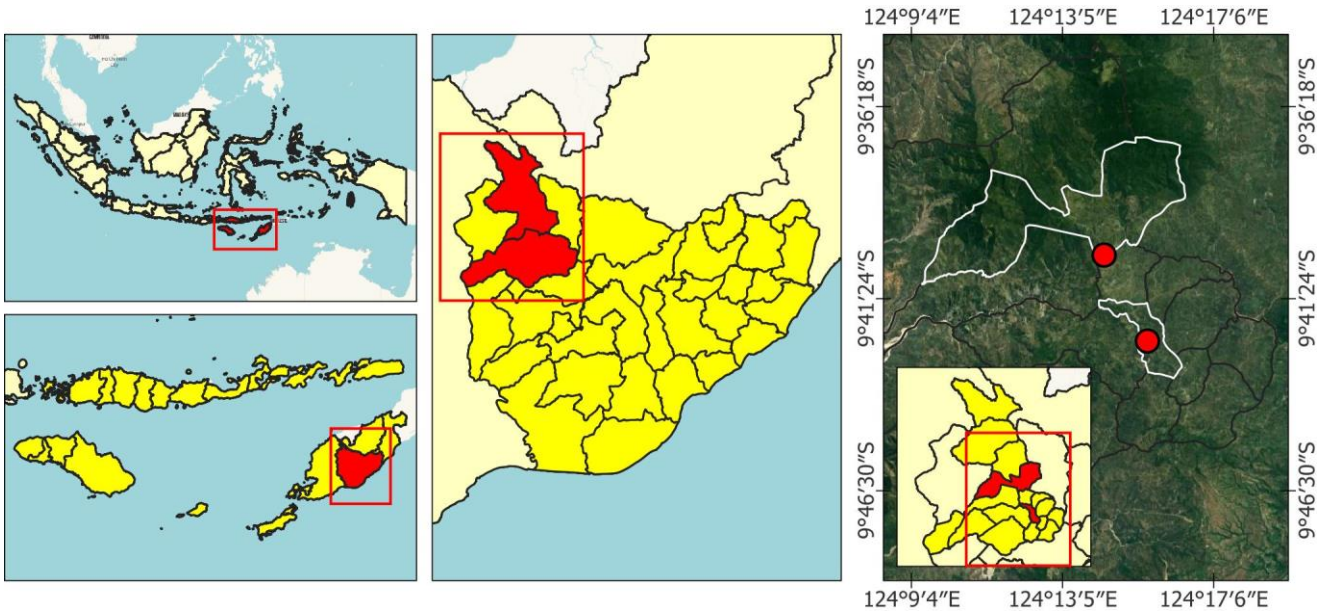

Figure 1. Map of the research location: red dots on the island showing the sampling sites in Kuanoel and Ajaobaki Villages, Fatumnasi and Mollo Utara Sub-district, South Central Timor District, East Nusa Tenggara Province, Indonesia

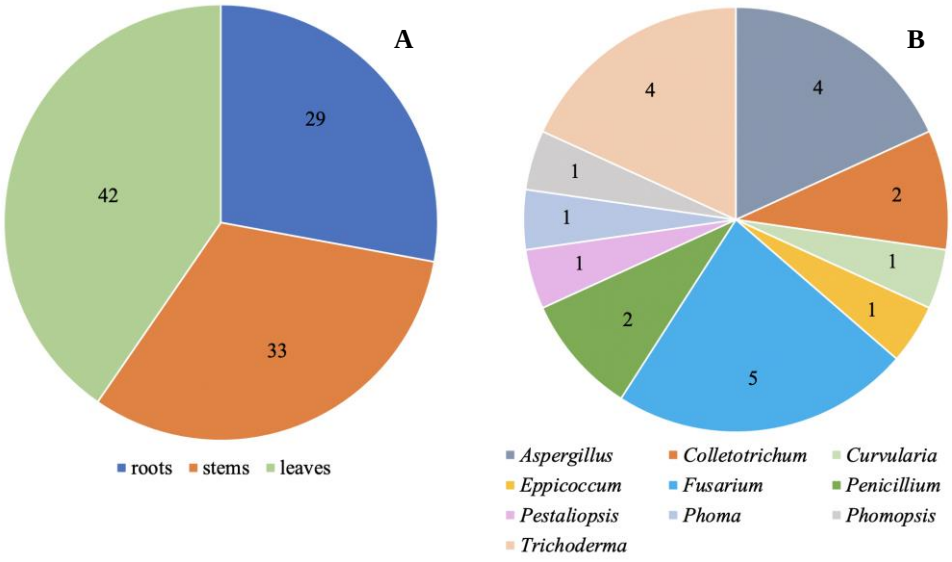

Figure 2. A: The number of fungal endophytes isolates from roots, stems, and leaves of sandalwood. B: Twenty-two morphospecies from 10 genera of fungal endophytes isolates from sandalwood's roots, stems, and leaves

Microscopic features were assessed to determine the presence or absence of conidia, the shape and size of conidia, conidial arrangement, conidiophores, and characteristics of hyphae, including whether they were septate or non-septate. The identification process involved staining the fungal colonies with lactophenol cotton blue using the wet mount technique, conducted per the established standards outlined in the identification manual (Barnett and Hunter 1998; Watanabe 2010).

Subculturing of *A. solani*, *F. oxysporum*, and *P. palmivora*

Alternaria solani, *F. oxysporum*, and *P. palmivora* isolates were obtained by the Plant Pathology Laboratory, Faculty of Agriculture, Universitas Nusa Cendana, Kupang, Indonesia. Isolates of *A. solani* and *F. oxysporum* were derived from diseased tomato plants, while *P. palmivora* was obtained from cocoa pods displaying symptoms of blackish-brown spots. All the cultures were subcultured onto fresh PDA medium and incubated at room temperature for three to seven days or until utilized.

Anti-phytopathogen activity test

The assessment of endophytic fungi's capacity to inhibit phytopathogen growth involved cultivating them in a 9 cm Petri dish on PDA media. The chosen phytopathogens included *A. solani*, *F. oxysporum*, and *P. palmivora*. Inoculums of phytopathogenic and endophytic fungi, aged seven days and measuring each 0.5 cm in diameter, were transferred from a pure culture Petri dish to a PDA medium Petri dish. The inoculum cut from the medium's edge was 3 cm, and the spacing between the two inocula was 2 cm. The antagonistic efficacy of endophytic fungi against phytopathogens was determined by measuring the colonies' diameter of both phytopathogenic and endophytic fungi. The colony diameters of phytopathogens and endophytic fungi were utilized to calculate the percentage of inhibition of endophytic fungi against phytopathogens. The obtained inhibition percentage data underwent calculation and statistical analysis for variance and continued with the Honestly Significant Difference (HSD) test.

RESULTS AND DISCUSSION

Isolation of endophytic fungi from root, stem, and leaves of sandalwood

The sandalwood plants cultivated in these areas are aged between 15-20 years. Based on the isolation process results, 104 endophytic fungal isolates were identified, comprising 29 isolates from the sandalwood plant roots, 33 isolates from sandalwood plant stems, and 42 isolates from sandalwood plant leaves. All obtained endophytic fungal isolates were classified into 22 morphospecies (Figure 2).

The isolated endophytic fungi reported in this study were consistent with earlier findings (Sun et al. 2014; Tapwal et al. 2016). Tapwal et al. (2016) confirmed the isolation of *F. oxysporum* among five endophytic species discovered in sandalwood. Additionally, all the endophytic fungi they obtained exhibited the presence of alkaloids, phenolics, tannins, flavonoids, sugars, glycosides, terpenoids, amino acids, and proteins. Sun et al. (2014) described 13 fungal isolates isolated from sandalwood roots, including *Phomopsis* and *Aspergillus*, with *Penicillium* being the most often isolated genera. In addition, Sun et al. (2014) also revealed that *Fusarium* was the most common genera isolated from sandalwood's host, *Kuhnia rosmarinifolia* Vent. Our study found that *Fusarium* was the most often isolated genera, followed by *Aspergillus* and *Trichoderma* (Figure 2B). The disparity in outcomes is believed to be influenced by environmental factors.

The variety, colonization rate, and frequency of endophyte occurrence were all impacted by environmental conditions. Several environmental elements can influence

the symbiosis between the endophyte and the plant. The weather is one of the most critical variables affecting how frequently endophytes occur. For instance, wind is the primary means that endophytes disperse their spores. Therefore, dispersal would intensify more in regions with stronger winds (Grabka et al. 2022). (Similarly, more precipitation is associated with higher endophyte occurrence, particularly for those that spread horizontally partly due to spore dispersal (Grabka et al. 2022). These endophytes depend on moisture for distribution, germination, and host plant colonization. Endophytes, which typically exist in specific temperature ranges, might find themselves in a situation that is either friendly or hostile depending on factors like temperature and sun radiation (Gomes et al. 2018).

Evidence indicates that endophyte diversity and colonization rate are dynamic. Seasonal variations, particularly in the spring, have revealed more diversity and colonization rates than in the fall (Sadeghi et al. 2019). The endophyte density can also be influenced by the age and location of the plants, with elderly leaves exhibiting more excellent resistance to colonization when compared to younger leaves (Manzar et al. 2022).

Macroscopic and microscopic characterization of endophytic fungi from root, stem, and leaves of sandalwood

The morphological characterization of selected fungi isolated from the root, stem, and leaves of sandalwood is presented in Table 1. Primary morphological characters such as texture, surface color, the reverse color of the colony in the plates, zonation, growth of the colony, and microscopic features, such as spore and hyphal appearance, were observed and used for genera identification (Barnett and Hunter 1998; Watanabe 2010).

A total of 22 morphospecies of fungal endophytes were isolated from sandalwood, comprising five identified as *Fusarium*, four as *Aspergillus*, four as *Trichoderma*, two each as *Colletotrichum* and *Penicillium*, and one each as *Curvularia*, *Epicoccum*, *Pestaliopsis*, *Phoma*, and *Phomopsis*. Furthermore, 20 isolates remained unidentified. Selected cultures of the obtained endophytic fungi are illustrated in Figures 3-5.

Anti-phytopathogen activity test

The endophytic fungal isolates were tested for antagonistic ability against three phytopathogens: *A. solani*, F. *oxysporum*, and *P. palmivora*. The results of the antagonist test are shown in Tables 2-4.

It can be seen from Tables 2, 3, 4 that the ability of endophytic fungal isolates varies to inhibit each type of pathogen. The lowest inhibition percentage was 26.7%, and the highest was 86.7%. The difference in inhibitory ability is related to the mechanism of inhibition of phytopathogens owned by each endophytic fungus. The mechanism of phytopathogen inhibition by endophytic fungi varies, for example, competition or mycoparasitism (Figure 6), and depends on the environmental conditions of the plant growth. The use of various mechanisms by endophytic microorganisms enhances the ability of plants to adapt. Endophytic fungi, for instance, utilize a range of mechanisms, including competition, antibiosis, and mycoparasitism, to inhibit pathogens directly. They can also indirectly induce resistance and activate plants' defense systems against diseases (Ahmed et al. 2020; Fadiji and Babalola 2020).

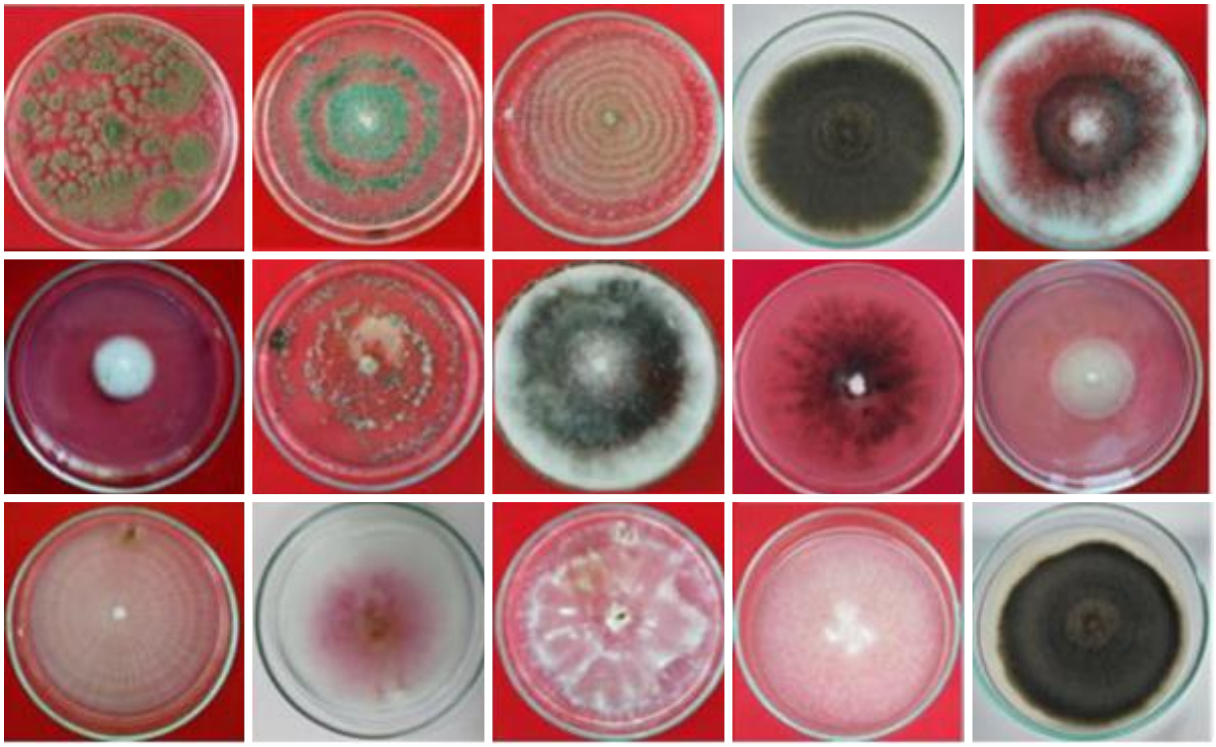

Figure 3. Examples of endophytic fungi isolated from the roots of sandalwood plant

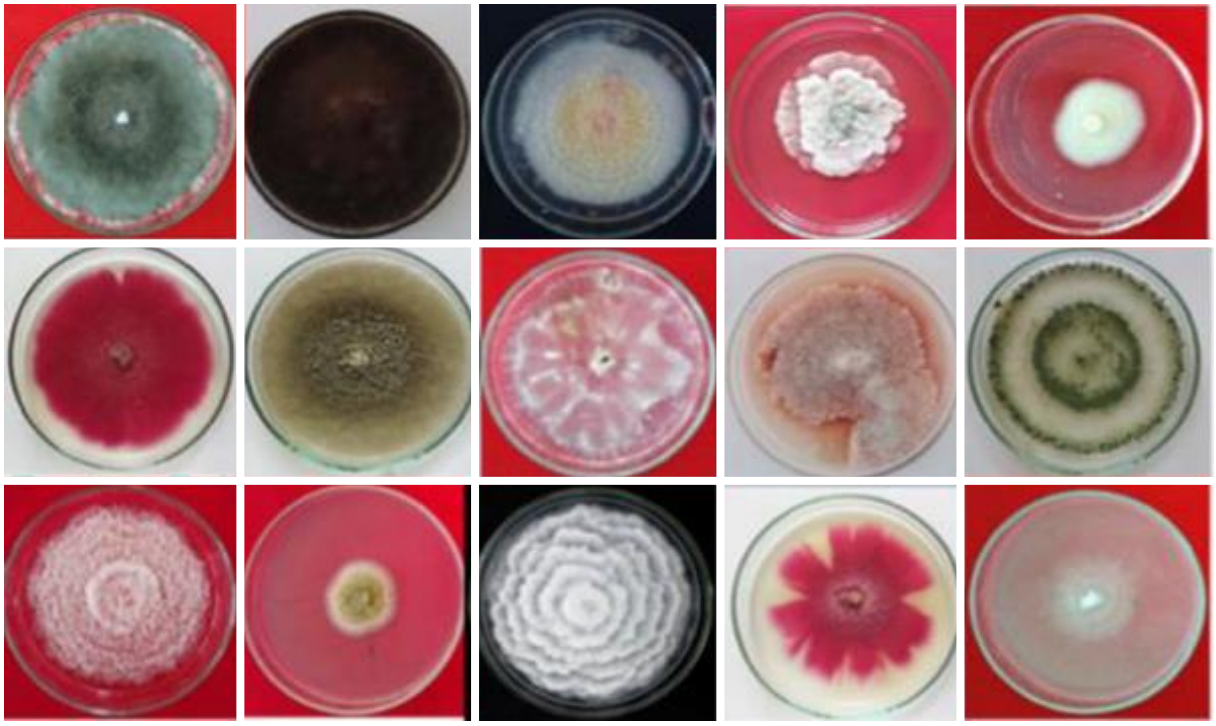

Figure 4. Examples of endophytic fungi isolated from the stem of sandalwood plant

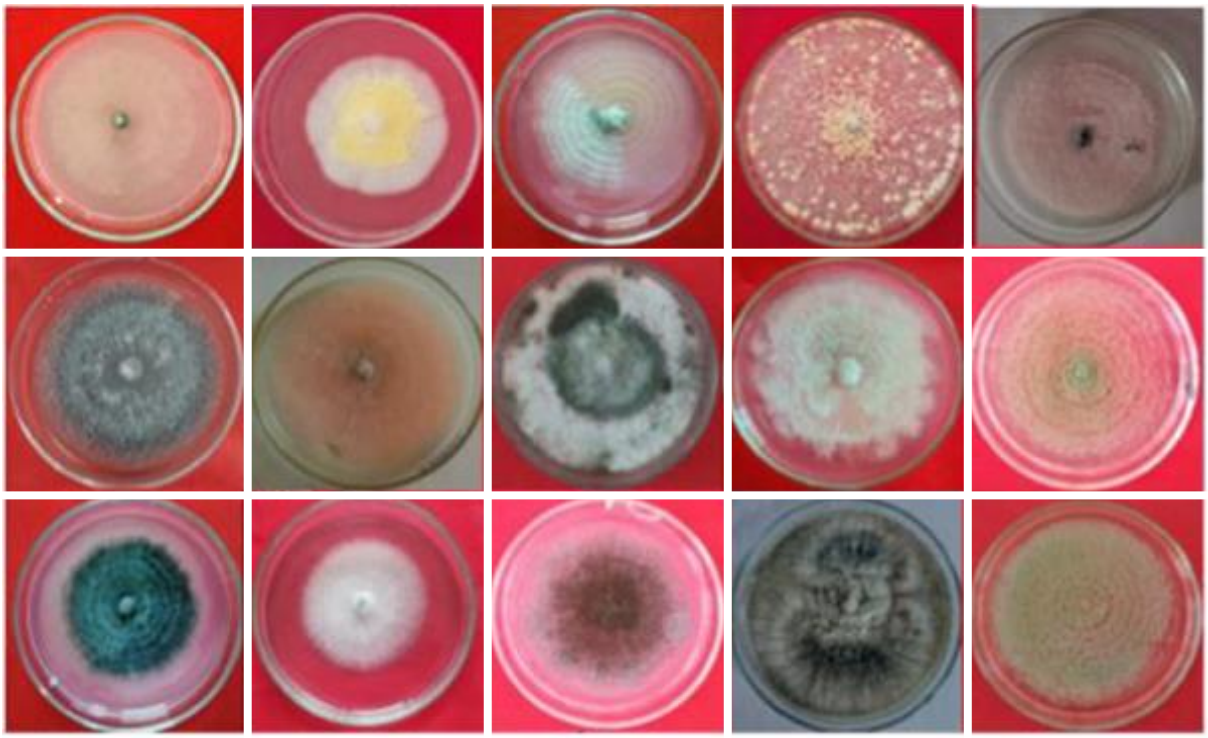

Figure 5. Examples of endophytic fungi isolated from the leaves of sandalwood plant

Table 1. Morphological characters of selected fungal isolates from sandalwood's root, stem, and leaves

Isolates	Characteristics of PDA medium		Genera
	Macroscopic	Microscopic	
Root 01	The colony center was yellow-green, while the outer edges were covered in white aerial mycelia. The colony had a grainy appearance and a relatively smooth surface.	It gave rise to conidiophores stipes with rough walls, uniseriate phialides on the uniseriate vesicles, and greenish conidial heads made up of catenulate conidia. The conidia were globose.	*Aspergillus*
Root 05	The colony was mainly deep green at the center and surrounded by a white edge with a regular margin, while yellowish-grey, pale yellow exudate droplets dominated the reverse.	Insulated hyphae with ramifications at the end of conidiophores, metulae, phialide, and conidia are attached and look like a chain. Conidiospores were green, varied in shape, round or obovoid.	*Penicillium*
Root 13	The colony was dark green and covered in dense white mycelia pustulates.	Conidiophores were narrow and flexuous, phialides were solitary and verticillate, and conidia were sub-globose.	*Trichoderma*
Root 20	The colony was grey and salmon in hue, giving it a cottony look. On the reverse side, the center was dark orange to pink.	Conidia had fusiform ends that tapered to a point.	*Colletotrichum*
Root 29	The colony was dark green with compact turfs of white mycelium in concentric ring-like zones.	Conidiophores were branched and produced spore masses at irregularly verticillate phialides. Conidia were formed in conidial heads clustered at the tips of the phialides, phialospores, and bearing ellipsoidal or oval clusters.	*Trichoderma*
Stem 07	The colony was dark brown, and there were aerial mycelia near the edge of the Petri dish.	It produced biseriate conidiophores with septate metulae and phialides and radiate conidial heads made up of catenulate conidia on dark brown globose vesicles. The conidia were clumped together, globoid, and brown.	*Aspergillus*
Stem 12	The colony was cottony and fast-growing with deep purple shades (obverse and reverse).	Microconidia were hyaline, one-celled, ellipsoidal, cylindrical, or reniform. Macroconidia were hyaline, usually three septate, slightly falcate to almost straight, and chlamydospores globose or sub-globose.	*Fusarium*
Stem 16	In its early growth, the mycelium is white. After two days, the color gradually changed to greyish-white and turned completely black due to pycnidia formation. The colony is circular, rough, and spreading outward.	Conidiomata were pycnidial, sub-globose to broadly ellipsoidal. Conidia were ellipsoidal to ovoid or sub-cylindrical.	*Phoma*
Stem 25	At first, the colony appeared dark brown-black and rather fluffy, but later, it became almost entirely black and dense. Following this, the formation of a black stroma was observed.	Conidia were brown, and unbranched were conidiophores discovered in the apical region with prominent conidiogenesis. The conidia were dark brown, curved to varying degrees, and tapered at both ends. There were three to four septa on each conidium.	*Curvularia*
Stem 29	The colony was expanding quickly. It was round, white, floccose, and light grey in the center with pale-reddish pigmentation.	After four weeks, many conidiomata were seen. Conidiomata were pycnidial and scattered. Conidia were smooth, hyaline, ellipsoidal to cylindrical.	*Epicoccum*
Leaf 04	The texture of the colony was fine and cotton-like, forming a circular pattern, white to brown. In addition, between day 7 and day 21 of the incubation process, black or dark brown acervuli started to grow.	The conidia each contained five cells with four septa. The conidia were fusiform in that the cell edges could either be straight or curved. There was a flagellum on both ends of the conidia.	*Pestaliopsis*
Leaf 09	The colony had a layered circular growth with wavy edges and a powdery, white-greyish-brownish texture. Its mycelium grows in a circular, wavy pattern that resembles a rose with uneven mycelium edges.	Hyphae were septate and hyaline. Conidia were subcylindrical to elliptical, hyalin unicellular, and dimorphic.	*Phomopsis*
Leaf 23	In the beginning stage, a dark green shade appeared in the colony's middle, gradually spreading outward to the edges. In the end, it looks like a bluish-green color.	Conidia were mostly globose and developed on phialides produced in opposite directions at each point.	*Trichoderma*
Leaf 25	White cottony with grey center becoming powdery with orange spots, reverse white then grey.	Setae was absent, while appressoria was observed. Appressoria were medium brown, aseptate, solitary, and elliptical. Conidia were hyaline and cylindrical.	*Colletotrichum*
Leaf 30	The colony center was a velvety dark green color. White aerial mycelia surrounded the edges.	It has greenish columnar conidial heads composed of catenulate conidia, and it has uniseriate phialides on the surface of the vesicle. The conidia were globose and phialospores in shape.	*Aspergillus*

Endophytes utilize the antibiosis mechanism by producing secondary metabolites with antibacterial and antifungal properties, thereby inhibiting the growth of phytopathogenic microorganisms (Khan et al. 2020). Figures 6.A, 6.B, and 6.D showed that the endophytes grew faster than the phytopathogens by the antibiosis mechanism. Numerous antimicrobial metabolites, including flavonoids, peptides, quinines, alkaloids, phenols, terpenes, steroids, and polyketides, are known to be produced by endophytes (Lugtenberg 2016; Munir et al. 2020). However, it is imperative to investigate the potential commercial applications of secondary metabolites produced by endophytic fungi (Egamberdieva and Jabborova 2020).

Endophytes primarily depend on competitive interactions to safeguard host tissues from pathogen invasion. They could systematically or locally inhabit various parts of the host plant. Achieving high growth speed (Figures 6.F and 6.G) was one of the main factors determining antagonistic fungi's ability to control phytopathogens (Köhl et al. 2019). However, the mechanisms employed by most endophytes for competition typically operate in conjunction with other mechanisms instead of acting independently. Since the control method used by endophytes is frequently local, they must systematically colonize the portion of the host where most pathogens attack (Fadiji and Babalola 2020; Nunna and Balachandar 2022; Akram et al. 2023).

Table 2. Percentage inhibition of phytopathogens by endophytic fungi isolated from roots of sandalwood

Fungal endophytes	Phytopathogens						Fungal endophytes	Phytopathogens				
	A. solani		*F. oxysporum*		*P. palmivora*			*A. solani*		*F. oxysporum*		*P. palmivora*	
Root 01	63.47	f	43.27	d	46.73	d	Root 16	53.30	c	53.30	g	33.33	b
Root 02	66.80	g	53.27	g	46.73	d	Root 17	70.03	h	70.30	j	73.30	j
Root 03	80.00	j	77.80	k	65.67	i	Root 18	83.23	k	53.30	g	83.33	k
Root 04	53.33	c	53.33	g	26.67	a	Root 19	53.27	c	49.97	f	53.30	f
Root 05	76.73	i	80.00	l	65.70	i	Root 20	83.27	k	83.30	m	83.33	k
Root 06	80.10	j	56.67	h	53.33	f	Root 21	83.27	k	83.33	m	83.33	k
Root 07	50.03	b	50.00	f	53.30	f	Root 22	76.73	i	83.30	m	83.30	k
Root 08	50,07	b	50.00	f	53.30	f	Root 23	83.27	k	83.30	m	83.33	k
Root 09	59.97	e	33.30	b	50.00	e	Root 24	83.30	k	83.27	m	83.30	k
Root 10	70.00	h	80.20	l	60.03	g	Root 25	83.33	k	83.27	m	83.33	k
Root 11	60.00	e	70.03	j	60.00	g	Root 26	83.30	k	83.30	m	83.30	k
Root 12	60.13	e	36.67	c	63.30	h	Root 27	26.67	a	26.70	a	26.73	a
Root 13	60.00	e	46.73	e	46.67	d	Root 28	83.27	k	83.33	m	83.33	k
Root 14	83.33	k	83.30	m	83.33	k	Root 29	63.27	f	83.30	m	43.33	c
Root 15	56.67	d	60.07	i	50.03	e							

Note: Numbers followed by different letters denote significant differences (HSD 0.05) among the treatment means

Table 3. Percentage inhibition of phytopathogens by endophytic fungi isolated from stems of sandalwood

Fungal endophytes	Phytopathogens						Fungal endophytes	Phytopathogens				
	A. solani		*F. oxysporum*		*P. palmivora*			*A. solani*		*F. oxysporum*		*P. palmivora*	
Stem 01	80.00	e	53.33	g	60.00	j	Stem 18	46.70	b	53,30	g	56.67	i
Stem 02	46.73	b	43.33	e	66.67	k	Stem 19	83.33	f	30,00	b	26.73	a
Stem 03	60.03	d	60.00	h	60.00	j	Stem 20	46.73	b	33,33	c	43.33	e
Stem 04	60.07	d	43.30	e	46.70	f	Stem 21	83.33	f	83,33	i	83.33	l
Stem 05	60.03	d	60.00	h	60.03	j	Stem 22	83.30	f	83,30	i	83.33	l
Stem 06	60.03	d	60.03	h	60.03	j	Stem 23	83.33	f	40,03	d	43.33	e
Stem 07	60.03	d	60.03	h	60.00	j	Stem 24	83.33	f	83,33	i	83.33	l
Stem 08	60.03	d	60.03	h	60.03	j	Stem 25	83.30	f	46,70	f	83.33	l
Stem 09	60.03	d	60.03	h	60.03	j	Stem 26	83.33	f	83,33	i	83.33	l
Stem 10	60.03	d	60.00	h	56.70	i	Stem 27	83.33	f	83,30	i	36.73	c
Stem 11	83.30	f	53.30	g	53.33	h	Stem 28	83.00	f	83,33	i	83.27	l
Stem 12	60.03	d	60.03	h	43.30	e	Stem 29	83.33	f	83,33	i	83.33	l
Stem 13	60.03	d	83.33	i	53.30	h	Stem 30	83.00	f	83,33	i	83.30	l
Stem 14	43.30	a	53.33	g	50.03	g	Stem 31	83.33	f	83,33	i	83.33	l
Stem 15	83.30	f	83.30	i	46.70	f	Stem 32	83.33	f	83,33	i	83.33	l
Stem 16	50.03	c	53.00	g	40.00	d	Stem 33	83.33	f	83,33	i	83.30	l
Stem 17	46,70	b	26.73	a	33.30	b							

Note: Numbers followed by different letters denote significant differences (HSD 0.05) among the treatment means

Mycoparasitism is another crucial mechanism in which endophytes shield the host ecology by directly attacking the identified pathogen or its reproductive structures (Huang et al. 2020). Endophytic fungi invade the hyphae of pathogenic fungi, coil around them, and lyse their cells to destroy their cell walls. Langa-Lomba et al. (2022) outlined that mycoparasitism has been characterized by diverse interactions and hyphal structures exhibited by antagonistic fungi. Remarkable structures observed during confrontations with phytopathogens, which were restrained in dual culture plate confrontation, include the formation of papilla-like structure, appressoria-like structure, the entangled hyphae, and the lysis of phytopathogen hyphae, as depicted in Figure 7.

Each endophyte isolate obtained exhibited varying abilities to suppress phytopathogens. Some isolates demonstrated the capability to suppress *A. solani* while not affecting *F. oxysporum* or *P. palmivora*. Typically, a single agent employs multiple concurrent mechanisms or distinct strategies to combat diverse infections. *Trichoderma harzianum*, for instance, usually suppresses pathogens via direct parasitism, which involves hyphae entanglement and degradation (Guzmán-Guzmán et al. 2023). Additionally, *T. harzianum* can impede the growth of a white mold agent through antibiosis and competition for place and or resources supply (Dutta et al. 2023).

Table 4. Percentage inhibition of phytopathogens by endophytic fungi isolated from leaves of sandalwood

Fungal endophytes	Phytopathogens						Fungal endophytes	Phytopathogens				
	A. solani		*F. oxysporum*		*P. palmivora*			*A. solani*		*F. oxysporum*		*P. palmivora*	
Leaf 01	80.03	j	80.00	l	80.03	n	Leaf 22	60.03	e	60.03	g	60.03	j
Leaf 02	66.70	f	63.33	h	56.67	i	Leaf 23	83.33	k	50.07	e	50.03	f
Leaf 03	50.03	b	66.73	i	46.67	e	Leaf 24	73.30	h	76.67	k	83.33	p
Leaf 04	60.03	e	60.00	g	66.67	l	Leaf 25	83.30	k	53.30	f	30.03	a
Leaf 05	60.03	e	43.30	c	60.03	j	Leaf 26	46.67	a	40.03	a	43.33	d
Leaf 06	56.73	d	43.33	c	46.67	e	Leaf 27	83.27	k	83.30	m	83.30	o
Leaf 07	50.03	b	50.03	e	50.07	f	Leaf 28	83.27	k	83.30	m	83.27	o
Leaf 08	76.73	i	76.73	k	53.33	h	Leaf 29	83.27	k	46.67	d	43.30	d
Leaf 09	56.67	d	40.03	b	40.03	c	Leaf 30	83.27	k	83.27	m	83.30	o
Leaf 10	76.73	i	76.67	k	46.67	e	Leaf 31	83.30	k	83.27	m	83.30	o
Leaf 11	60.03	e	60.00	g	33.30	b	Leaf 32	83.27	k	83.27	m	83.27	o
Leaf 12	60.00	e	60.03	g	60,03	j	Leaf 33	66.73	g	40.03	a	63.33	k
Leaf 13	60.00	e	43.30	c	60.03	j	Leaf 34	83.30	k	83.30	m	83.27	o
Leaf 14	51.13	c	46.67	d	51.13	g	Leaf 35	83.27	k	83.30	m	83.30	o
Leaf 15	60.03	e	60.03	g	60.00	j	Leaf 36	83.30	k	83.30	m	83.27	o
Leaf 16	66.67	f	60.03	g	60.03	j	Leaf 37	83.30	k	83.30	m	83.30	o
Leaf 17	60.03	e	40.03	a	40.00	c	Leaf 38	83.27	k	43.33	c	53.30	h
Leaf 18	80.03	j	73.30	j	73.30	m	Leaf 39	83.30	k	36.67	a	83.27	o
Leaf 19	60.00	e	86.67	n	60.03	j	Leaf 40	83.30	k	83.27	m	83.30	o
Leaf 20	60.03	e	60.00	g	60.00	j	Leaf 41	83.27	k	83.27	m	83.30	o
Leaf 21	86.67	l	86.67	n	53.33	h	Leaf 42	83.30	k	83.27	m	83.30	o

Note: Numbers followed by different letters denote significant differences (HSD 0.05) among the treatment means

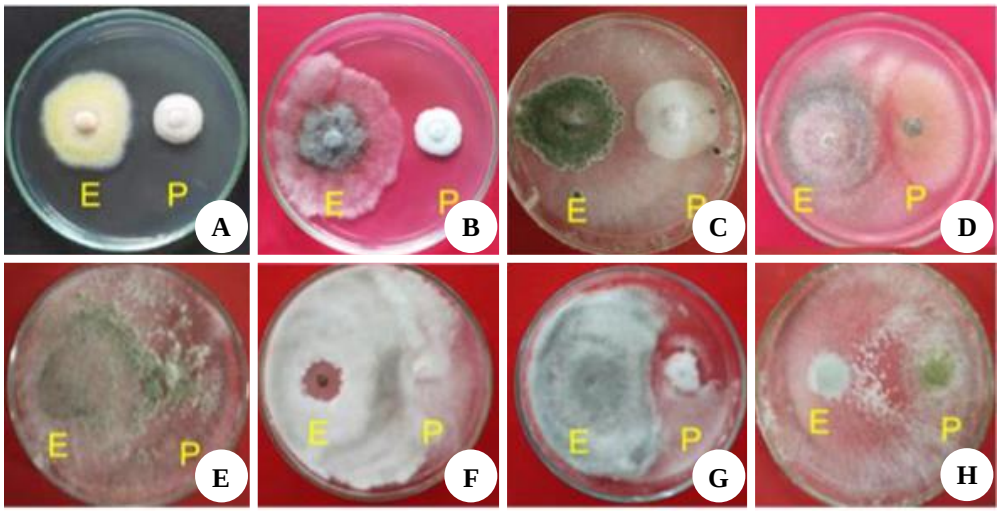

Figure 6. The mechanism of inhibition of endophytic fungi isolates (E) against phytopathogens (P). The antibiosis mechanism occurs in Figures 6.A, B, D; the competition mechanism occurs in Figures 6f and 6g, while the mycoparasitism mechanism occurs in the others

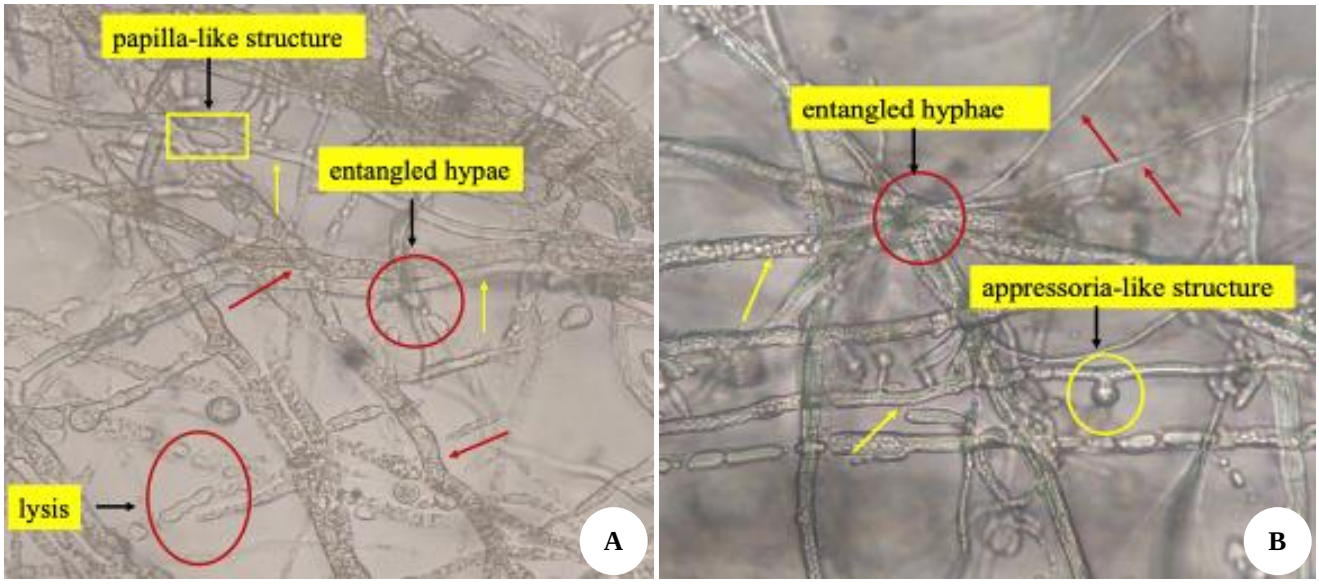

Figure 7. The interactions between fungal endophytes and phytopathogens in dual culture confrontation: A. Papilla-like structure, entangled hyphae, and the lysis of phytopathogen; B. Entangled hyphae and appressoria-like structure. Red arrow indicates the phytopathogen hyphae, and the yellow arrow indicates the fungal endophyte hyphae

While in vitro screening provides a controlled and standardized environment and for initial evaluations, it might not capture the intricate interplay between endophytes and their host plants in real-world. The artificial conditions of in vitro studies may oversimplify the interactions and fail to account for biological complexities that can impact the success of endophytic fungi as biocontrol agents. Therefore, it is essential to approach the interpretation of in vitro screening results with caution and to complement them with in vivo studies to better assess the true potential of endophytic fungi in controlling phytopathogens in the natural context. Integrating both types of research can offer a more comprehensive understanding of the efficacy and practical applicability of these fungi in agricultural and environmental settings (Kashyap et al. 2023).

Apart from providing benefits, endophytic fungi also have several weaknesses. The weaknesses need to be addressed well, need to be considered, and need to be anticipated beforehand so that work related to endophytic fungi can run well. In some cases, endophytic fungi can turn into pathogens, or have negative effects on plant growth, including inhibiting seed germination and flowering (Soesanto and Mugiastuti 2023). Endophytes can outcompete phytopathogens by utilizing available resources more efficiently, denying them essential nutrients. Nonetheless, there are examples where endophytes may face challenges in effective competition, resulting in limited success in controlling phytopathogens (Kashyap et al. 2023). This study can be a reference for further research in the agricultural sector. Research on the role of sandalwood endophytic fungi and the secondary metabolites they produce will be tested in the field as agents for controlling plant pests and diseases, promoting plant growth, and biofertilizers.

Recent advances have also shown that the most important and valuable drugs from plants have been also produced by their associated endophytic fungi. For example, paclitaxel known as natural product from the plant *Taxus brevifolia* has been found in its endophytic fungus *Taxomyces andreanae* isolated from the plant *Taxus brevifolia*. Moreover, at least 20 species of endophytic fungi have been reported to produce paclitaxel (Ola 2020). In addition, the metabolites of the Madagascar plant of periwinkle (*Catharanthus roseus*), namely vincristine and vinblastine, were also reported to be produced by the endophytic fungi *Talaromyces radicus* (Palem et al. 2015) and *Fusarium oxysporum* (Kumar et al. 2013). Therefore, there is a close relationship between the plant and its endophytic fungi in shaping secondary metabolites production. Based on this recent finding, we hypothesized that the endophytic fungi inside the sandalwood tree are also responsible for the secondary metabolites production by sandalwood tree including the essential oil production

This investigation has proposed the potential utility of endophytic fungi derived from sandalwood in controlling phytopathogens. Various fungal endophytes have demonstrated promise as biocontrol agents against *A. solani*, *F. oxysporum*, and *P. palmivora* when assessed through the dual culture method. These research avenues present compelling and explored prospects for addressing fundamental inquiries essential for developing fungal endophytes in pest and disease management. Furthermore, they contribute to understanding fungal biodiversity, fungal-plant interactions, and intricate multipartite relationships.

The interactions between endophytic fungi and phytopathogens present promising opportunities for developing sustainable disease management strategies. Utilizing the biocontrol potential of endophytes offers a

reasonable approach to diminishing reliance on chemical fungicides and advocating environmentally responsible practices. Furthermore, the exploration and characterization of novel bioactive compounds derived from endophytic fungi obtained from sandalwood hold the potential for the development of innovative antimicrobial agents aimed at protecting crops. Subsequent research in the field employing endophytic fungal isolates demonstrating in vitro inhibition levels exceeding 70% against plant disease is imperative.

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