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DIVERSITY OF ENDOPHYTIC FUNGI IN ASSOCIATION WITH TERMINALIA TOMENTOSA

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ABSTRACT

Endophytic fungus have the potential to yield new natural compounds that could be used in the medicinal, agricultural, and environmental fields. The current work concentrated on isolating and identifying endophytic fungi from Terminalia tomentosa Roxb. in the Chhattisgarh Tiger Reserve known as Achanakmar. The diverse activities of the plants include antifungal and antibacterial properties. A 30m-tall deciduous tree from the Combretaceae family, Terminalia tomentosa is a regular sight in Chhattisgarh's Achanakmar Tiger Reserve. The Baiga tribes of the Reserve utilizes the tree as a traditional medicine. The Terminalia tomentosa plant has simple, uppermost alternate or subopposite, thick coriaceous, ovate, oblong, softly textured immature leaves and more or less hairless older leaves. The current investigation concentrated on isolating and identifying endophytic fungi from Terminalia tomentosa Roxb. leaves. According to our research, host plant type, location and season all have an impact on the endophytic community in Terminalia species. These fungal endophytes may be future producers of a steady supply of bioactive and chemically unique chemicals for application in industry, agriculture, and possibly medicine.

KEYWORDS: *Endophytic, Fungi, Terminalia Tomentosa, Achanakmar Tiger Reserve, Chhattsgarh.*

INTRODUCTION

The term "*endophytic fungi*" (Endo = inside; Phytes = plants) refers to a class of fungi that live inside the living tissues of their host plants without actually generating illness or symptoms that are indicative of disease. In the plants they dwell, endophytic fungus play significant ecological and physiological roles. Despite the fact that several species of temperate plants from various habitats have had their endophyte assemblages examined (Rodrigues and Petrini 1997; Stone *et al*.,, 2000; Suryanarayanan *et al*., 2001), no attention has been paid to the study of fungi that live as endophytes on tropical plants in light of what is known about these fungi. According to Hawksworth (1991), the endophytes would show the existence of various novel and distinctive fungi that were significant for the economy. While examining the endophytes of a single host, Rodrigues and Samuels (1992, 1994) described two new *Idriella* species and a new Loculoascomycete genus. Because this type of fungi represents a potential source of novel bio-chemicals, some of which are important for agriculture and medicine, research on fungal endophytes has been more active recently (Monaghan *et al*., 1995).

Numerous grass endophytes create anti-herbivore substances that keep pest insects away and are spread through the host plant's seeds. Certain endophytes produce in culture antibiotics that are effective against animal pathogens and phytophagous insects (Johnson and Whitney, 1994, Findlay *et al*., 1997). Fisher *et* *al*. (1984a) found that more than 30% of the endophytes they examined exhibited anti-fungal and antibacterial action and they were successful in isolating a broad-spectrum antibiotic from an endophyte of *Vaccinium* sp. (Fisher *et al.*1984b). Certain plant species' seed germination is influenced by the species of *Epicoccum* and *Aureobasidium* which produce phytohormones (Petrini *et al*., 1992). Li *et al*., (1998) described a *Taxus* endophyte that generates the anticancer medication Taxol. Some tropical fungal endophytes have been demonstrated by Lumyong *et al.*, (1998) to manufacture an enzyme with anti-tumor action.

Studies concentrating on endophytic relationships with tropical plants are just being started. Microbiologists have good cause to focus on the diversity of endophytes, especially in Chhattisgarh, where a wide range of adaptations to high temperatures have long been anticipated. The state's high rates of growth, breakdown, and nutrient cycling also imply that endophytes must contend with difficult ecological difficulties such fierce competition for scarce resources. In order to perform preliminary research on endophytes of tree species, *Terminalia tomentosa* (Saja), which is significant from an ethnobotanical standpoint, will be the subject of this study. *Rhabdocline parkeri*, a species of endophyte that defends plant needles from gall midge assault, is one example of an endophyte suppressing a pest. Numerous endophytic fungi serve as a source of cutting-edge biochemicals with potential for use in agriculture or medicine. Because these fungi are incorporated into the host system, the current study will further our understanding of fungal endophytes used in biological control programmes against insect-pests of plants like Sal and their wild relatives, trees.

Achanakmar Tiger Reserve (ATR) in Chhattisgarh is home to the 30 m-tall deciduous tree *Terminalia tomentosa*. The Baiga tribes of the reserve utilize the tree as a traditional medicine. The vegetation and animals of Achanakmar Tiger Reserve (ATR) are highly diverse. One of the world's most endangered "hot-spots of biodiversity" is ATR. This is situated between 383 and 800 metres above sea level in the centre of India. The Reserve, which has a total size of 552 square kilometres, is located in the Mungeli district of Chhattisgarh, India, between latitudes 22° 15' and 22° 58' N and longitudes 81° 25' and 82° 5' E. The Deccan Peninsula Central Highlands biogeographic zone of India corresponds to the terrain of ATR. With regard to some biotic groups and the Baiga tribal people, who have created a pharmacopoeia based on plant products, the upland plateaus serve as a pristine habitat. This is a significant forest site in the Bilaspur division, where the local natural vegetation differs. The northern tropical deciduous forests are represented by the ABR region. This is well renowned for containing a variety of edaphic and climatic variables at varying heights. *Shorea robusta*, *Anogeissus latifolia*, *Buchanania lanzen*, *Cleistanthus collinus*, *Diospyros melanoxylon*, *Eugenia jambolana*, *Lagerstroemia parviflora*, *Terminalia bellerica, T. chebula , T. tomentosa, T. arjuna, Madhuca indica*, and *Butea monosperma* are among the tree species with the greatest vegetational variability.

Essentially a hilly state, Chhattisgarh has ranges that extend from the east to the west and from the north to the south. This state actually has Sal forests, which are classified as either tropical moist Sal forests or tropical dry Sal forests. The great diversity of tree species may be found in the three National Parks and eleven wildlife sanctuaries. However, there hasn't been any research done so far to show the relationships between different tree species and insects, microbes, wild animals and tribal people. There's also no knowledge of the interaction between plant endophytes and insect management.

Endophytic microorganisms

Endophytic microorganisms are microorganisms that live in the plant tissue beneath the epidermal cell layers without causing damage to or infecting the host plant. Endophytic microorganisms that live in the intercellular space of tissues and therefore they can invade living cells. Endophytic microorganisms that live in the intercellular space of tissues and therefore they can cause damage to or infect the host plant.

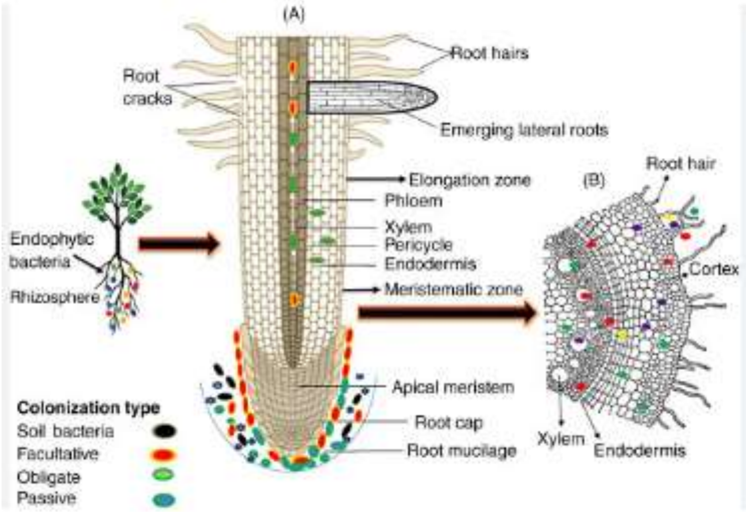

Fig. 1 Entry, colonization, and distribution of endophytic microorganisms in plants

Endophytic fungi

Endophytic fungi make up a disproportionately large portion of the total population of endophytic microorganisms found in plants. They are thought to be the origin of a large number of novel chemicals, including a large number of active substances that have fascinating biological effects. These fungal forms can be seen growing naturally in the deeper tissues of host plants when the plants are healthy and growing normally. They are native to the plant that serves as their host and, as a result of the robust biosynthetic capacity they possess, are able to create a significant quantity of metabolites. This has the potential to result in the discovery of new bioactive compounds and holds the possibility of advancing manufacturing to an industrial level. Additionally, the compounds that are produced by endophytic fungi are regarded to be an agent that helps to balance the microflora on the host plant in order to prevent diseases from growing on the plant.

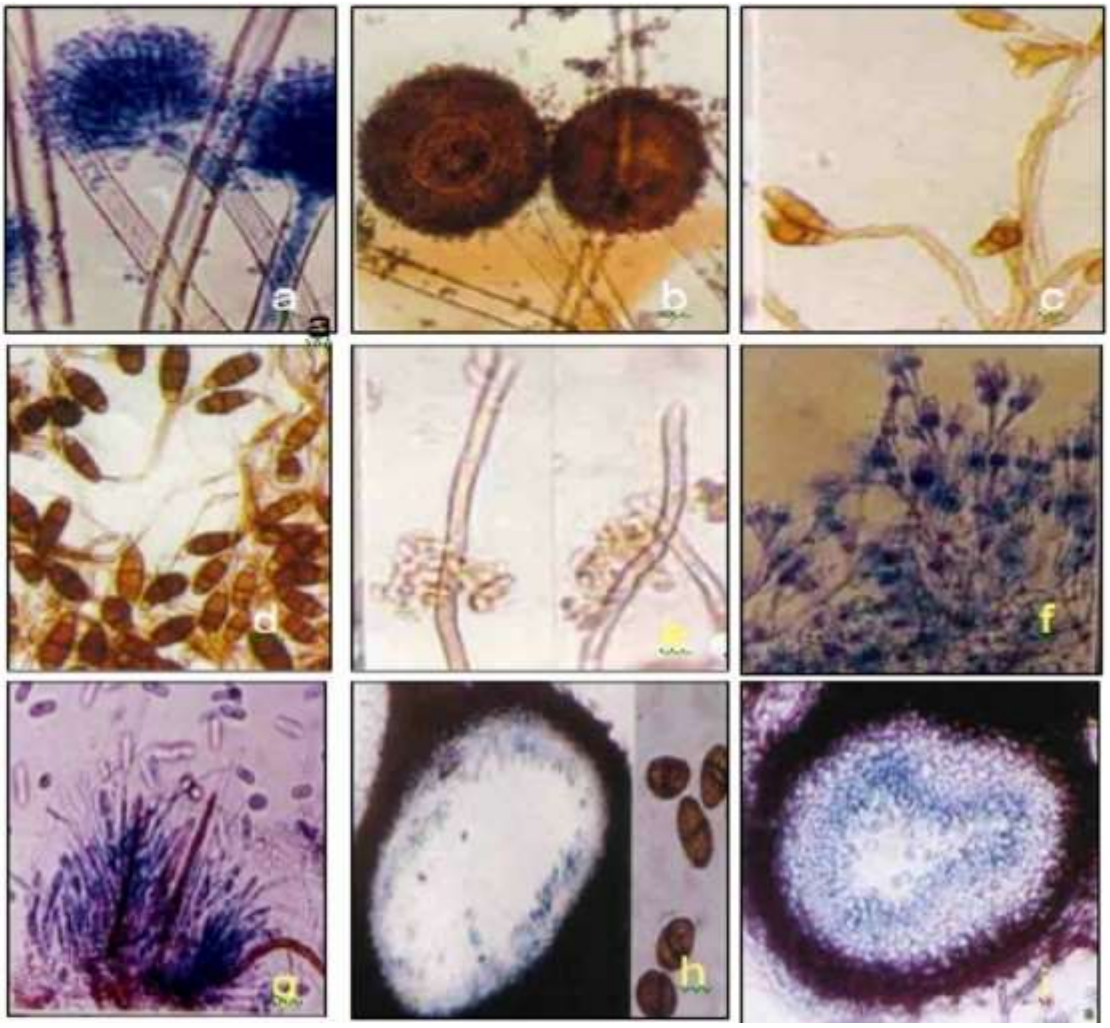

Fig.2 endophytic fungi identified from selected medicinal plants

Diversity of endophytic fungi

According to the findings of a study that was carried out in 1971 by Matsushima on a variety of angiosperms and conifers in North America and Panama, endophytic fungi are quite common. The study also showed that the majority of endophytic fungus are members of the class known as Ascomycetes.

In 2008, Huang *et al*. discovered that endogenous fungus were present in 27 out of 29 medicinal plants that were investigated. Endophytic fungi are found in rather high numbers, with the most common genera being *Fusarium* (27%), *Colletotrichum* (20%), *Phomopsis* (11%), *Alternaria* (9%), *Aspergillus* (5%), and others. (Figure 1).

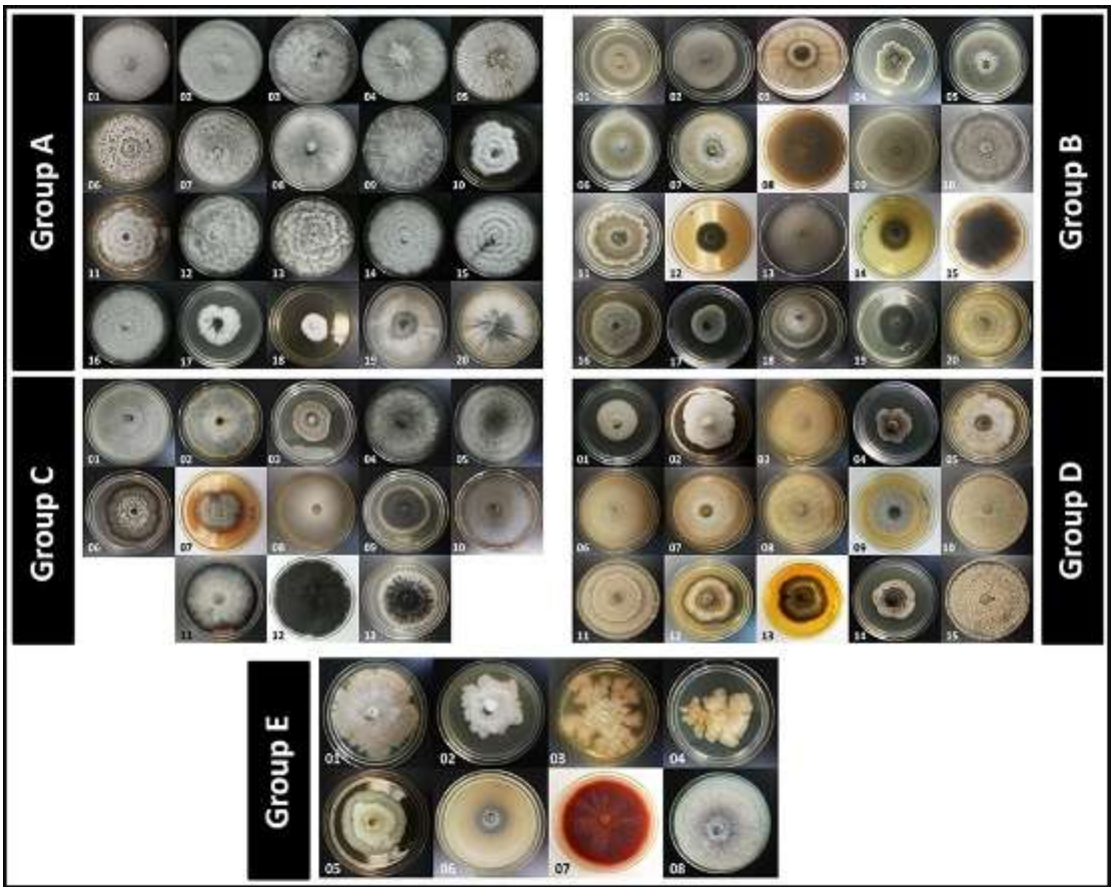

Figure 1. Ascomycete strains that were isolated from the leaves of angiosperms and conifers in Panama and North America (source: Selim *et al*., 2012).

Biodiversity of Endophytic Fungi Isolated from Different *Terminalia* Species

One of the most diverse types of life that can be found on this planet are the fungi. After decades of research on fungal endophytes, it is now clear that they are present in all taxonomic groups of the plant kingdom, vegetation types (ranging from alpine to tropical), and ecological types (ranging from hydrophytes to xerophytes) in a great diversity. This is the case despite the fact that fungal endophytes were previously thought to be an exception. Arnold *et al.* (2000) found that the tropics have an extremely high number of different fungal endophytes. As a result, we should be able to anticipate that *Terminalia* spp., which are found predominantly in tropical regions of the world, could be the host of endophytic fungus communities that contain a wide variety of species.

OBJECTIVE

1. To evaluate the ethno-botanical importance and ecological sustenance of *Terminalia tomentosa* (Saja) tree species in Chhattisgarh.
2. Isolation and identification of endophytic fungi from *Terminalia tomentosa* .

MATERIALS AND METHODS

The root samples of *Terminalia tomentosa* were gathered from two distinct collection sites, namely, Collection Site I- and Collection Site II- of Achanakmar Tiger Reserve, during two distinct time periods. These collection sites and times were referred to as "Collection Site I- " and "Collection Site II" respectively. For periods I and II, meteorological data was collected, and physicochemical study of the soil at both of the collecting locations was investigated.

Isolation of fungal endophytes from the root of *Terminalia tomentosa* was carried out from each of the collection locations for two separate time periods, and a total of 27 unique fungal endophytes were recovered, of which 41% were sporulating and 59% were nonsporulating. On Potato Dextrose Agar (PDA) medium, pure cultures of a variety of distinct sporulating fungal isolates were kept alive and well. On the basis of its colony form and a microscopic investigation of the fungal spore using Lactophenol Cotton Blue [LCB] staining, a preliminary identification of the fungal isolates was carried out. In order to get secondary metabolites from each fungal isolate, the isolates were allowed to grow for a total of 21 days in Potato Dextrose Broth (PDB).

METEOROLOGICAL DATA

Meteorological data such as Mean Maximum and Minimum temperature, Relative Humidity, and Total Rainfall were obtained from the Regional Meteorological station in College Road, Chhattisgarh, during the period of sample collection for both plantation sites. These data were used to compare the two plantation sites.

PHYSICO-CHEMICAL CHARACTERISTICS OF SOIL

Physicochemical factors such as soil type and texture, pH, Electrical Conductivity (EC), Organic Carbon (OC), N, P, K, Ca, Mg, Na, Fe, Humus, and total minerals were analysed at the Research Centre using the procedures that are standard. Soil samples were collected from both locations at both times during the plant sample collection. The analysis's mean values were displayed below for your perusal.

STATISTICAL ANALYSIS

Each experiment was conducted in triplicates. Results are shown as mean ± standard error. Experimental results were analysed following appropriate methods such as Analysis of Variance (ANOVA), (One way ANOVA and Two-way ANOVA),Student's t-test and Duncan's Multiple Range Test (DMRT) using statistical software SPSS 20.0.

RESULTS AND DISCUSSION

Antimicrobial Activity

- **Antifungal activity**

By employing cell free extract (CFE), the researchers were able to acquire the maximum zone of inhibition against all of the test phytopathogens at a dose of 100 g/mL for all of the extracts.

In TTM1, the greatest zone of inhibition achieved against *Fusarium solani* measured 30.27 0.12 mm; however, when CFE was used at a dose of 100 g/mL, no zone of inhibition was identified against *Macrophomina phaseolina*. The highest zone of inhibition against *Fusarium oxysporum* was achieved in

TTM2 (29.070.09mm), however no zone of inhibition was detected against *Macrophomina phaseolina* when CFE was used at a dose of 100 g/mL. In TTM4, the greatest zone of inhibition achieved against *Fusarium solani* was 25.270.09mm; however, when CFE was used at a concentration of 100 g/mL, there was no zone of inhibition identified against *Curvularia oryzae*. In TTM6, the largest zone of inhibition was achieved against *Fusarium oxysporum* (30.270.09mm). On the other hand, the least zone of inhibition was discovered against *Curvularia oryzae* (8.800.15mm) when CFE was present at a concentration of 100 g/mL. Maximum zone of inhibition against *Fusarium solani* was observed in TTM7 (25.430.09mm), however no zone of inhibition was detected against *Fusarium oxysporum* when CME was used at a dose of 100 g/mL. Using CFE at a concentration of 100 g/mL, the highest zone of inhibition was achieved against *Rhizoctonia solani* in TTM8 (28.43 0.20 mm); however, no zone of inhibition was identified against *Alternaria alternata*. In TTB1, the greatest zone of inhibition was achieved against *Fusarium solani* (30.200.06mm). However, when applying CFE at a concentration of 100 g/mL., no zone of inhibition was identified against *Alternaria alternata* and *Curvularia oryzae*. In TTB2, the largest zone of inhibition was achieved against *Fusarium oxysporum* (31.600.06mm). On the other hand, the least zone of inhibition was discovered against *Alternaria alternata* (5.470.24mm) when CFE was used at a dose of 100 g/mL. The highest zone of inhibition against *Rhizopus stolonifer* was achieved in TTB3 (21.400.10mm); however, no zone of inhibition was detected against *Botrytis cinerea*, *Curvularia lunata*, *Fusarium oxysporum*, or *Macrophomina phaseolina* when CFE was used at a dose of 100 g/mL. When CFE was used at a dose of 100 g/mL. in TTB5, the largest zone of inhibition was achieved against *Fusarium oxysporum* (33.97 0.09 mm), while the least zone of inhibition was discovered against *Botrytis cinerea* (6.93 0.12 mm). When applying CFE at a dose of 100 g/mL in TTB6, the largest zone of inhibition was observed against *Rhizopus stolonifer* (21.40 0.10 mm), but no zone of inhibition was identified against *Curvularia oryzae*, *Curvularia lunata*, *Fusarium oxysporum*, or *Macrophomina phaseolina*. A difference that was shown to be statistically extremely significant (p 0.01) was found between the fungal endophytic extracts of varying concentrations.

- **Antibacterial activity**

By employing cell free extract (CFE), the researchers were able to acquire the maximum zone of inhibition against all of the bacterial pathogens tested at a concentration of 100 g/mL. for all of the extracts.

In TTM1, the largest zone of inhibition achieved was against *Escherichia coli* (20.130.09mm). On the other hand, the minimal zone of inhibition was discovered against *Pseudomonas aeruginosa* employing CFE at a dose of 100 g/mL. Using CFE at a concentration of 100 g/mL., TTM2 achieved the highest zone of inhibition against *Escherichia coli* (18.170.12mm), while no zone of inhibition was identified against *Bacillus subtilis*. When employing CFE at a dose of 100 g/mL. in TTM4, the largest zone of inhibition was achieved against *Bacillus subtilis* (18.100.10mm), while the least zone of inhibition was discovered against *Escherichia coli* (6.000.00mm). In TTM6, the largest zone of inhibition against *Escherichia coli* was achieved (23.050.05mm), but the smallest zone of inhibition was discovered against *Pseudomonas aeruginosa* (8.100.10mm) when CFE was used at a dose of 100 g/mL. Using CME at a dose of 100 g/mL, TTM7 achieved the highest zone of inhibition against *Pseudomonas aeruginosa* (8.030.03mm), while no zone of inhibition was identified against *Proteus vulgaris*. When CFE was used at a dose of 100 g/mL in TTM8, the largest zone of inhibition was achieved against *Escherichia coli* (19.200.10mm), while the least zone of inhibition was discovered against *Pseudomonas aeruginosa* (7.000.00mm). Utilizing CFE at a concentration of 100 g/mL, the highest zone of inhibition was obtained against *Staphylococcus aureus* in TTB1 (22.000.00mm), however no zone of inhibition was identified against *Proteus vulgaris*. This was done using CFE. When employing CFE at a dose of 100 g/mL in TTB2, the largest zone of inhibition was achieved against *Bacillus subtilis* (14.000.00mm), while the least zone of inhibition was discovered against *Escherichia coli* (2.000.00mm). In TTB3, the largest zone of inhibition was achieved against *Bacillus subtilis* (17.040.03mm). On the other hand, the least zone of inhibition was discovered against *Proteus vulgaris* (4.000.00mm) when CFE was used at a dose of 100 g/mL. When CFE was used at a dose of 100 g/mL in TTB5, the largest zone of inhibition was achieved against *Pseudomonas aeruginosa* (20.000.00mm). On the other hand, the least zone of inhibition was discovered against *Bacillus subtilis* (10.050.04mm). Using CFE at a concentration of 100 g/mL, the highest zone of inhibition was observed against *Pseudomonas aeruginosa* in TTB6 (15.03 0.09 mm), however no zone of inhibition was identified against *Bacillus* subtilis or Proteus vulgaris. A variation in concentration that was statistically significant (p 0.01) was found to exist between the fungal endophytic extracts of varied concentrations.

EFFECT OF THE FUNGAL ENDOPHYTES ON GERMINATION ANDGROWTH USING VIGNA RADIATA

Five of the fungal isolates, namely TTM1, TTM6, TTM7, TTB2 and TTB5, had a beneficial influence on the germination and growth of *Vigna radiata*. These isolates are listed in alphabetical order. When compared to the seeds that had been treated with fungal suspension, it was discovered that those that had been given treatment with cell-free broth produced significantly better results. In the germination test, the cell free broth treatment exhibited a germination rate of one hundred percent. On the other hand, the germination rate of seeds treated with fungal suspension was suppressed following treatment for twenty-four hours. After twenty-four hours, the control had a germination rate of 87 percent. After 72 hours, the development of lateral root and leaf was noticed on the seeds that had been treated with cell free broth culture. On the other hand, no such growth was identified in the seeds that had been treated with sterilised water. When compared to the control group, the effects of TTM1, TTM6, TTM7, TTB2 and TTB5 on the development of *Vigna radiata* were shown to be statistically significant (p 0.01). However, the highest levels of growth were seen in *Vigna radiata* seeds after they had been treated with the fungal broth of TTB2 (3.500.06 cm), followed by TTB5 (3.270.07 cm) (Table 1).

Table 1: Effect of Fungal cell free extract and fungal suspension on *Vigna radiata*

FungalIsolates	Growth of *Vigna radiata* (cm)		t- Value	P Value
	cell free exract	Fungal suspension		
Control	1.30 ± 0.06^{b}	1.30 ± 0.06^{f}	0.000	Not significant
TTM1	2.33 ± 0.12^{c}	0.10 ± 0.00^{a}	18.582	<0.001**
TTM6	0.83 ± 0.03^{a}	0.92 ± 0.02^{c}	2.236	Not significant
TTM7	2.70 ± 0.06^{d}	0.34 ± 0.02^{b}	38.622	<0.001**
TTB2	3.50 ± 0.06^{f}	1.07 ± 0.07^{d}	27.591	<0.001**
TTB5	3.27 ± 0.07^{e}	1.12 ± 0.01^{e}	46.842	<0.001**

F Value	227.348	189.832
P Value	<0.001**	<0.001**

Note :

1. . The value refers to Mean ± SE
2. ** denotes significant at 1% level.
3. Different alphabet among treatments denotes significant at 5% level using Duncan Multiple Range Test (DMRT)

CONCLUSION

We arrived to the conclusion that TTB2 (*Aspergillus niger*) and TTB5 (*Aspergillus tamaraii*) were the most potentially active isolates after conducting an analysis of the bioactive potential of all of the identified fungal endophytes. These fungi have the potential to be utilized efficiently in order to replace the chemical pesticides, fertilizers, and artificial growth regulators (growth hormones) that have a negative impact on environmentally sustainable agriculture. In order to get the highest possible level of efficacy from these powerful isolates, the growing conditions necessary for the development of bioactive chemicals and antifungal activity were fine-tuned. The optimized growing conditions guarantee the highest possible production of both bioactive chemicals and fungal biomass; this will, without a doubt, be of assistance in the process of commercializing the powerful fungal isolates, which is regarded as an essential need.

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