STUDY ON THE ENDOPHYTIC FUNGI FROM BANANA SPECIES OF NAGALAND AND THEIR EFFECT AGAINSTTHE FUSARIUM WILT PATHOGEN

Thesis

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of

Doctorof Philosophy

in

Plant Pathology

by

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I, Bendangsenla, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis had not been submitted by me for any research degree in any other university/institute.

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This is to certify that the thesis entitled **"Study on the endophytic fungi from banana species of Nagaland and their effect against the Fusarium wilt pathogen"**submitted by Miss. Bendangsenla, Admission No. Ph-225/18, Registration No. Ph.D./PPL/00245 to the NAGALAND UNIVERSITY in partial fulfilment of the requirements for the award of degree of Doctor of Philosophy in Plant Pathology has been examined by the Advisory Board and External examiner on …….....

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Dedicated to my

niece and nephews

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ACRONYMS AND ABBREVIATIONS

^{o}C	-	Degree Celsius
%	-	Percentage
@	-	At the rate of
µ	-	Micron
&	-	and
bp	-	Base pair
CD (p=0.05)	-	critical difference at 5 per cent probability
cm	-	centimetre
CRD	-	Complete Randomized Design
CV	-	Coefficient of variation
DNA	-	Deoxyribose Nucleic Acid
et al.	-	and others
etc.	-	etcetera
Fig.	-	Figure
f. sp.	-	Forma specialis
g	-	Gram
h	-	hours
i.e.	-	that is
in vitro	-	in laboratory
L	-	Litre
ml	-	millilitre
mg	-	milligram
mM	-	millimolar
min	-	minutes
max	-	maximum
MSL	-	Mean Sea Level

No.	-	number
P	-	Phosphorous
PCR	-	Polymerase Chain reaction
PDI	-	Per cent disease index
pH	-	Potential of hydrogen
PDA	-	Potato Dextrose Agar
psi	-	Pounds per square inch
rpm	-	Revolution per minute
R.H.	-	Relative humidity
SEm	-	Standard error of mean
sp., spp.	-	species (singular and plural)
viz.	-	namely

ABSTRACT

The present study was conducted to explore the diversity of fungal endophytes in the banana species of four districts of Nagaland *viz*., Chumoukedima (earlier under Dimapur district), Kohima, Peren and Mokokchung district, their growth promotional and antagonistic activities against the Fusarium wilt pathogen. A total of 281 fungal isolates were isolated from the wild and cultivated banana species of Nagaland, with 166 isolates from leaf sample and 115 isolates from root samples. Morphological characterization was carried for all the fungal endophytes and 135 isolates belonging to 15 genera were identified which were, *Penicillium* sp. (29), *Trichoderma* sp. (11), *Fusarium* sp. (16), *Colletotrichum* sp. (16), *Diaporthe* sp. (30), *Apiospora* sp. (6), *Aspergillus* sp. (6), *Pestalotiopsis* sp. (6), *Mucor* sp. (3), *Phomopsis* sp. (2), *Botrytis* sp. (1), *Helminthosporium* sp. (1), *Alternaria* sp. (6), *Beauveria felina* (1) and *Cladosporium tenuissimum* (1). In growth promotional test, all the 281 isolates produced IAA with a concentration range of 9.38 to 114.12 µg/ml with FEB75 (*Apiosporalongistroma*), FEB83 (*C.horii*), FEB178 (*C.tenuissimum*), FEB192, FEB194 and FEB222 (*Penicillium* sp.) producing maximum concentration of 114.12 µg/ml. In Gibberellic Acid production test, all the isolates produced GA3 with a concentration range of 7.95 to 113.36 µg/ml with FEB186 (*M.circinelloides*) (113.36 µg/ml), FEB251 (*M.circinelloides*) (109.64 µg/ml) and FEB269 (*C.gloeosporioides*) (99.94 µg/ml) as the best three performing isolates. For phosphate solubilization test, out of 281 isolates, only 44 isolates were found to be positive. For amylase test, 73 isolates showed positive reaction. Chitinase activity test of the isolated endophytes revealed that all the 281 isolates showed negative reaction. For siderophore production test, 92 isolates were found to be positive. The pathogen causing Fusarium wilt in

banana was isolated from the pseudostem of symptomatic banana plants and microconidia, macroconidia and chlamydospores were observed. Pathogenicity test using detached leaf assay was also carried out to confirm the pathogen and it was found that the tested pathogen treated leaves showed typical yellowing symptoms on the leaves after 10 days of incubation, however, no symptoms were observed in negative control. Molecular identification of the isolated pathogen using ITS primers identified the pathogen as *Fusarium oxysporum* with 99.64% similarity. Antagonistic activity of the isolated fungal endophytes against the pathogen revealed that for dual culture, FEB116 (*T.asperellum*) at 61.90% was the best performing isolate. For volatile metabolite test, FEB81 (*C.kahawae*) at 54.81% was the best performing isolate. For non-volatile metabolite production test, FEB3 (*F.haematococcum*) at 69.21% was the best performing isolates. Molecular characterization of the best three performing fungal endophyte isolates from all the experiments was carried out using the ITS primers and 24 isolates were identified through BLAST sequence in the NCBI database and they are *A.longistroma* (FEB75), *C.horii* (FEB83), *C.tenuissimum* (FEB178), *C. gloeosporioides* (FEB269), *M.circinelloides* (FEB186), *M. circinelloides* (FEB251), *T.asperellum* (FEB46), *D.phaseolorum* (FEB27), *Phomopsis* sp. (FEB129), *B.felina* (FEB143),*A.clavatonanicus*(FEB51), *P.citrinum* (FEB187),*Phomopsis* sp. (FEB254), *C.fructicola* (FEB65), *C.gloeosporioides* (FEB68), *A. versicolor* (FEB23), *D.chromolaenae*(249), *T.asperellum* (FEB116), *A.hydei* (FEB80), *C.kahawae*(FEB81), *D.fructicola*(FEB115), *F.haematococcum*(FEB3), *T. hamatum* (FEB5) and *F.solani* (FEB9).

Keywords: Fungal Endophytes, Fusarium Wilt, Banana, Plant Growth Promotion, Antagonism.

CHAPTER I

INTRODUCTION

INTRODUCTION

Bananas belong to the genus *Musa* (Musaceae, Zingiberales), which are monocotyledonous plants. These are enormous herbs that may grow up to 3 meters tall and lack the lignifications and secondary stem thickening that trees are known for. (Tomlinson, 1969). South-East Asia is the centre of origin of this group, where they can be found from Polynesia to India (Simmonds, 1962). Malaysia or Indonesia have been designated as the centre of diversity (Daniells *et al*., 2001), although significant diversity is recognized throughout the range. Banana (*Musa* spp.) continues to control fruit market in the world, with cultivation taking place in more than 135 nations (FAO, 2021). Its production and cultivated areas have grown throughout the years (FAO, 2021). Estimated banana exports in 2020 were 22.2 million tons, increased by 1.7% from 2019 (FAO, 2021). Among fruit crops in India, bananas rank first and are the most produced and occupy the third most area. With an average productivity of 34.2 tonnes, India produces over 26.5 million tonnes of bananas annually from 0.76 million hectares of area. Out of all the major fruit crops, bananas account for 32.6% of output, mango and citrus follow with 22.1 and 12.4% of production, respectively (Anon, 2013). The broad major part of bananas that are cultivated are the result of inter- and intraspecific crosses between *Musa acuminata* and *Musa balbisiana*, two diploid (2n = 2x = 22) wild species (Simmonds and Shepherd, 1955). These chromosomal sets are classified as having either the BB (*M. balbisiana*) or AA (*M. acuminata*) genomic constitution. They typically grow in woodlands and are seedy, non-pulpy, and inedible. The species that is most widely distributed is *M. acuminata* (Daniells *et al*., 2001).

The majority of wild *Musa* species can be found in low rainfall zones of deciduous woods, wet evergreen forests and some tropical rain forests. In addition to areas of southeast India, the Andaman and Nicobar Islands, the

northeastern Indian Himalayas, which include parts of Nagaland, Assam, Arunachal Pradesh, and Meghalaya, are home to tropical rain forests. The wild *Musa* species can be found in the Khasi, Jaintia, Naga, Patkai, and Garo hills in northeastern India, at both lower and higher elevations. In the group of five key sections, at least 11 species and the majority of edible bananas are found in Eumusa. In this section, *Musa acuminata* and *Musabalbisiana* are the ancestors of the majority of edible bananas. There are 7 species of Eumusa that may exist in India out of the 11 or 12 species that possibly exist: *Musaacuminata*, *Musa balbisiana*, *Musa itinerans*, *Musa nagensium*, *Musa aurantiaca*, and perhaps *Musa sikkimensis* and *Musa cheesmani*. Simmonds (1962) reported the last two species and the eighth species, *Musa flaviflora*, which was all reported from Assam. In India's northeast region, *Musa acuminata* has been found in the native habitats the Kaziranga forest range in Assam, and the state Meghalaya Khasi hill ranges (Subbaraya*et al*., 2006).

A few wild species found in Nagaland include *M. flaviflora* (Simmonds, 1956), collected from Zunheboto District, Nagaland. This species is extremely rare and was only collected in one high-altitude location of Nagaland (Joe *et al*., 2013). One of the most elegant species in the genus, *Musa aurantiaca*G.Mann ex Baker, is a wild species that is found in Nagaland. It has bright orange buds and a lot of possibilities for ornamentation (Joe and Sabu, 2016). Nagaland is home to another wild banana species, *Musa markkui* Gogoi & Borah. The distribution is confined to the Minkong forests in the Mokokchung district (Joe and Sabu, 2016). *Musa velutina*H.Wendl. & Drude, is found in Myanmar and India. In India, it is commonly found in Arunachal Pradesh, Assam, Meghalaya, and Nagaland (Joe and Sabu, 2016). *Musa velutina* H. Wendl & Drude subsp.*markkuana*, M. Sabu, A. Joe, and Sreejith, *M. velutina* subsp. *markkuana* are native to North-East India. Northeastern India's Arunachal Pradesh and Nagaland are home to this taxon. It usually occurs in Mokokchung'sMinkong Forests in Nagaland (Joe and Sabu, 2016).

Dey *et al*. (2014) reported on a new *Musa* sect. known as *Musa nagalandiana* S. Dey & Gogoi, discovered from the Zunheboto district of Nagaland, India. The species was named after the Indian state of Nagaland, where it was found.

Fusarium wilt, generally known as Panama wilt, is one of the major destructive diseases of banana out of all the pests and diseases that affect bananas (Moore *et al*., 2001). *Fusarium oxysporum*Schlect f. sp. *cubense* (E. F. Smith) Snyd. & Hans. (Foc) is a pathogenic fungus that enters the plant through the roots and obstructs the vascular system, causing the wilt of plant and eventually the whole plant dies (Moore *et al*., 2001). Australia reported the first occurrence of Fusarium wilt in plantains and bananas (Bancroft, 1876 and Ploetz *et al*., 2003). The fungal pathogen is often divided into four races: races 1, 2, and 4 are harmful to bananas of which, race 1 infects almost all the banana cultivars, with the exception of the "Cavendish group" (Ploetz, 1990; Ploetz *et al*., 2003; Pérez-Vicente and Dita, 2014). The disease is widespread throughout banana-growing regions of India, with race 1 being the most common and it is followed by race 2 (Sivamani and Gnanamanickam, 1988; Prasadji and Smith. 2007), while there have been reports of race 4 in some pockets (Thangavelu *et al*., 2011). Many of the current methods for managing the diseases, like crop rotation with rice and injecting 2% carbendazim into rhizomes, are laborious. It is still not possible to control the disease in a way that is economical. Biological control is an additional strategy for treating Fusarium wilt in a harmonious way, and the hunt for antagonistic microorganisms has produced a number of very active antagonistic fungi and bacteria (Saravanan *et al*., 2004 and Getha *et al*., 2005). Plants are thought of as intricate micro-ecosystems where a wide range of microorganisms, including endophytes, can be exploited with distinct niches (Azevedo *et al*., 2000). Endophytes are found in plant tissues; they do not harm plants or develop exterior shapes that appears from the tissues of plant (Azevedo and Araujo, 2007). Endophytes are typically bacteria and fungi that are crucial to a

plant's ability to adapt to its environment (Mendes and Azevedo, 2007). De Bary (1866) was the first to report non-pathogenic fungus that reside inside plants, referring to them as endophytes. About 40 years ago, the endophytic microbiota began to be recognized as advantageous to their plant hosts, shielding plants from diseases brought on by pathogenic bacteria, fungi, and nematodes as well as insect pests (Souza *et al*., 2014; Thangalevu& Gopi, 2015a; Su *et al*., 2017; Kavino& Manoranjithan, 2018). Additional advantageous characteristics were introduced, such as the ability of endophytes to produce growth hormones, fixation of nitrogen from the atmosphere, and cause solubilization of phosphate (Ting *et al*., 2008; Muthuri *et al*., 2012; Souza *et al*., 2013; Andrade *et al*., 2014; Benzon *et al*., 2014; Karthik *et al*., 2017; Souza *et al*., 2017). Endophytes have been isolated from a broad range of plants, and from many plant sections, including roots, stems, nodes, leaves, and fruits, including several that are of agricultural relevance, like tomatoes (Pillay and Nowak, 1997), rice (Stolzfus*et al*., 1997), cotton (Quadt-Hallmann *et al*., 1997), maize (Araújo *et al*., 2000), wheat and sorghum (Zinniel *et al*., 2002) and banana plants (Weber *et al*., 2007).

It has been demonstrated and proved that endophytes are abundant producers of novel natural compounds with a wide range of biological activity and considerable structural diversity (Pimentel *et al*., 2011). In present day, the utilization of endophyte biocontrol agents has been demonstrated to be an eco-friendly approach to managing disease (Xue *et al.,* 2015, Deltour*et al*., 2017, Fu *et al.,* 2017). The vascular system that serves as a home to endophytes, and which shares the same habitat as Foc could likely be candidates for development of biocontrol techniques. Hence, the plan of this investigation was to isolate, select and to know the diversity of endophytic fungi, to understand their growth promoting ability in plants and their antagonistic activity from *Musa* sp. cultivars that are cultivated in the state of Nagaland with the following objectives.

1. To study the diversity of fungal endophytes in wild banana plant
2. To assess plant growth promotion activities of the endophytes
3. To assess biocontrol activities of the endophytes against the Fusarium wilt pathogen

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Literature relevant to the different aspects of the proposed investigation entitled **"Study on the endophytic fungi from banana species of Nagaland and their effect against the Fusarium wilt pathogen"** has been reviewed in this chapter under the following heads.

2.1 Endophytes

Endophytes are fungi that colonize healthy plant tissues and either persist in a dormant phase or comprise more extensive, but symptomless infections (Petrini, 1991).

The first person to describe non pathogenic fungi living inside plants was De Bary (1866) who named them as endophytes. Endophytic fungi are usually defined as fungal isolates growing within the tissues of their host plants without showing any disease symptom (Schulz *et al*., 1999). Fungal endophyte is also referred to as fungi within apparently healthy, functional root tissues at the 'moment' of sample collection (Sieber, 2002).

Endophytes are mutualistic symbionts that live asymptomatically for a whole or part of their life cycle in plant tissues, receiving nutrition and structural refuge from the host, while benefiting the host with enhanced growth and health (Faeth and Fogain, 2002; Sikora *et al.,* 2003; Paparu*et al.,* 2004; Ju *et al*., 2006).

2.2 Collection, isolation, identification of fungal endophytes from healthy banana species

2.2.1 Endophytic fungi from banana

Photita*et al*. (2000) isolated endophytic fungi from 7500 samples of wild *Musa acuminata* collected from five sites at Doi Suthep Pui National Park, Thailand during December 1998 to July 1999. Sixty-one different fungal taxa were isolated.

Cao *et al*. (2002) isolated 163 endophytic fungal cultures from 200 leaf samples of *Musa acuminata* and 68 endophytic fungal cultures were isolated from 100 root samples.

Zakaria and Rahman (2011) isolated endophytic *Fusarium* species from roots of wild banana (*Musa acuminata*) collected randomly in several locations in Penang Island, Malaysia. A total of 54 isolates of *Fusarium* were recovered from 100 root fragments.

Garoe*et al*. (2013) isolated 15 fungal endophytes from banana corm that belonged to four different genera taxa (*Aspergillus*, *Penicillium*, *Fusarium* and *Chaetomium*).

Dita *et al*. (2014) isolated root-associated endophytic microorganisms (360 bacteria and 143 fungi) from 20 *Musa* sp. genotypes of the ex-situ collection in Corbana, Guapiles, Costa Rica.

Zakaria *et al*. (2016) isolated endophytic fungi from roots of wild banana (*Musa acuminata*). A total of 31 isolates of endophytic fungi were isolated from 80 root fragments.

Baruah *et al*. (2018) isolated a total of 30 native fungalrhizospheric microbes and seven fungal endophytes from rhizospheric soil samples and from roots of healthy banana plants, collected from different banana growing areas of Assam.

Souza Junior *et al*. (2018) isolated the cultivable endophytic bacterial and fungal community associated with leaves of an organic banana plantation in the Brazilian Amazon state of Roraima. A total of 24 fungi and 27 bacteria isolates were selected. The taxonomical classification showed that the cultivable endophytic fungi community is affiliated to the following 11 genera: *Aspergillus, Peniophora, Meyerozyma, Saccharicola*,*Nigrospora, Byssochlamys*, *Periconia*, *Myrothecium*, *Acrocalymma*and *Peroneutypa*. Regarding the bacterial isolates 13 genera were found: *Serratia*, *Pantoea*, *Streptococcus*, *Neisseria*, *Bacillus*, *Arsenicicoccus*, *Sphingobacterium*,

Herbaspirillum, Lactococcus, Variovorax, Pseudorhodoferax, Stenotrophomonas and *Brevibacterium*.

Henao *et al*. (2019) studied endophyte populations present in Manzano - apple bananas- affected by *Fusarium oxysporum*f. sp. *cubense*race 1. Endophytes were isolated in two commercial farms in Urabá-Colombia, taking leaf, pseudostem, corm and root tissues from healthy and diseased plants. One hundred forty three isolates with 11 genera were obtained from healthy plants.

Savani *et al*. (2021) analysed fungal and bacterial endophytes that were isolated from leaf, pseudostem and root of banana and a total of 330 endophytes were isolated out of which 220 were bacterial and 110 were fungal endophytes and tested against the Panama wilt disease of banana.

Mohanty and Gupta (2021) isolated fungal endophytes from the leaves, petioles, and roots of different banana var. grown in Odisha. They isolated 36 fungi from different cultivated varieties and some of which are *Alternaria alternata, Aspergillus niger, Cladosporium cladosporioides, Colletotrichum gloeosporioides, Penicillium citrinum* and *Fusarium* sp.

Panda *et al*. (2023) isolated 139 fungal endophytes from 143 samples which were 62 roots, 18 fruit and 54 leaf samples of 15 different varieties of banana from 10 locations in Assam, India during 2018-19.

2.2.2 Morphological Identification of endophytes

Photita*et al*. (2001) identified endophytic fungi from 7500 samples of wild *Musa acuminata* collected from five sites at Doi Suthep Pui National Park, Thailand during December 1998 to July 1999. Sixty-one different fungal taxa were isolated. Fewer isolates were recovered from younger than older samples. Xylariaceous taxa and *Guignardiacocoicola*were the most frequently isolated endophytes from leaves and were either absent or rare in midrib, petiole and pseudostem. *Colletotrichum gloeosporioides*, *C. musae*, *Guignardiacocoicola*, various sterile mycelia and xylariaceous spp. were common at all sites. The endophyte fungal communities at the five sites were

found to differ. *Deightoniellatorulosa*was the most frequent isolate at the Ban Suthep site and was either absent or rare at other sites. *Colletotrichum* species were most common in the midribs and petioles at all sites, while *Pyriculariopsis parasitica* and *Dactylaria*sp. were most common in the pseudostems.

Cao *et al*. (2002) identified one hundred and sixty-three endophytic fungal cultures from 200 leaf samples of *Musa acuminata* plants. They belonged to the genera of *Gloeosporiummusae*(45%),*Myxosporium* sp. (11%), *Deightoniellatorulosa* (8.5%), *Alternaria tenuis* (7.9%), *Sphaceloma* sp. (7.4%), *Aureobasidium* sp. (4.3%), *Melida* sp. (1.8%), *Uncinula* sp. (1.8%), *Penicillium* sp. (1.8%), *Aspergillus* sp. (1.2%), *Sarcinella* sp. (1.2%), *Cladosporium* sp. (0.6%), *Cephalosporium* sp. (0.6%) and sterile mycelium (6.7%). Sixty-eight endophytic fungal cultures were also identified from 100 root samples. They belonged to the genera of *Aspergillus* sp. (31%), *Paecilomyces* sp. (16%), *Penicillium* sp. (15%), *Fusarium* sp. (10%), *Gloeosporiummusae* (6%), yeast (3%), *Deightoniellatorulosa* (3%), *Spicaria* sp. (1.4%), *Cephalosporrium* sp. (1.4%), *Meliola* sp. (1.4%) and sterile mycelium (10%).

Xia *et al*. (2011) investigated the dispersal of diverse species of endophytic and epiphytic *Trichoderma* corresponding with the banana roots. One hundred eighty nine endophytic and epiphytic *Trichoderma* were isolated. Largest group comprised of *T. asperellum, T. virens* and *Hypocrealixii*, isolated from both the outside and inside of banana roots, followed by *T. atroviride* and *T. koningiopsis*, found only on the surface, lastly, *T. brevicompactum* which was isolated from the inside of the roots.

Zakaria and Rahman (2011) identified endophytic *Fusarium* species from roots of wild banana (*Musa acuminata*) collected randomly in several locations in Penang Island, Malaysia. A total of 54 isolates of *Fusarium* were recovered from 100 root fragments. Based on morphological features of

macroconidia, microconidia and conidiogenous cells, three *Fusarium* species were identified in which the most common species was *F. oxysporum*(41.5%) followed by *F. solani*(32.1%) and *F. semitectum*(24.5%).

Henao *et al*. (2019) studied endophyte populations present in Manzano - apple bananas- affected by *Fusarium oxysporum*f. sp. *cubense*race 1 from leaf, pseudostem, corm and root tissues from healthy and diseased plants. One hundred forty three isolates with 11 genera were obtained from healthy plants with the following frequencies: *Fusarium* sp. (18.67%), *Nigrospora*sp. (8%), mycelia sterilia (48%), among others. Also, eight genera were found in diseased plants, *Fusarium* sp. (23.53%), *Colletotrichum* sp. (17.76%), mycelia sterilia (47.06%). All endophytic fungi were found to be ascomycetes, except for *Pythium* sp., oomycete that was isolated only from diseased plants. *Pythium* sp. which, was isolated from healthy plants, constitutes the first reports in musaceas.

Mohanty and Gupta (2021) isolated fungal endophytes from the leaves, petioles, and roots of different banana var. grown in Odisha. They isolated 36 fungi from different cultivated varieties and some of which are *Alternaria alternata, Aspergillus niger, Cladosporium cladosporioides, Colletotrichum gloeosporioides, Penicillium citrinum* and *Fusarium* sp.

Panda *et al*. (2023) isolated and identified forty different fungal endophyte belonging to 14 genera including *Absidia, Arthrinium, Aspergillus, Bipolaris, Cladosporium, Curvularia, Dendrophion, Fusarium, Humicola, Mortierella, Mucor, Penicillium, Paecilomyces, Verticillium* and one mycelium sterile.

2.2.3 Molecular characterization and identification of the fungal endophytes

Dita *et al*. (2014) identified root-associated endophytic microorganisms (360 bacteria and 143 fungi) from 20 *Musa* sp. genotypes of the *ex situ* collection in Corbana, Guapiles, Costa Rica. Analyses of specific genome

regions (16S rDNA for bacteria and tefa-1_ or ITS for fungi) revealed 21 different bacterial genera, with *Klebsiella*, *Enterobacter*, *Bacillus*, *Acinetobacter* and *Burkholderia*as the most frequent. *Trichoderma* spp. and *Fusarium oxysporum* prevailed among the 12 genera of fungi identified.

Zakaria *et al*. (2016) identified endophytic fungi from roots of wild banana (*Musa acuminata*). A total of 31 isolates of endophytic fungi were initially sorted based on morphological characteristics and identified using sequences of TEF-1α gene for *Fusarium* spp. and ITS regions for other fungi. The most common fungal species isolated was species from the genus *Fusarium* in which the isolates were identified as *F. proliferatum*, *Fusarium* sp., *F. solani*species complex and *F. oxysporum*. Other endophytic fungi isolated were *Curvularialunata, Trichoderma atroviride, Calonectriagracilis, Rhizoctonia solani, Bionectriaochroluca*and *Stromatoneurospora phoenix* (Xylariceae). Several of the fungal genera such as *Fusarium, Trichoderma, Rhizoctonia* and Xylariceae are among common fungal endophyte reported in plants. Their present study showed that roots of wild banana harbour diverse group of endophytic fungi.

Zakaria and Aziz (2018) isolated endophytic fungi from banana leaves which were identified using ITS (Internal Transcribed Spacer region) sequences of which 10 genera comprising 17 species were molecularly identified. Endophytic fungal species identified were *Nigrosporaoryzae*, *Nigrosporasphaerica*, *Colletotrichum gloeosporioides*, *Colletotrichum siamense*, *Fusarium equiseti*, *Fusarium chlamydosporum*, *Phomasorghina*, *Pestalotiopsis oxyanthi,Pestalotiopsis theae, Pestalotiopsis eugeniae*, *Penicillium steckii, Penicillium purpurogenum,Bipolarispapendorfii, Bipolaris*sp., *Lasidiodiplodiatheobromae, Cochliobolus intermedius* and *Aspergillus niger.*

Tanapichatsakul*et al*. (2019) isolated and identified 11 fungal endophytes from *Cinnamomum loureiroi* leaves using ITS4 and ITS5 primers.

The identified isolates belonged to 6 genera which are *Botryosphaeria, Colletotrichum, Diaporthe, Fusarium, Neopestalotiopsis* and *Pestalotiopsis*.

Malubag*et al*. (2021) isolated and identified nine fungal endophytes were identified through cultural, morphological and DNA sequencing of the ITS1 and ITS4 regions. These were *Cladosporium cladosporioides* with 99.14% identity, *Fusarium chlamydosporium* with 99.80% identity, *Fusarium keratoplasticum* with 98.46% identity, *Fusarium solani* strain f2-f6 with 98.87% identity, *Fusarium solani* strain ZB11263612 with 98.07% identity, *Fusarium solani* strain F10-3 with 99.24% identity, *Geotrichumcandidum* with 96.32% identity, *Nigrospora oryzae* with 100.00% identity and *Schizophyllum commune* with 99.66% identity.

Ramanujam *et al*. (2021) reported on the molecular identification of an entomopathogen that was isolated as one of the potential fungal endophytes, which was found to naturally infect the fall army worm in the Karnataka state of India. It was done using ITS1 and ITS4 primers and identified as *Beauveria felina*.

Ferdous *et al*. (2022) isolated and molecularly identified fungal endophytes from *Zingiber officinale*Rosc. using ITS4 and ITS5 primers and they were *Fusarium proliferatum, Fusarium solani* and *Cladosporium cladospoiroides*.

Liao *et al*. (2023) collected and studied endophytic *Apiospora* species from *Wurfbainiavillosa* and grasses in Guangdong and Yunnan provinces in China. Molecular identification was done using ITS1 and ITS4 primers, the large subunit nuclear rDNA, the partial elongation factor 1-α and d β-tubulin were also done to give a clarified phylogenetic affinity of the genus *Apiospora* and its various species. One hundred ninety one strains of *Apiospora* species were identified and some of which were *A. endophytica, A. guangdongensis, A. wurfbainiae, A. yunnanensis, A. guizhouensis, A. hysterina, A. longistroma, A. sorghi* etc.

2.3 Plant growth promotion activities of fungal endophytes

Hassan (2002) and Waqas *et al*. (2012) reported that most of the fungal endophytes including *Aspergillus flavus, A. niger, Fusarium oxysporum, Penicillium corylophilum, P. cyclopium, P. funiculosum and Rhizopus stoloniferi*solated from diverse kinds of plants have ability to produce different kinds of plant growth promoting hormones like indole acetic acid (IAA) and gibberellic acid (GA).

Wakelin *et al*. (2004) and Souchie*et al*. (2006) reported that *Aspergillus* and *Penicillium* are two important endophytic fungal genera having very efficient phosphate solubilizing activity.

Genus *Streptomyces* is reported to promote plant growth by producing indole-3-acetic acid (IAA) to help root growth (Merckx *et al*., 1987), a number of antibiotics that are secondary metabolites (Doumbou*et al*., 2001) and siderophores to improve nutrient uptake (Khamna*et al*., 2009).

Hamayun*et al*. (2010) reported on the GA3 production and growth promotion by fungal endophytes isolated from roots of soil grown cucumber where all the 19 isolates were found to have growth promoting ability and *Cladosporium* sp. MH-6 was found to produce the highest amount of GA3.

Khan *et al*. (2011) reported that endophytic fungi can produce phytohormones, particularly gibberellins (GAs), that enhance crop growth and alleviate the harmful effects of abiotic stresses.

Sunitha *et al*. (2012) reported on the amylase activity of fungal endophytes isolated from *Alpinia calcarata* (Haw.) Roscoe and found that 11 fungal endophytes showed positive results for amylase activity test, some of which includes *Fusarium* sp., *Colletotrichum* sp., *Alternaria* sp., *Cladosporium* sp. Maximum amylase production was found to be at 30º C and at pH 7.0 of the growth medium.

Nath *et al*. (2015) reported on the plant growth promoting factors of endophytic fungi isolated from the root, stem and leaves of tea (*Camellia sinensis*) shrubs collected from different tea gardens of Assam, India. Out of ten different endophytic fungi isolated, the highest IAA (indole acetic acid) activity was observed for *Aspergillus niger* followed by *Penicillium sclerotiorum*. The highest GA3 activity was exhibited by the fungus *Fusarium oxysporum*followed by *P. chrysogenum*. Nine isolates could solubilize phosphate with the highest being *Penicillium sclerotiorum* followed by *Penicillium* sp., *A. niger* and *A. fumigatus*. *A. niger*was also found as the highest potassium solubilizer.

Potshangbam*et al*. (2017) studied on the plant growth promotion activities of endophytic fungi isolated from healthy maize and rice plants against phytopathogens, *viz, Pythium ultimum*, *Sclerotium oryzae, Rhizoctonia solani,* and *Pyricularia oryzae*. Most dominating fungal endophyte associated with both the crops belonged to genus *Fusarium*, *Sarocladium*, *Aspergillus,* and *Penicillium*.

Mahfooz*et al*. (2017) reported on the enzymatic amylase activity of fungal endophytes that were isolated from *Cupressus torulosa* D. Don and found that 8 isolates showed positive reaction to amylase activity test and some of which are *Penicillium oxalicum, Alternaria alternata, Fusarium circinatum, Pestalotiopsis versicolor* and *Penicillium megasporum*.

Junaidi and Bolhassan (2017) reported on the isolation of 10 fungal endophytes from *Phyllathus niruri* Linn. which were all identified as *Fusarium oxysporum*. Screening of IAA was carried out for all the isolates and it was found that only 2 isolates gave high amount of IAA production and they are FO9 and FO10 with a concentration of 23.52 µg/ml and 5.95 µg/ml, respectively.

Mehmood *et al*. (2018) explored the role of indole-3- acetic acid (IAA) as a signalling molecule for chemical dialogue between endophytic fungus and

host plant roots. The endophytic fungus was isolated from the leaves of drought stressed *Withaniasomnifera* and was identified as *Aspergillus awamori* Wl1. The isolated Wl1 strain was capable of producing important secondary metabolite, IAA. The strain efficiently colonized the maize roots and enhanced the growth of host plant.

Gusmiaty*et al*. (2019) reported that plant growth can be influenced by the diversity of microbes that exist in the rhizosphere, e.g., fungi. Their study was aimed to identify rhizosphere fungi and evaluate the ability of IAA production. Fungus identification observed five fungus genera (*Aspergillus, Trichoderma, Rhizopus, Penicillium*, and *Fusarium*) to have plant growth promotion activity. IAA production ability test showed that *Fusarium* has the highest concentration, which was 38,611 ppm. Fusarium isolates have the potency to be developed as biological fertilizers.

Turbat*et al*. (2020) reported on the plant growth promoting role of fungal endophytes that were isolated from different parts of a medicinal plant (*Sophora flavescens*) important in Mongolia and China. Fifteen isolates belonging to the genera *Alternaria, Didymella, Fusarium* and *Xylogone* were isolated and it was found that all of the isolates could produce IAA, five of the isolates possessed phosphate solubilization activities and twelve secreted siderophores.

Khalil *et al*. (2021) isolated 15 fungal endophytes, belonging to 3 genera, *Penicillium, Alternaria* and *Aspergillus* that were obtained from leaves of *Ephedra pachyclada* to test their plant growth promotion activity. *Penicillium commune* was found to produce maximum IAA. They also reported that phosphorous is one of the important macronutrients required by plants in higher amount for their growth promotion and endophytes are capable of converting it from an unavailable to abled source for plant uptake. They tested their ability to solubilize phosphate and found that only *Penicillium crustosum, P. chrysogenum* and *Aspergillus flavus* could solubilize phosphate.

Savani *et al*. (2021) studied the endophyte potential in the growth promotion activity against the Panama wilt disease of banana and they isolated a total of 220 bacterial and 110 fungal endophytes from leaves, pseudostems and roots of banana. Only 3 endophytes could produce IAA and out of these, only 1 fungal endophyte gave the highest production, which was *Trichoderma reesei* UH EF.

Malubag*et al*. (2021) isolated 9 fungal endophytes from *Musa paradisiaca* (plantain banana) and identified through cultural, morphological and DNA sequencing of the ITS1 and ITS4 regions. and were identified belonging to genus *Cladosporium, Fusarium, Geotrichum, Nigrospora* and *Schizophyllum*. Eight out of nine fungi were able to degrade starch and some of them are *F. chlamydosporium, F. keratoplasticum* and the three different strains of *F. solani*.

Reyes *et al*. (2021) reported on the amylase activity of fungal endophytes that were isolated from *Citrofortunella macrocarpa* (Bunge) and all the 11 identified isolates could produce amylase and some of which were *Fusarium oxysporum, Colletotrichum fructicola, Colletotrichum gloeosporioides*.

Hawar (2022) reported on the amylase activity of fungal endophytes isolated from *Ziziphus spina* leaves, a medicinal plant and 5 isolates were found to show amylase activity and some of which are *Aspergillus niger, A. flavus, Cladosporium* sp., and *Mucor* sp.

Kumar and Prasher (2023) reported that *Colletotrichum gloeosporioides* and *Aspergillus fumigatus* isolated from *Dillenia indica* rhizosphere could solubilize phosphate.

2.4 Chitinase activity and siderophore production of the fungal endophytes

Meenavalli*et al*. (2011) studied the chitinase enzyme production by fungal endophytes isolated from different host species where out of the 162

isolates, only 31 isolates showed chitinase production. Genera like *Alternaria, Nigrospora, Cladosporium, Pestalotiopsis* and *Phyllosticta* and some species of *Colletotrichum, Fusarium, Cladosporium* and *Phomopsis* did not produce chitinase.

Dolatabad*et al*. (2017) reported that *T. harzianum* TH 5-1-2, *T. atroviride* TA 2-2-1 and *T. harzianum* TH 10-2-2 could produce chitinase enzyme, however, *Byssochlamys nivea, Chaetomium interruptum, Fusarium incarnatum-equiseti, F. acuminatum, F. tricinctum* etc, could not produce chitinase.

Mahfooz*et al*. (2017) reported on the chitinase activity of some fungal endophyte, out of which *Penicillium oxalicum, A. alternata, Daldinia sp., Fusarium circinatum* and *Penicillium megasporum* were found to be positive for chitinase test and only *Pestalotiopsis versicolor* was found to be negative.

Puig and Cumagun (2019) reported on the isolation of 155 fungal endophytes from 20 plants from Mt. Apo rainforest, Davao, Philippines and the best 5 isolates based on the antagonistic test against *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (FocTR4) were tested for their production of chitinase enzyme. *Pestalotiopsis* CGP117 did not produce chitinase and the rest were found to produce chitinase enzyme.

Gateta*et al*. (2023) reported on the study of fungal endophytes for their plant growth promotion activities isolated from Maled Phai rice seeds, from Thailand and *Trichoderma pinophilus* PBMP28 and *Aspergillus flavus* KKMP34 were found to be the isolates producing siderophore.

Toghueo*et al*. (2023) reported that out of 22 *Diaporthe* sp. isolated from the roots of *Festuca rubra* subsp. *pruinosa*, 20 strainscould produce siderophore.

2.5 The Pathogen

Banana wilt caused by the fungal pathogen *Fusarium oxysporum*Schlect f. sp. *cubense*(E. F. Smith) Snyd. & Hans. (Foc), widely

referred to as Panama wilt disease, is a devastating disease of bananas and plantains (*Musa* sp.) throughout the world (Stover, 1962 and Pérez-Vicente and Dita, 2014). The pathogen is broadly categorised into four races, of which races 1, 2 and 4 are pathogenic to banana, with race 1 infecting most banana cultivars excluding "Cavendish group" (Ploetz, 1990; Ploetz *et al*., 2003; Pérez-Vicente and Dita, 2014). In India, the disease is prevalent in all banana growing areas with race 1 forming the most common one followed by race 2 (Sivamani and Gnanamanickam, 1988; Prasadji and Smith. 2007), while race 4 has been reported in certain pockets (Thangavelu *et al*., 2011). The first description of *Fusarium* wilt in banana and plantains came from Australia (Bancroft, 1876 and Ploetz *et al*., 2003). The pathogen is soil-borne invading the roots and obstructing the water and nutrient flow through vascular colonization. Consequently, leaves turn yellow with the oldest ones succumbing first. The plants eventually wilt and collapse, thus causing serious crop losses (Bancroft, 1876 and Ploetz *et al*., 2003).

2.6 Isolation, morphological characterization, pathogenicity, identification of the Fusarium wilt pathogen of banana

2.6.1 Isolation and morphological characterization of the pathogen

The Fusarium wilt, also known as Panama wilt is the most destructive disease of banana. The initial symptoms include, yellowing of leaves, longitudinal splitting of the stem at the base and then eventually, the younger leaves wilt and in severe cases, the whole leaves dry up and collapse around the stem. When cut opened, reddish colour discoloration of the vascular tissues is observed, which is one of the major characteristic symptoms of Fusarium wilt disease of banana. The above statement is observations recorded by earlier workers such as Ploetz (2006), Leong *et al*. (2009), Li *et al*. (2011), Kai-li *et al*. (2019).

The characteristic morphological features of the pathogen have been described by several authors. The microconidia are found in abundance in false

heads on monophialides, 5 to 16 × 2.4 to 3.5 µm in size, with the conidia being one- or two-celled and oval- to kidney-shaped. Macroconidia were produced sparsely and were four- to six-celled, 27 to 55 × 3.3 to 5.5 µm and slightly sickle-shaped with an attenuated apical cell and a foot-shaped basal cell. Chlamydospores were produced singly or in pairs, both in the hyphae and inside macroconidia. The above characters are reported by Leslie and Summerell (2006), Ploetz (2006), Thangavelu *et al*. (2019).

2.6.2 Pathogenicity test of the pathogen

Udompongsuk and Soytong (2016) and Patel and Jampala (2018) conducted pathogenicity test on the banana leaves by detached leaf method. The fungus was isolated from an infected pseudostem of a banana plant. The pathogenicity test proved that the fungus caused wilt symptoms on the inoculated banana leaves and no symptoms were observed on the control leaves.

2.6.3 Molecular characterization and identification of the pathogen

Kai-li *et al*. (2019) isolated and identified 12 *Fusarium oxysporum* f. sp. *cubense* strains from the 79 soil samples that were collected from four regions of Zhangzhou City, the primary banana production area in Fujian, China, based on internal transcribed spacer (ITS) sequence analysis, PCR amplification by using Foc-specific primers and pathogenicity assays. Their analysis indicated that 11 isolates belong to Foc race 1, and 1 isolate belongs to the Foc tropical species race 4 (TR4). This is the first report of TR4 isolated from the soil in Fujian Province.

Prakash *et al*. (2023) isolated 8 infected samples of Fusarium wilt disease of banana from 8 different fields. These isolates were identified based on 18s rRNA sequencing using ITS1 and ITS4 primers. They were identified and confirmed as *Fusarium oxysporum*.

2.7 To assess biocontrol activities of the endophytes against Fusarium wilt pathogen of banana

2.7.1 Antagonistic effect of endophytic fungi against the pathogen by dual culture

Dagamac*et al*. (2008) reported on the antagonistic effect of fungal endophytes isolated from the roots of banana. Out of the 75 fungal endophytes isolated, 25 isolates were screened for their antagonistic effect against*Fusarium oxysporum* f. sp. *cubense* and it was found that 3 *Aspergillus* species tested against the wilt pathogen could inhibit the growth of the pathogen.

Garoe*et al*. (2013) evaluated 15 fungal endophytes from banana corm that belonged to 4 different genera (*Aspergillus*, *Penicillium*, *Fusarium* and *Chaetonium*). Endophytic fungi were observed to have antagonistic effects against the *Fusarium* wilt. Three endophytic fungi (2 *Aspergillus* spp. and 1 *Penicillium* sp.) inhibited mycelia growth of the pathogen.

Thangavelu and Gopi (2015) reported on the inhibitory effect of *Trichoderma* isolates against Foc. *Trichoderma* isolates with a total of 20 from rhizosphere and 43 endophytes were isolated. They found that 6 *Trichoderma* isolates from rhizosphere and 10 endophytic *Trichoderma* isolates could inhibit the growth of the pathogen *in vitro*. Under greenhouse condition, *Trichoderma* sp. NRCB3 + endophytic *Trichoderma asperellum* Prr2 could completely control the growth of the pathogen when tested against the Foc infected cv. Grand Naine (AAA) variety.

Ribeiro *et al*. (2018) reported that fungal endophytes belonging to *Diaporthe* genus from *Pachystachys lutea* were found to be antagonistic against *F. oxysporum*.

Hidayat *et al*. (2019) reported on the antagonistic effect of fungal endophytes *Aspergillus* sp. isolated from the banana plant against the Fusarium wilt disease. Antagonistic assay by dual culture method showed that the *Aspergillus* sp. strain PD2, strain PD4, and strain PD5 inhibited the growth of Foc isolate by 37.31%, 26.52%, and 12.04%, respectively.

Lalngaihawmi and Bhattacharyya (2019) reported on the screening of 54 native rhizospheric microbes against *Fusarium oxysporum* f. sp. *cubensein vitro* and it was found that the effect of all the rhizospheric microbes significantly differed in terms of inhibition of radial growth of the wilt pathogen. After 120 hours of incubation, *Trichoderma reesei* (RMF-25) was found most promising as antagonist against Foc with 71.08 per cent inhibition of radial growth followed by *Trichoderma reesei* (RMF- 13) with 70.55 per cent and *T. harzianum* (RMF-28) with 70.15 per cent inhibition of radial growth of Foc.

Abramczyk *et al*. (2022) reported that *Diaportheeres* from *Prunus dulcis* showed antagonistic activity against *Fusarium avenaceum*.

2.7.2 Volatile and Non-volatile metabolite production

Kumar and Kaushik (2013) reported that fungal endophyte, *Colletotrichum truncatum* isolated from *Jatropacurcas*, an oil seed crop could effectively control *Fusarium sclerotiorum* through the production of volatile compounds.

Raza *et al*. (2013) reported on the non-volatile metabolite production of *Trichoderma harzianum* SQR-T037 against the wilt disease of watermelon, *Fusarium oxysporum* f. sp. *niveum* and it was found that *Trichoderma harzianum* could inhibit the growth of the pathogen significantly when tested *in vitro*.

Rabha *et al*. (2014) also reported that the endophyte *Colletotrichum gloeosporioides* isolated from *Camellia sinensis*, Assam, India showed inhibitory effect as a result of volatile compound production against the pathogen, *Pestalotiopsis theae* with a per cent inhibition of 64%.

Li *et al*. (2014) studied the fungal endophytes isolated from the leaf, roots and stems of cotton plants and investigated the activity of the isolated endophytes for their non-volatile metabolite production. CEF-325 (*Fusarium solani*) was found to completely inhibit the growth of *Verticillium dahliae*.

Monggoot*et al*. (2017) reported that fungal endophytes that belonged to the genus *Colletotrichum* sp. MFLUCC16-0047, *Colletotrichum* sp. MFLUCC16-0048,*Arthrinium* sp. MFLUCC16-0042 and *Diaporthe* MFLUCC16-0051 which were isolated from *Aquilaria subintegra*, Thailand, produced a wide range of volatile compounds like β-agarofuran, α-agarofuran, δ-eudesmol, oxo-agarospirol, and β-dihydro agarofuran that had bioactivities against plant diseases.

Thoyajakshi Bai *et al*. (2018) reported on the non-volatile metabolite production of rhizospheric microbes against chilli wilt, *Fusarium oxysporum*, where *Trichoderma* sp. was found to produce non-volatile compounds against the pathogen. All the tested fungal antagonists showed positive results by significantly reducing the growth of the pathogen.

Lalngaihawmi and Bhattacharya (2019) reported on the non-volatile metabolite production activity of three rhizospheric*Trichoderma* isolates and it was found that all the three isolates could greatly inhibit the growth of Foc due to the metabolite production and the highest per cent inhibition was found in *T. reesei* (RMF 25) with 35.96% inhibition followed by *T. reesei* (RMF 13) with 35.22% and *T. harzianum* (RMF 28) with 34.72% inhibition, under *in vitro*.

Song *et al*. (2019) isolated fungal endophyte*Diaportheapiculatum* strain FPYF 3052 from *Leucaena leucocephala* which was found to be inhibit 8 plant pathogens through the production of volatile metabolites with a per cent inhibition range of 23.80% to 66.70%.

Ahmed *et al*. (2023) reported on the production of secondary metabolites by *Fusarium* sp., a fungal endophyte isolated from the roots of *Mentha longifolia* L. (Labiatae) and found that it inhibited the growth of several pathogens like *C. albicans, C. glabrata, C. krusei* and *A. fumigatus* through the production of cyclodepsipeptidefusaripeptide A.

Santra and Banerjee (2023) reported on a fungal endophyte, *Diaporthe* sp. CEL3 which was isolated from leaves of an ethnomedicinal plant, from

Arunachal Pradesh, and the endophytic isolate was found to produce volatile compounds inhibiting several important plant pathogens like *Moniliniafructicola*, a causal agent of cherry fruit rot, in VOC-exposed cherry fruits. *Rhizoctonia solani, Botrytis cinerea, Pythium ultimum*, and *M. fructicola* were maximally inhibited up to 51.5%, 55.8%, 61.9%, and 78.5%, respectively, in comparison to control by the volatiles.

CHAPTER III

MATERIALS AND METHODS

MATERIALS AND METHODS

The proposed investigation entitled **"Study on the endophytic fungi from banana species of Nagaland and their effect againstthe Fusarium wilt pathogen"** was carried out under *in vitro* condition in the laboratory of the Department of Plant Pathology, SAS, Nagaland University.

3.1 General information

3.1.1 The school is located at Medziphema, Nagaland under Chumoukedima District. The place is located at 25° 45′ 45′′ North latitude and 93° 51′ 45" East longitudes at an elevation of 310 m above mean sea level.

3.1.2 In all the experimental studies, Borosilglasswares were used. The glasswares were washed with detergent and rinsed. All the glasswares used in the study were sterilized in hot air oven at 180^{o}C for 2 hours. Both solid and liquid media were sterilized in an autoclave at 121^{o}C and 15psi (pound per square inch) for 15 minutes.

3.2 Details of sample collections site

The sample collection for isolation of fungal endophytes from the banana species of Nagaland was carried out during the year 2019-2021 from four districts of Nagaland; Chumoukedima (earlier under Dimapur district), Kohima, Peren and Mokokchung,the details of which have been given in Table 3.1 and Fig 3.1. Twelve samples each from leaf and roots of wild banana and one sample each from leaf and roots of cultivated banana was collected from each of the four districts of Nagaland (Plate 1).

Table 3.1. Sites of collection of banana samples for isolation of endophytes

District	Collection Site	Longitude	Latitude	Altitude in meters (msl)
Chumoukedima (earlier under Dimapur district)	Patkai Christian College (PCC)	93.8003ºE	25.8017ºN	248
	Ruzaphema (RZP)	93.7873ºE	25.7144ºN	486
	Medziphema (MDZ)	93.8816°E	25.7594°N	489
	Kukidolong (KKD)	93.8172°E	25.7698°N	275
Kohima	Viswema Village (VSM)	94.1450ºE	25.5615ºN	1679
	Dzulakie (DLK)	93.9557ºE	25.6205ºN	1817
	Kiruphe, Basa Village (KBV)	94.0081ºE	25.7381ºN	1595
	Sechu Zubza (SCZ)	94.0353ºE	25.7099ºN	1081
	Lerie Colony (LCZ)	94.1083ºE	25.6492ºN	1456
	Kohima village (KHV)	94.1086º E	25.6751°N	1489
Peren	Sector – B, Old Jalukie (OJL)	93.4306ºE	25.3415ºN	503
	Mhainamtsi, Jalukie (MJL)	93.7027ºE	25.6327ºN	1117
	Punglwa (PGW)	93.8418ºE	25.6792ºN	709
Mokokchung	Chuchuyimlang (CCY)	94.4599ºE	26.4064ºN	1137
	Yisemyong (YSY)	94.6325ºE	26.4382ºN	1117
	Changtongya (CTY)	94.6827ºE	26.5345ºN	820
	Alichen (ALC)	94.4555ºE	26.2693ºN	1197

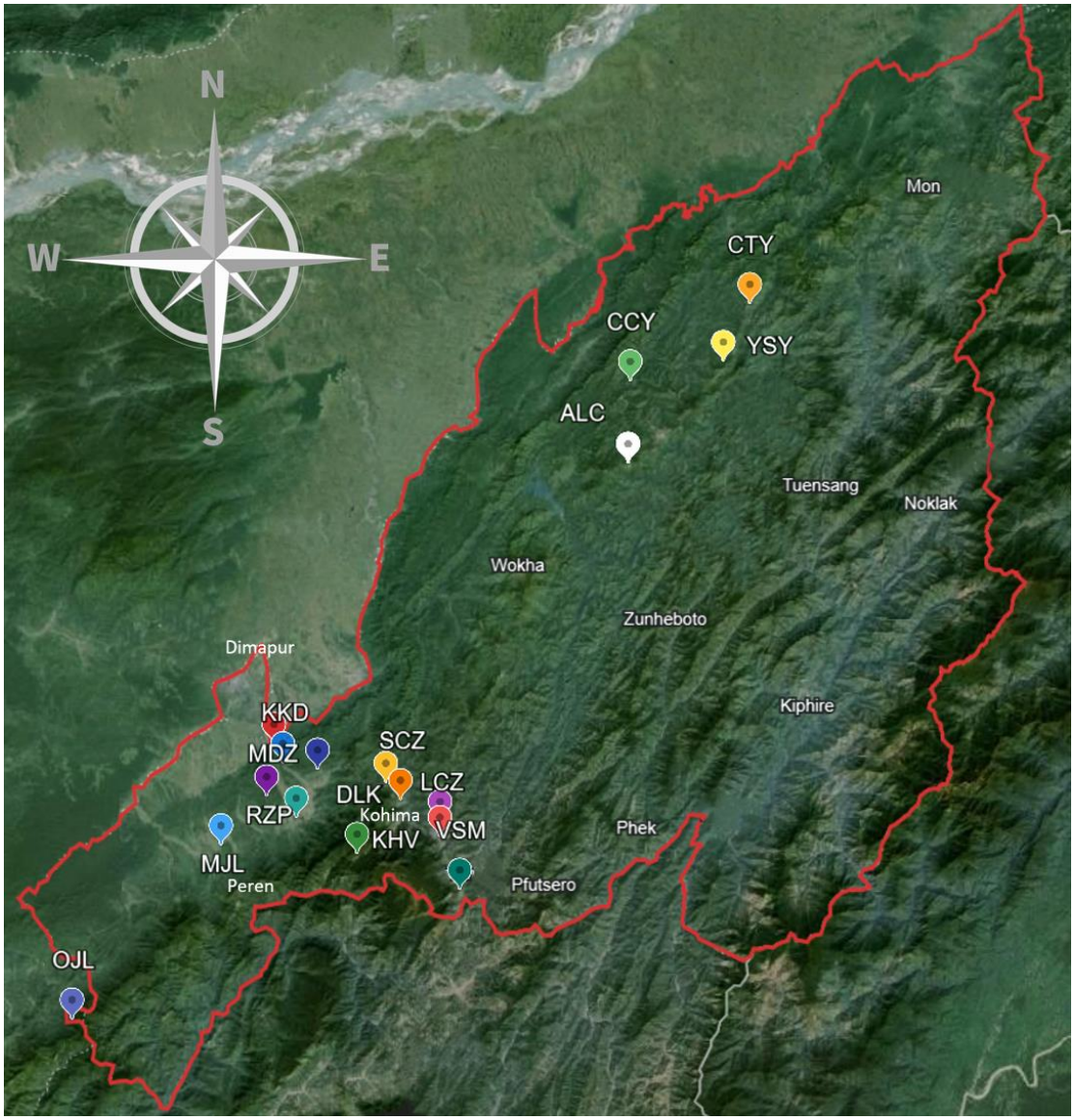


Fig 3.1. Mapping of collection sites from the 4 districts of Nagaland

Plate 1. Some wild banana germplasm collected from the four districts of Nagaland

3.3 Isolation and identification of fungal endophytes from the leaves and roots of healthy banana plants.

3.3.1 Culture medium

Potato Dextrose Agar (PDA) medium was prepared and used for the isolation of fungal endophytes from the healthy banana plants. The media composition is mentioned in the Appendix.

3.3.2 Isolation and purification of the fungal endophytes

3.3.2.1 Isolation from leaves

Isolation of endophytic fungi from leaves of healthy and symptomless banana plant collected from four districts of Nagaland was carried out using surface sterilisation technique. The leaf sample from banana plant was washed with distilled water and air dried, and then 1 cm segment of the leaf was cut using a sterilized scalpel. The cut bits of banana leaf were sterilized in 2% sodium hypochlorite solution for 3 min, followed by rinsing 3 times in sterile distilled water for 1 minute each, and blot drying using sterilised filter paper to eliminate excessive water (Zakaria and Aziz, 2018).

Once the leaf bits were completely dried, imprinting procedure was done by pressing the sterilized leaf bits onto the surface of Potato Dextrose Agar (PDA) to affirm the effectiveness of the surface sterilisation method and to affirm that only endophytic fungi are isolated. If there is no fungal growth on the impression plate, the surface sterilization method used was successful in eliminating the surface fungi or epiphytes (Schulz *et al*., 1993). The leaf bits were then transferred onto PDA plates and incubated at 25±1°C. A total of four leaf bits were placed onto a PDA plate. The PDA plates with leaf bits were incubated for 1–4 days or until growth of mycelium from the leaf bits were observed. The mycelium that was arising from the leaf bits was sub-cultured onto new plates of PDA.

3.3.2.2Isolation from roots

Banana root samples were randomly collected from healthy and symptomless banana plant from four districts of Nagaland. The roots were taken by digging the soil around the banana plant. All the root samples were put in plastic bag according to their respective location.

The roots were washed with running water to remove the soil that was adhered to them. Surface sterilization method was carried out in order to isolate endophytic fungi. The roots were cut into tiny pieces (between 2.0 and 3.0 cm), immersed in 70% ethanol for 30 seconds, 1% sodium hypochlorite for three minutes, and 95% ethanol for five minutes. Finally, the roots were rinsed three times with sterile distilled water. After drying with sterile filter paper, the root fragments were sliced into much smaller pieces (1.0–1.5 cm) and placed on potato dextrose agar (PDA) (Zakaria *et al*., 2016).

On each of the PDA plate, four root fragments were placed. The root fragments were imprinted on PDA plate prior to plating in order to identify any epiphytes present on the root pieces (Schulz *et al*., 1993). A daily observation log was kept while the plates were incubated at 27±1°C to monitor for any fungal growth from the fragmented roots. On fresh PDA plates, mycelial growths from the roots were sub-cultured.

3.3.3 Morphological characterization and identification of the fungal endophytes

The pure culture of the endophytes isolated from leaves and roots were observed under microscope to study the morphological characters. Cultural characteristics of the isolates were also recorded. Photomicrographs of each isolate was taken and measurements of the conidia were also taken.

3.3.4 Molecular characterization and identification of the fungal endophytes

3.3.4.1Growth of fungal endophytes

A mycelial disc (5 mm diameter) from 7 days old cultures were transferred to 100 ml of sterilized potato dextrose broth medium containing 10 µl of Tween 80 and incubated at shaker incubator at 28^{o}C for 7 days. The mycelium was filtered through a sterile Whatman filter paper No. 1, and the excess broth was drained out. Mycelium weighing about 5-10 g was taken and ground using a mortar and pestle in liquid nitrogen (DNA extraction kit, HiMedia).

3.3.4.2Extraction of DNA and PCR amplification

For species confirmation of morphologically identified and the best performing isolates (24 no.), sequencing of ITS regions was carried out. ITS region is regarded as DNA barcode marker for identification of fungi (Schoch *et al*.,2012) and therefore, the region was used in the present study.

DNA extraction of the selected fungal endophyte isolates (24 no.) was carried out using the HiMedia DNA extraction kit.After DNA extraction, PCR amplification was carried out using HiMedia PCR amplification kit and PCR was performed in Thermal Cycler (Bio-Era, Model ADEPT, India).

The PCR program followed is mentioned below:

1. Initial denaturation was kept at 94^{0}C for 10 minutes.
2. Denaturation was done at 94^{0}C for 45 seconds, followed by annealing at 55^{0}C for 30 seconds and extension at 72^{0}C for 30 seconds.A total of 35 cycles was carried out.
3. Final extension was carried out at 72^{0}C for 10 minutes.

The universal primers used for amplification of the extracted DNA were ITS1 (Forward) (5’ -TCC GTA GGT GAA CCT GCG G- 3’) and ITS4 (Reverse) (5’ -TCC TCC GCT TAT TGA TAT GC- 3’) (White *et al*., 1990).

3.3.4.3Agarose gel electrophoresis of PCR products

After PCR, electrophoresis was run to detect the PCR product by using 1.0% agarose gel. One hundred bp ladder was used as a molecular size standard marker. The PCR products were separated by electrophoresis (at 75 V for 60 min) using agarose gel with 1x Tris acetate EDTA buffer. The gel was stained with ethidium bromide (0.5 μg ml^{-1}) before pouring. The stained gel was viewed and the image was captured using a gel documentation system.

3.3.4.4Sequencing and bioinformatic analysis

PCR products of 18S rRNA gene was sent for purification and sequencing to BioKart, Bangalore. After sequencing, the sequences were aligned by using BioEdit Sequence Alignment Editor Version 7.0.5 software by Hall (1999) to obtain consensus sequences. The consensus sequences were then compared with other DNA sequences in GenBank using Basic Local Alignment Search Tool (BLAST) in National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). Identification of the endophytes was done based on the highest similarity of the BLAST search. Phylogenetic tree was constructed using the MEGA11 software. The nucleotide sequences of ITS 18S rRNA gene were deposited in NCBI GenBank for acquiring the accession number.

3.4 Indole acetic acid (IAA) production

The IAA production by fungal endophyte isolates was evaluated as per the protocol given by Gordon and Weber (1951). The fungal endophyte isolates were cultured in ISP2 medium (Appendix) for seven days at 30°C. Four mm diameter of agar discs was cut with the help of a sterilized cork borer and inoculated into 100 ml of ISP-2 broth (Appendix) that contained 0.2% L-tryptophan. The cultures were then incubated with continuous shaking at 125 rpm at 30°C for 14 days. After incubation, the suspensions were centrifuged for 15 mins at 11,000 rpm and the supernatant (1 ml) was mixed with Salkowski's reagent (2 ml) and incubated further for 25 mins in darkness at

30°C. The production of IAA was distinguished with the pink-red color development and the measurement of absorbance was done at 530 nm using a spectrophotometer (UV-VIS Spectrophotometer, Model No. LMSP- UV1200) and comparison was also done with the IAA standard curve and the quantity of IAA was expressed in µg/ml.

3.5 Gibberellic acid (GA3) production

To 100 ml of Murashige and Skoorg (MS) medium (Appendix) amended with 1000 µg/ml of tryptophan, a spore suspension of 2 $x10^6$ spores per ml was inoculated directly into the flasks. The flasks were then incubated at 25º C for 6 days. The quantity of GA3 found in the culture supernatant was calculated using the standard procedure (Uthandi*et al*., 2010). To separate the particulate matter, that includes the fungal hyphae, 30 millilitres of the broth culture that has been cultured for six days was taken from each flask and centrifuged at 3000 rpm. A 40 ml test tube containing 25 ml of culture supernatant was added with 2 ml of zinc acetate (1M). Two ml of potassium ferrocyanide was added after 2 minutes, and centrifuged for fifteen minutes at 1000 rpm. An equal amount of 30% HCL was gently added to 5 ml of the supernatant, and it was incubated at 20ºC for 75 minutes. As a reference, a blank sample with uninoculated broth was taken and treated similarly, and its absorbance was measured spectrophotometrically (UV-VIS Spectrophotometer, Model No. LMSP- UV1200) at a wavelength of 254 nm.

3.6 Phosphate solubilisation

The endophytic fungal isolates were screened for their phosphate solubilizing activity in Pikovskya's agar medium (Appendix) plates. The fungal endophyte isolates that showed clear zones around the growing colonies after incubation for 72 hrs at 25^0C was taken as positive reaction for P solubilization activity (Gour, 1990).

3.7 Screening for secretion of amylase

For amylase production test, fungal endophyte isolates were inoculated on glucose yeast extract peptone (GYP) agar medium (Appendix) that contained 1% soluble starch. After incubation for 5 days, 1% iodine in 2% potassium iodide was flooded in the fungal colony plates. Appearance of clear zone surrounding the fungal colony was observed to be positive for amylase test (Bhardwaj *et al.,* 2015).

3.8 Chitinase activity

The selected isolates were tested for their chitinase enzyme activity on the colloidal chitin agar medium (Appendix). Positive chitinase activity was measured based on the clear halo zone on colloidal chitin agar medium (Skujins*et al*., 1965).

3.9 Siderophore production

The fungal endophyte isolates were inoculated on Chrome Azurol S (CAS) agar medium (Appendix) and incubated for 5 days at 28^0C (Schwyn and Neilands, 1987). The colonies with orange zones were considered as siderophore producing isolates.

3.10 Isolation and identification of the Fusarium wilt pathogen of banana

3.10.1 Collection of disease specimen

Banana plant pseudostem infected with typical symptoms of the wilt disease was collected from farmers' field, Dimapur. The specimen was collected in paper bag and brought to the laboratory and it was isolated in Potato Dextrose Agar (PDA).

3.10.2 Cultivation medium

Potato Dextrose Agar (PDA) was used for isolation, purification and maintaining the pathogen.

3.10.3 Isolation and purification of the pathogen

The portion banana plant infected with the wilt disease was sliced along with the healthy part of the stem into tiny pieces, and surface sterilized with 1% sodium hypochlorite solution for 1 minute, followed by rinsing 3 times with sterilized distilled water and sterilized blotting paper was used to blot dry. The stem pieces were aseptically transferred on Petri plates containing PDA medium. The Petri plates inoculated with the infected stem pieces were incubated at 28±1^{o}C. After the growth was initiated, the culture was purified by transferring it to a new medium. The culture was maintained in PDA slants and stored at 4^{o}C (Saravanan *et al*., 2004).

3.10.4 Morphological characterization and identification of the pathogen

Identification of the pathogen was done culturally and morphologically by observing the spores under microscope (Debro Microscope, Model No. DX-600). Spore measurement with an average of 100 spores (both length and breadth) was also carried for all the isolated endophytes under 40x.

3.10.5 Pathogenicity test using detach leaf assay

Healthy leaves of banana were detached and surface sterilized with 70% ethanol. The leaves were cut into 5 x 5 cm bits and placed in a sterilized Petri plate that contained sterilized moist filter paper to maintain humidity. The cut bits of the leaves were wounded with the help of a sterile needle so that the test pathogen can have easy access to the leaves.

The test pathogen was grown in PDA for 7 days and the mycelial disc was cut with the help of a cork borer and was inoculated on the wounded leaf bits (Udompongsuk and Soytong, 2016). Non inoculated negative controls were inoculated with an agar plug without the fungus and with *Fusarium oxysporum* f. sp. *lycopersici*, the pathogen causing wilt disease in tomato plant. Comparison was also made with the *Fusarium oxysporum* f. sp. *cubense* treated leaves as a positive control, that was procured from ITCC (Indian Type Culture Collection), IARI, New Delhi. The Petri plates were incubated at room

temperature for 10 days to observe symptom development. Five replications were maintained for each treatment.

3.10.6 Molecular identification of Fusarium wilt pathogen of banana

The culture of the pathogen was grown in potato dextrose broth (PDB) amended with 10 µl of Tween 80 and incubated at shaker incubator at 28^{o}C for 7 days as per the method given in the HiMedia DNA extraction kit. The genomic DNA of the isolate was extracted using DNA extraction kit from HiMedia. After DNA extraction, PCR amplification was carried out using HiMedia PCR amplification kit and PCR was performed in Thermal Cycler (Bio-Era, India). The universal primers used for amplification of the extracted DNA were ITS1 (Forward) (5' -TCC GTA GGT GAA CCT GCG G- 3') and ITS4 (Reverse) (5' -TCC TCC GCT TAT TGA TAT GC- 3') (White et al., 1990). After PCR, electrophoresis was run to detect the PCR product by using 1.0% agarose gel.

PCR products of 18S rRNA gene was sent for purification and sequencing to BioKart, Bengaluru. After sequencing, the sequences were aligned by using BioEdit Sequence Alignment Editor Version 7.0.5 software by Hall (1999) to obtain consensus sequences. The consensus sequences were then compared with other DNA sequences in GenBank using Basic Local Alignment Search Tool (BLAST) in National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). Identification of the isolates was done based on the highest similarity of the BLAST search. Phylogenetic tree was constructed using the MEGA11 software. The nucleotide sequences of ITS 18S rRNA gene were deposited in NCBI GenBank for acquiring the accession number (Kai-li*et al.*, 2019).

3.11 Antagonism assays of fungal endophytes of banana against the Fusarium wilt pathogen

3.11.1 *In vitro* screening through dual plate culture

The isolated fungal endophytes were tested against Fusarium wilt pathogen of banana under *in vitro* condition by dual culture technique and per cent inhibition was calculated after Vincent (1947) as given below:

$$\text{Per cent inhibition} = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100$$

3.11.2 Volatile metabolites production

The fungal endophyte isolates were assessed for their potential to produce volatile compounds as per the method given by Dennis and Webster (1971). The fungal endophyte isolates were inoculated on the sterilized Petri plates that contained PDA. Additional plate of equal diameter was inoculated with actively growing mycelial discs of Fusarium wilt pathogen and then inverted over the first plate. The joint of both the Petri plates were secured firmly with parafilm. PDA medium with the pathogen at the upper and lower lid was maintained as control. Three replications were maintained for the control as well as the treated plates and was incubated for 7 days at 25°C. The pathogen growth after incubation was measured and per cent inhibition of mycelial growth of the pathogen was calculated according to Vincent (1947).

$$\text{Percent Inhibition} = \frac{(C - T)}{C} \times 100$$

Where,

C = Diameter of fungus in control plates (mm)

T = Diameter of fungus on the plate inoculated with antagonist (mm)

3.11.3 Non-volatile metabolites production

The test on non-volatile compounds production by the fungal endophyte isolates was done using the method given by Dennis and Webster (1971). The fungal endophyte isolates were set on a sterilized cellophane paper which were placed on top of solidified PDA media in Petri plates. The fungal endophyte isolates were separately placed onto the cellophane paper and incubated at 25±2°C. Control plates were also maintained with cellophane paper only without inoculating the test pathogen. Three replications were maintained for all the antagonist and were incubated for 7 days at 25°C. The plates were then incubated for seven days to check whether the fungal endophyte isolates were able to produce diffusible non-volatile compounds. After 7 days, the cellophane paper was carefully removed along with the isolates and the same plates were inoculated with the tested pathogen for additional 5 days at 25±2°C. The pathogen growth after incubation was measured and per cent inhibition of mycelial growth of the pathogen was calculated according to Vincent (1947).

3.12 Statistical Analysis

The data obtained in the present investigation were subjected to appropriate statistical analysis. The differences exhibited by treatments in various experiments were tested for their significance by employing Completely Randomized Design (CRD) as per the details given by Panse and Sukhatme (1967). The percentage values were converted to arc sine values wherever required. Results of the measurements were subjected to analysis of variance (ANOVA) by Least Significant Difference (LSD) using WASP 2.0 (WebAgrilStatPackage) software.

CHAPTER IV

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

The results recorded during the course of the investigation entitled "Study on the endophytic fungi from banana species of Nagaland and their effect against the Fusarium Wilt Pathogen" are presented and discussed in this chapter. All the results are analyzed statistically to evaluate the effectiveness of different treatments applied. The findings thus obtained are discussed under the following heads with appropriate tables.

4.1Collection and isolation of fungal endophytes from healthy banana species

The collection of leaf and roots samples for isolation of endophytic fungi from the wild and cultivated banana species of Nagaland was done randomly from four districts of Nagaland, *viz*., Chumoukedima (Dimapur), Kohima, Peren and Mokokchung during the course of the research investigation.

Isolation of fungal endophytes from the leaves and roots was done in the laboratory and all the isolated endophytes were purified and maintained in PDA slants for further investigation.

A total of 281 fungal endophytes were isolated from the leaves and roots of healthy banana plants as per the method given by Zakaria and Aziz (2018) and Zakaria *et al*. (2016) respectively, and out of these, 246 isolates were acquired from the wild banana and 35 isolates from cultivated banana species of four districts of Nagaland. A total of 166 isolates were obtained from leaf samples and 115 isolates from root samples (Table 4.1; Plate 2).

Similar studies on the diversity of fungal endophytes were also carried out by other researchers. Photita*et al*. (2001) isolated fungal endophytes from 7500 samples of wild *Musa acuminata* that were assembled from 5 location sites sites at the National Park called Doi Suthep Pui, Thailand. Cao *et al*.

(2002) isolated 163 fungal endophytes from 200 leaf sample and 68 fungal endophytes

Table 4.1. Number of fungal endophytes isolated from banana samples collected from different districts of Nagaland

District	Sample Source	Wild Banana		Cultivated Banana		Total
Chumoukedima (earlier under Dimapur district)	Leaves	38	FEB1 to FEB38	5	FEB39 to FEB43	43
	Roots	12	FEB44 to FEB55	3	FEB56 to FEB58	15
Kohima	Leaves	43	FEB59 to FEB101	8	FEB102 to FEB109	51
	Roots	29	FEB110 to FEB138	5	FEB139 to FEB143	34
Peren	Leaves	31	FEB144 to FEB175	4	FEB175 to FEB178	35
	Roots	30	FEB179 to FEB208	3	FEB209 to FEB211	33
Mokokchung	Leaves	33	FEB212 to FEB244	4	FEB245 to FEB248	37
	Roots	30	FEB249 to FEB278	3	FEB279 to FEB281	33
Total		**246**		**35**		**281**

FEB- Fungal Endophytes of Banana

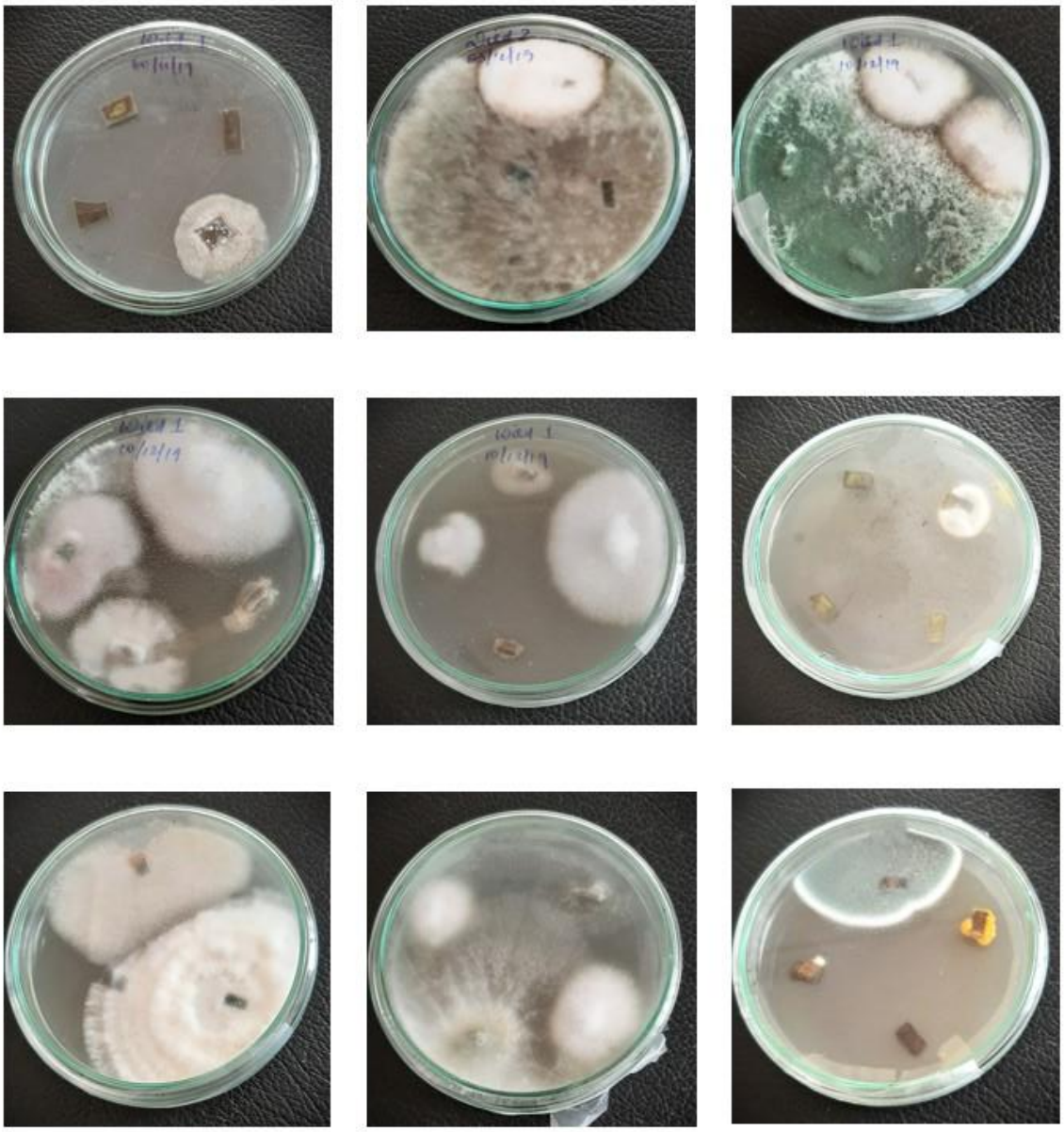


Plate 2. Some of the fungal endophytes isolated in PDA medium

from 100 root samples of *Musa acuminata*. Zakaria and Rahman (2011) isolated 54 endophytic *Fusarium* species from 100 sample of roots from different sites of Penang Island, Malaysia. Zakaria *et al*. (2016) isolated and characterized fungal endophytes from the roots of *Musa acuminata*, a wild banana andisolated 31 isolates from root fragments of 80 nos.

Savani *et al*. (2021) analysed fungal and bacterial endophytes which were isolated from leaf, pseudostem and root of banana and a total of 330 endophytes were isolated out of which 220 were bacterial and 110 were fungal endophytes and tested against the fungal pathogen causing Panama wilt disease of banana. Panda *et al*. (2023) also reported on the investigation of endophytic fungi from banana cultivars of Assam, India, where an entirety of 139 isolates were recorded from 134 (62 roots, 54 leaves and 18 fruits) samples of banana consisting of 15 different varieties from 10 locations during the year 2018-2019.

Other researchers such as Garoe*et al*. (2013); Dita *et al*. (2014); Souza Junior *et al*. (2018) and Henao *et al*. (2019) also studied the diversity of fungal endophytes from various segments of the banana plant.

In the current exploration, majority of the fungal endophytes were isolated from the leaf samples than the roots. Isolation was done using the conventional method for the leaf and root samples with a slight modification for root samples. This variation of the fungal endophytesisolates from the leaf and roots samples of the banana plants can be perhaps justified by the report given by Henao *et al*. (2019). They isolated fungal endophytes from leaf, roots, pseudostems and corms of banana cv. Manzano based on two disinfection methods; conventional (2% sodium hypochlorite + 70% ethanol) and chlorine gas (6.25% sodium hypochlorite + 37% hydrochloric acid). They reported that leaf consisted of the largest diversity of fungal endophytes more so on the healthy tissues. Roots on the other hand, though consisted of large diversity of fungal endophytes but more from the disease tissues and not the healthy

tissues. The method of disinfection regulated the percentage of strains obtained during isolation. When gaseous chlorine (GCD), a methodical way to eliminate surface contaminants was used as a disinfection method, 30% less isolates were isolated from the healthy and symptomatic plants in contrast to the traditional disinfection method. This method is needed for tissues from roots, pseudostems and corms that consist of porous surfaces which may create air chambers that may block GCD from thoroughly cleaning them. They stated that *Fusarium* species were isolated from leaves of healthy plants, but, they were not isolated from the tissues when gas chlorine was used. In contradiction, *Sordaria* and *Stachybotrys* were found in leaves and corms respectively and *Pythium* on roots of symptomatic plants when the tissues were disinfected with GCD. Thus, this record proposes suggests that disinfection method for isolation of endophytes plays a vital role in the proportion of endophytes that are isolated from various parts of a plant.

4.2Morphological Characterization and Identification of the endophytic fungi

On the ground of the cultural and morphological characters studied, the fungal endophytes were identified in the laboratory. All the identified and non-identified fungal isolates are mentioned in Table 4.2. Out of the 281 fungal endophytes isolated, identification of 135 fungal isolates in entirety belonging to 15 genera was done,of which 119 isolates were from the wild and 16 isolates were from cultivated banana. Of these 15 genera, 14 belonged to the Phylum Ascomycota and 1 belonged to Mucoromycota. The identified fungal endophytes belonged to the genera *Penicillium* sp., *Trichoderma* sp., *Fusarium* sp., *Colletotrichum* sp., *Diaporthe* sp., *Apiospora* sp., *Aspergillus* sp., *Pestalotiopsis* sp., *Mucor* sp., *Phomopsis* sp., *Botrytis* sp., *Helminthosporium* sp., *Alternaria* sp., *Beauveria felina*, and *Cladosporium tenuissimum*. The details of the cultural and morphological characters have been given in Table 4.2 and Plate 3.

Table 4.2. Morphological Characterization of Fungal Endophytes Isolated from the wild and cultivated Banana Species of Nagaland

Isolate number	Characteristics				
	Colony Color	**Colony Aspects or Texture**	**Spores**	**Microscopic Characteristics (40x)**	**Identification**
FEB1	Light pink	Powdery	Present	Conidia are minute, globose or ovoid in shape, 10 – 12 µm in diameter, formed in chains in basipetal succession, formed on a specialized conidiogenous cell called the phialide which were observed to be branched. Conidiophores were branched or unbranched, long, slender, hyaline	*Penicillium* sp.
FEB2	White	Cottony	Absent	Hyaline, septate and branched	Mycelia sterilia
FEB3	White	Light cottony	Present	Conidia are single celled, hyaline, oblong or cylindrical or fusiform, straight or slightly curved or bent, 67.07 x 24.09 µm in size. Phialides were not observed. Chlamydospores are globose to ellipsoidal, thick walled, intercalary or terminal.	*Fusarium haematococcum*
FEB4	Dark grey	Velvety	Present	Conidia are oval in shape, light brown in color, 46.05 x 19.00 µm. Conidiophore are light brown, septate and branched	Unidentified
FEB5	Green	Light cottony	Present	Conidia are ellipsoidal, smooth, greenish in color, 16 x 12 µm in size. Conidiophores were not observed.	*Trichoderma hamatum*
FEB6	Dark green	Cottony	Present	Conidia were minute, globose to subglobose, yellow green pigmentation, 10.72 – 12.17 µm in size. Conidiophores were not observed under the microscope	*Trichoderma* sp.
FEB7	White	Cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB8	Light pink	Powdery	Present	Conidia are minute, globose or elliptical in shape, bluish green on color, 10 – 12 µm in diameter, formed in chains in basipetal succession, formed on a specialized conidiogenous cell called the	*Penicillium* sp.

				phialide which were observed to be branched. Conidiophores were branched or unbranched, long, slender, hyaline	
FEB9	White	Cottony	Present	Microconidia are single celled, hyaline, oval or cylindrical, straight or slightly curved or bent, 67.07 x 24.09 µm in size. Conidiophore are hyaline, septate and branched. Chlamydospores were in abundant, rough walls, globose to subglobose, singly, pairs or in chains, 44.20 µm in size	*Fusarium solani*
FEB10	Grey	Powdery	Present	Conidia are minute, globose or ovoid in shape, 10.14 – 12.07 µm in diameter, they form in chains in basipetal succession. The conidia are formed on a specialized conidiogenous cell called the phialide. Conidiophores were branched or unbranched, long, slender, hyaline	*Penicillium* sp.
FEB11	Greyish	Powdery	Present	Conidia are minute, globose or elliptical in shape, bluish olive green in color, they are borne on a stalk called a conidiophore that emerges from hyphae, 20.08 – 24. 04 µm in size. The conidiophores are thin, slender, long or short, smooth, 320 – 600 µm in length. Metulae were observed and at the end of each metula, conidium bearing structures called the phialides were also observed	*Penicillium* sp.
FEB12	Olive green	Powdery	Present	Conidia are minute, globose or elliptical in shape, bluish to olive green in color, 15 – 20 µm in size, formed at the tip of conidiophore which are long, thin, slender, smooth walled, septate and hyaline. The metulae and phialides were thin and slender	*Penicillium* sp.
FEB13	Off white	Fluffy	Present	Conidia are ovoid to oval in shape, hyaline, 37.35 µm in size. The hyphae were aseptate and hyaline	Unidentified
FEB14	Light pink	Powdery	Present	Conidia are minute, globose or elliptical in shape, bluish green on color, 10 – 12 µm in diameter, formed in chains in basipetal succession, formed on a specialized conidiogenous cell called the phialide which were observed to be branched. Conidiophores were branched or unbranched, long, slender, hyaline	*Penicillium* sp.
FEB15	Off white	Light	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia

		cottony			
FEB16	Off white	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB17	White	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB18	Light brownish to off white	Light cottony	Absent	No conidia were observed. Conidiophores were brown, long, thin, slender, with tapered tips, septate, 1340.79 µm in length	Unidentified
FEB19	Light brown to white	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB20	Brownish center with white margin	Light cottony	Absent	Hyaline, septate and branched hyphae	*Diaporthe* sp.
FEB21	Dark bluish grey	Velvety	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB22	White	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB23	Bluish green	Powdery	Present	Conidia are single celled, globose to subglobose, hyaline to greenish in color, 10 – 12 µm in size. Conidiophores were not observed	*Aspergillus versicolor*
FEB24	White	Light cottony	Present	Conidia are globose or subglobose, olive to dark brown, 58.61 µm in size, found in clusters under the microscope. Conidiophores were not observed.	*Apiospora* sp.
FEB25	Grey	Cottony	Absent	Light brown, highly septate and branched hyphae	Mycelia sterilia
FEB26	Light yellow	Cottony	Present	Microconidia are single celled, ovoid, straight or slightly curved, hyaline, 47.39 x 23.20 µm in size. Chlamydospores were globose to subglobose, smooth or rough walled, hyaline, 42.40 µm in size	*Fusarium* sp.
FEB27	White	Light cottony	Absent	Hyaline, septate and branched hyphae	*Diaporthephaseolorum*
FEB28	Dark grey	Light	Absent	Conidia were not observed. Conidiophores were brown, septate, thin,	Unidentified

		cottony		long, slender, smooth egdes, tapering towards the edges, 280 – 460 µm in length	
FEB29	Off white	Light cottony	Absent	Hyaline, septate and branched hyphae	*Diaporthe* sp.
FEB30	White	Light cottony	Absent	Hyaline, septate and branched hyphae	*Diaporthe* sp.
FEB31	White	Velvety	Absent	Hyaline, branched hyphae	Mycelia sterilia
FEB32	Whitish black with brown margin	Light cottony	Absent	Hyaline, septate and branched hyphae	*Diaporthe* sp.
FEB33	Grey	Cottony	Present	Conidia are hyaline, ovoid to oblong in shape formed at the tip of the conidiophore which are septate and branched. The size of the conidia is 180.42 x 50.57 µm	Unidentified
FEB34	Initially white to beige color	Cottony	Present	Macroconidia are hyaline, falcate shaped tapering towards the edge, 3-8 septation, 53.35 x 6.14 µm in size. Microconidia not formed. Chlamydospores found to be singly, thick walled and globose	*Fusarium* sp.
FEB35	Off white to light grey	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB36	Blackish off white	Dense cottony	Present	Conidia are single celled, ovoid to oblong in shape, hyaline and 114.64 x 35.16 µm in size	*Colletotrichum* sp.
FEB37	Blackish off white	Fluffy	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB38	Greyish black	Velvety	Absent	Brown, septate and branched hyphae	Mycelia sterilia
FEB39	Off white with orange coloracervuli	Light cottony	Present	Conidia are one celled, ovoid to oblong in shape, hyaline, oil globule at the center, 85.10 x 35.86 µm in size	*Colletotrichum* sp.
FEB40	Brownish white	Velvety	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB41	Off white	Light	Present	Conidia are globose, borne singly on the conidiophore, dark brown to	Unidentified

		cottony		black in color, 115.39 μm in size. Conidiophore are hyaline and branched	
FEB42	Off whitish to light grey	Velvety	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB43	Grey	Light cottony	Present	Conidia are one celled, ovoid to oblong in shape, hyaline, oil globule at the center, 60.45 x 32. 41 μm in size	*Colletotrichum* sp.
FEB44	Green	Light powdery	Present	Conidia were globose to subglobose, found singly or in clusters, light green in color, 18 μm in size. Conidiophore were not observed	*Trichoderma* sp.
FEB45	White	Light cottony	Present	Conidia were single or 2 celled, ovoid, straight or slightly curved, hyaline, 117.42 μm in size	Unidentified
FEB46	Whitish dark green	Cottony	Present	Conidia were globose to oval, bluish green in color, 10 – 15.20 μm in size. Conidiophore were not observed. Chlamydospore were found in abundant, subglobose to globose, hyaline to light green, double walled, thick, around 40 – 42 μm in size.	*Trichoderma asperellum*
FEB47	Whitish dark green	Cottony	Present	Conidia are globose to oval in shape, bluish green in color, 10 – 16.30 μm in size, found singly or in clusters. Conidiophore were not observed. Chlamydospores were observed and they were globose to subglobose, double walled, thick, hyaline to light green in color	*Trichoderma* sp.
FEB48	Yellow	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB49	Black	Cottony	Present	Conidia are globose to subglobose, dark brown in color, rough texture, 16 – 20 μm in diameter. The conidiophore are protrusions from a septate and hyaline hypha. They are smooth and hyaline. The dark globose vesicles are formed at the tip of the conidiophore which is 120 – 300 μm in diameter. The vesicles produce the metulae which supports the phialides on the conidiophore. The phialides produces the conidia	*Aspergillus niger*
FEB50	White	Velvety	Present	Conidia are minute, oval to globose, hyaline, 8 – 10 μm in size. Conidiophores were branched, thin, long and slender	Unidentified

FEB51	Bluish-grey green	Velvety	Present	Conidia are elliptical, smooth and comparatively thick-walled, 12-18 x 10-18 μm in size. Conidiophores are coarse, smooth walled, hyaline and 6-12 mm in length.	*Aspergillus clavatonanicus*
FEB52	Dark grey	Velvety	Absent	Brown, septate and branched hyphae	Mycelia sterilia
FEB53	Off white	Cottony	Present	Minute globose hyaline conidia, 15.21 μm in size	Unidentified
FEB54	Dark grey	Light cottony	Absent	Brown, septate and branched hyphae	Mycelia sterilia
FEB55	Dark grey	Light cottony	Present	Conidia are oval to ovoid shape, hyaline, 40.14 x 21.02 μm. Light brownish, septate and branched hyphae	Unidentified
FEB56	Greyish green	Powdery	Present	Conidia are ellipsoidal, chains of single celled conidia are produced from a specialized conidiogenous cell called a phialide, 8-15 μm in size	*Penicillium* sp.
FEB57	Off white	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB58	Grey to brown	Velvety	Present	Conidia are globose, light brownish, formed in clusters at the tip of the conidiophore, 30.70 μm in size. Conidiophore are erect, branched, septate and brownish in color.	*Botrytis* sp.
FEB59	White with black acervuli at the center	Light cottony	Present	Spores are 5 celled with 4 septation. The spores are fusiform with straight or curved cell edges and has flagellum at both ends. The median cells are yellow to brown and apical and basal cells are hyaline. It is about 127.92 x 28.02 μm in size	*Pestalotiopsis* sp.
FEB60	White	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB61	Greyish white	Dense cottony	Absent	Brown, septate and branched hyphae	Mycelia sterilia
FEB62	White	Cottony	Present	Macroconidia are hyaline, falcate shaped tapering towards the edge, 3-7 septation, 138.94 x 10.14 μm in size. Microconidia not formed.	*Fusarium* sp.
FEB63	Grey	Cottony	Present	Conidia are one celled, ovoid to oblong in shape, hyaline, 90.99 x 32. 41 μm in size	*Colletotrichum* sp.

FEB64	Off white with black coloracervuli at the center	Light cottony	Present	Spores are 5 celled with 4 septation. The spores are fusiform with straight or curved cell edges and has flagellum at both ends. The median cells are yellow to brown and apical and basal cells are hyaline. It is about 127.92 x 28.02 μm in size	*Pestalotiopsis* sp.
FEB65	Greyish white	Dense cottony	Absent	Conidia are single celled, cylindrical, smooth walled, hyaline, 62. 02 x 24.40 μm in size. Conidiophores and setae were not observed	*Colletotrichum fructicola*
FEB66	Greyish off white	Light cottony	Absent	Brown, septate and branched hyphae	Mycelia sterilia
FEB67	Greyish brown	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB68	Light brown with light brownish yellowish conidial masses	Light cottony	Present	Conidia are one celled, ovoid to oblong in shape, hyaline, oil globule at the center, 100.15 x 29.07 μm in size	*Colletotrichum gloeosporioides*
FEB69	Light brown	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB70	Greyish white	Cottony	Present	Conidia are 3-7 septate, falcate shape, hyaline, 25.62 x 7.94 μm	*Colletotrichum* sp.
FEB71	Light grey with greyish black margin	Dense cottony	Present	Conidia are straight or pyriform, brown in color, multiseptate, 167.30 x 67.25 μm in size. Conidiophores were simple or branched and bent at the point where the conidia originated.	*Alternaria* sp.
FEB72	White	Cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB73	Light grey with light brownish margin	Light cottony	Absent	Hyaline, aseptate, branched hyphae with thick globose structures	Mycelia sterilia
FEB74	Light brown	Cottony	Present	Conidia are one celled, ovoid to oblong in shape, hyaline, 86.02 x 28.44 μm in size	*Colletotrichum* sp.

FEB75	Off white	Light cottony	Present	Conidia are globose or subglobose, olive to dark brown, 116.65 µm in size. Conidiophores are light or pale brown, smooth.	*Apiosporalongistroma*
FEB76	Grey with white margin	Light cottony	Absent	Brown, septate, thick and branched hyphae	Mycelia sterilia
FEB77	White	Light cottony	Absent	Brown, septate, thick walled and branched hyphae	Mycelia sterilia
FEB78	Light grey	Light cottony	Absent	Brown, septate, thick walled and branched hyphae	Mycelia sterilia
FEB79	Blackish white	Light cottony	Present	Conidia are globose or subglobose, olive to dark brown, 107.25 µm in size. Conidiophores are light or pale brown, smooth.	*Apiospora* sp.
FEB80	White	Light cottony	Present	Conidia are globose or subglobose, olive to dark brown, 116.65 µm in size. Conidiophores are light or pale brown, smooth.	*Apiosporahydei*
FEB81	Grey color with white margin	Cottony	Present	Conidia are one celled, ovoid to oblong in shape, hyaline, oil globule at the center, 100.15 x 29.07 µm in size	*Colletotrichum kahawae*
FEB82	Dark grey	Cottony	Absent	Brown, septate and branched hyphae	Mycelia sterilia
FEB83	Light grey	Light cottony	Present	Conidia are one celled, ovoid or oblong in shape, slightly curved, hyaline, smooth, 89.32 x 24. 22 µm in size. Conidiophore were not observed under the microscope	*Colletotrichum horii*
FEB84	Off white	Cottony	Present	Conidia are minute globose shape, hyaline, 10 – 12 µm in size. Hyaline, aseptate, branched hyphae were observed	Unidentified
FEB85	Off white with light greyish center	Light cottony	Absent	Hyaline, septate and branched hyphae. No conidia or conidiophores were found	Mycelia sterilia
FEB86	Greyish center with light	Fluffy	Present	Conidia are brown color, 5-8 septations, obclavate, truncate and cicatrized at base, straight or slightly curvy, 456.39 x 109.25 µm in	*Helminthosporium* sp.

	brownish margin			size. Conidiophore are brown, highly septate, protruding slightly towards where it bears the conidia, branched or unbranched, bears conidia at the top or from the sides, 622.41 – 961.35 µm in length	
FEB87	Yellowish green	Velvety	Present	Conidia are minute, globose, 10 – 20 µm in diameter, formed in chains in basipetal succession, formed on a specialized conidiogenous cell called the phialide which were also observed under the microscope. Slight branching of the phialides were also observed. Conidiophores were hyaline.	*Penicillium* sp.
FEB88	White	Light cottony	Present	Conidia are globose or subglobose, olive to dark brown, 113.17 µm in size, found in clusters under the microscope. Conidiophores were not observed.	*Apiospora* sp.
FEB89	Light brown	Cottony	Present	Conidia are one celled, oval to ovoid or oblong in shape, hyaline, 84.33 x 35.94 µm in size. Conidiophores were not observed under the microscope	*Colletotrichum* sp.
FEB90	Off white	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB91	Light brown	Light cottony	Absent	Brown, septate and branched hyphae	Mycelia sterilia
FEB92	Off white with blackish margin	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB93	Light yellowish to Dark pink	Fluffy	Present	Macroconidia are hyaline, falcate shaped tapering towards the edge, formed in clusters from the tip of the conidiophore, 3-7 septation, 326.05 x 38.44 µm in size. Microconidia not formed.	*Fusarium* sp.
FEB94	Whitish black	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB95	Whitish black	Light cottony	Present	Minute globose conidia, hyaline and 10 – 15 µm in size	Unidentified
FEB96	Grey	Cottony	Absent	Brown, septate and branched hyphae	Mycelia sterilia

FEB97	Dark grey	Cottony	Present	Conidia are one celled, ovoid or oblong or cylindrical, slightly curved, hyaline, 102.93 x 25. 93 µm in size. Conidiophore were not observed under the microscope	*Colletotrichum* sp.
FEB98	Light yellowish white	Light cottony	Present	Conidia are brown to dark brown, thick walled, globose or sub globose, sometimes ellipsoidal or slightly curved, 107.79 µm in size	*Apiospora* sp.
FEB99	Light grey	Light cottony	Present	The conidia are obclavate, obpyriform, sometimes ovoid or ellipsoidal, with a cylindrical beak or beakless, pale brown to light brown, 3-5 septations, transverse and longitudinal septations were also observed in some conidia, 205.44 x 91.05 µm in size. Conidiophore are pale brown to olive brown, straight or flexous, septate	*Alternaria* sp.
FEB100	Light grey	Cottony	Present	Conidia are one celled, ovoid or oblong in shape, hyaline, 101.03 x 42.31 µm in size. Setae was dark brown and acicular	*Colletotrichum* sp.
FEB101	Off white	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB102	Brownish or olive grey	Light cottony	Present	Conidia are brown, obclavate or ovoid or obpyriform, 2-5 longitudinal septations, some with 1-2 transverse septations, with or without beak, in acropetal chains, 240.31 x 53.20 µm in size. Conidiophore are brown, septate and branched	*Alternaria* sp.
FEB103	Light yellowish brown	Light cottony	Present	Conidia are brown, obclavate to ovoid in shape, 1-4 longitudinal septations, 1-2 transverse septations, with or with beak, 273.57 x 64.96 µm in size. Conidiophores are brown, septate and branched, 150 – 200 µm in length	*Alternaria* sp.
FEB104	Light grey	Light cottony	Absent	Light brown, septate and branched	Mycelia sterilia
FEB105	Light greyish brown	Light cottony	Present	Conidia are brown, oval or ovoid or obclavate, 1-4 longitudinal septations, 1-2 transverse septations, size variation, 189.74 x 83.30 µm in size. Conidiophore are brown, septate and branched	*Alternaria* sp.
FEB106	Yellow	Light	Present	Conidia are globose to subglobose, light brown to dark brown in color,	*Aspergillus* sp.

		cottony		produced in chains, they were found singly or in clusters, 17 – 20 µm in size. Conidiophores are light brown, thicker at the edge, aseptate, slender, long, and they give rise to the vesicle which are spherical, elliptical or club shaped structure and forms a layer of phialide where the conidia are produced in chains	
FEB107	Brownish or olive grey	Light cottony	Present	Conidia are brown, obclavate or ovoid or obpyriform, 2-5 longitudinal septations, some with 1-2 transverse septations, with or without beak, in acropetal chains, 242.02 x 50.14 µm in size. Conidiophore are brown, septate and branched	*Alternaria* sp.
FEB108	Grey	Cottony	Present	Conidia are light brown, oblong or elongate, straight or slightly curved, slightly tapering towards the edge, 4-7 septations, 270.20 x 96.43 µm in size. Conidiophores are light brown and septate	Unidentified
FEB109	Dark grey	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB110	Beige white	Velvety	Present	Conidia are minute, globose or oval in shape, hyaline, 10 – 12 µm in size	Unidentified
FEB111	Off whitish grey	Light cottony	Absent	Light brown, septate and branched	*Diaporthe* sp.
FEB112	Dark grey	Velvety	Absent	Brown, septate and branched hyphae	Mycelia sterilia
FEB113	Off white	Velvety	Absent	Hyaline, septate and branched hyphae	*Diaporthe* sp.
FEB114	Greyish white	Velvety	Absent	Hyaline, septate and branched	*Diaporthe* sp.
FEB115	Off white	Light cottony	Absent	Hyaline, septate and branched	*Diaporthefructicola*
FEB116	Dark green	Light cottony	Present	Conidia were minute, globose to subglobose, yellow green pigmentation, 10.15 – 12.07 µm in size. Conidiophores were observed to be branched producing lateral side branches, phialides arising directly from the main axis near the tip	*Trichoderma asperellum*
FEB117	Off white to light brown	Light cottony	Absent	Hyaline, aseptate and branched	*Diaporthe* sp.

	margin				
FEB118	Grey center to off whitish margin	Light cottony	Absent	Hyaline, aseptate and branched hyphae	*Diaporthe* sp.
FEB119	White	Velvety	Absent	Hyaline, aseptate and branched hyphae	*Diaporthe* sp.
FEB120	Light brown with light brown conidial mass	Velvety	Present	Conidia are minute, globose or oval in shape, hyaline, 15 – 18 μm in size	Unidentified
FEB121	Dark brown	Velvety or suede	Present	Conidia are light brown to dark brown in color, globose to subglobose and produced in chains, they were found singly or in clusters, 16 – 20 μm in size. Conidiophores are light brown, thicker at the edge, aseptate, slender, long, and they give rise to the vesicle which are spherical, elliptical or club shaped structure and forms a layer of phialide where the conidia are produced in chains	*Aspergillus* sp.
FEB122	Light brown with light brown conidial mass	Velvety	Present	Conidia are minute, globose to oval in shape, olive green in color, 23.92 μm in size, formed in clusters. Conidiophore are hyaline with thich rough edges, aseptate, branched	Unidentified
FEB123	Light yellow	Cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB124	White	Cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB125	Pink	Light cottony	Present	Macroconidia are hyaline, falcate shaped tapering towards the edge, formed in clusters from the tip of the conidiophore, 3-7 septation, 302.05 x 35.46 μm in size. Microconidia not formed. Chlamydopsore were found in abundant, they are thick walled, spherical, hyaline and 74.61 μm in size	*Fusarium* sp.

FEB126	Light greyish white	Light cottony	Present	Conidia are brown in color, globose or oval in shape, forms in chains, thick walled, 90.30 µm (globose), 125.32 x 68.97 µm (oval) in size	Unidentified
FEB127	Olive green with brownish reddish background	Powdery	Present	Conidia are minute, globose or oval, olive green in color, 23.48 µm in diameter, formed in chains in basipetal succession, formed on a specialized conidiogenous cell called the phialide which were also observed under the microscope. Slight branching of the phialides were also observed. Conidiophores were hyaline, thick, septate and long	*Penicillium* sp.
FEB128	Light brown	Light cottony	Absent	Light brown, septate and branched hyphae	Mycelia sterilia
FEB129	White	Light cottony	Absent	Hyaline, septate and branched	*Diaporthe*sp.
FEB130	White with greyish background	Light cottony	Absent	Hyaline, septate and branched hyphae	*Diaporthe* sp.
FEB131	Light grey	Cottony	Present	Conidia are minute, globose or oval in shape, hyaline, 10 – 15 µm in size	Unidentified
FEB132	Bluish green	Velvety	Present	Conidia are minute, globose or oval, olive green in color, 8 - 10 µm in diameter, formed in chains in basipetal succession, found singly or in clusters, formed on a specialized conidiogenous cell called the phialide which were also observed under the microscope. Branching of the phialides were also observed. Conidiophores were hyaline, thin, slender, long and some short, septate and long, branching were also observed with a length of 1101.54 µm. Branching of metulae was also observed	*Penicillium* sp.

FEB133	Dark brown	Fluffy	Present	Dark brown fruiting body which are globose to ovoid in shape covered with hyphalike hair, borne on long, slender, brown structure that are aseptate and smooth	Mycelia sterilia
FEB134	Off white	Cottony	Absent	Hyaline, septate and branched	Mycelia sterilia
FEB135	Off white with light colored conidial pigments in concentric growth	Velvety	Present	Conidia were minute, globose, light bluish green in color, 52.94 µm in size. Large unknown fruiting body were observed which were dark brown to black in color	Unidentified
FEB136	Bluish green with reddish background	Powdery	Present	Conidia are minute, globose or oval, olive green in color, 22.15 µm in diameter, formed in chains in basipetal succession, formed on a specialized conidiogenous cell called the phialide which were also observed under the microscope	*Penicillium* sp.
FEB137	White	Velvety	Absent	Hyaline, septate and branched	*Diaporthe* sp.
FEB138	Olive green	Powdery	Present	Conidia are minute, globose or oval, bluish to olive green in color, 20 - 24 µm in diameter, formed in chains in basipetal succession, formed on a specialized conidiogenous cell called the phialide which were also observed under the microscope Branching of the phialides were also observed. Conidiophores were hyaline, thin, slender, long and some short, septate and long, branching were also observed with a length of 1045.22 µm	*Penicillium* sp.
FEB139	Light pink	Light cottony	Present	Microconidia are single celled, ellipsoidal or cylindrical, straight or curved, hyaline, 46 x 14 µm in size. Macroconidia was not observed. Chlamydospores were globose to subglobose, smooth or rough walled, hyaline, 40.10 µm in size	*Fusarium* sp.
FEB140	Light brownish to white margin	Velvety	Present	Conidia are globose or subglobose, hyaline, smooth walled, 17 – 20 µm in size. Conidiophores were not observed	Unidentified

FEB141	Light yellow with white margin	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB142	Grey	Light cottony	Absent	Light brown, septate and branched hyphae	Mycelia sterilia
FEB143	White with hair like structures	Velvety	Present	Conidia are single celled, oval to ellipsoidal, hyaline, appearing at the tip of conidiogenous cell, 10 x 14 µm in size. Conidiophores are hyaline, arises from the hyphae with abundant long erect clusters of conidiogenous cells	*Beauveria felina*
FEB144	Light Brown to Whitish in color	Light cottony	Absent	Hyaline, branched hyphae	Mycelia sterilia
FEB145	Light yellowish with black postules		Present	Oval brown color conidia, conidiophores are brown and septate, size of the spore are 83.01 x 25.19 µm	Unidentified
FEB146	Greyish color	Dense cottony	Absent	Hyphae branched and septate	Mycelia sterilia
FEB147	Light brown	Light cottony	Absent	Hyaline septate hyphae	Mycelia sterilia
FEB148	Light greyish	Light fluffy	Absent	Light brown, septate and branched hyphae	Mycelia sterilia
FEB149	White	Light cottony	Absent	Hyaline hyphae	Mycelia sterilia
FEB150	White	Light cottony	Absent	Brown and septate hyphae	Mycelia sterilia
FEB151	Off white	Velvety	Present	Minute hyaline circular spores 15-20 µm in size, hyaline and branched hyphae	Unidentified
FEB152	Grey	Fluffy	Absent	Hyaline, branched and septate hyphae	Mycelia sterilia
FEB153	Light brown	Light	Present	Spores are brown colored, oval shape with pointed edge, 72.11 x 51.	Unidentified

		cottony		99 μm in size, conidiophores are brown and septate, hyaline hyphae	
FEB154	White	Light velvety	Absent	Hyaline, branched hyphae	Mycelia sterilia
FEB155	Off white	Light cottony	Present	Ovoid shaped hyaline spores, 43.92 x 15.05 μm in size	Unidentified
FEB156	White	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB157	White	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB158	Off white to light grey	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB159	Off white	Velvety	Absent	Hyaline, septate and branched hyphae	*Diaporthe* sp.
FEB160	Off white to light brown	Velvety	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB161	Off white	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB162	Light brown	Light cottony	Absent	Hyaline hyphae	Mycelia sterilia
FEB163	White with black acervuli	Light cottony	Present	Spores are 5 celled with 4 septation. The spores are fusiform with straight or curved cell edges and has flagellum at both ends. The median cells are yellow to brown and apical and basal cells are hyaline. It is about 127.92 x 28.02 μm in size	*Pestalotiopsis* sp.
FEB164	White	Fluffy	Absent	Hyaline, septate hyphae	Mycelia sterilia
FEB165	White with black acervuli	Light cottony	Present	Spores are 5 celled with 4 septation. The spores are fusiform with straight or curved cell edges and has flagellum at both ends. The median cells are yellow to brown and apical and basal cells are hyaline. It is about 27.59 x 5.37 μm in size	*Pestalotiopsis* sp.
FEB166	Off white with black acervuli	Light cottony	Present	Spores are 5 celled with 4 septation. The spores are fusiform with straight or curved cell edges and has flagellum at both ends. The	*Pestalotiopsis* sp.

				median cells are yellow to brown and apical and basal cells are hyaline. It is about 27.59 x 5.37 µm in size	
FEB167	Off white to light grey	Velvety	Absent	Brown color conidiophore with no septation, hyaline hyphae	Mycelia sterilia
FEB168	Off white, concentric growth	Velvety	Present	Oval, brown colored spores, 51.55 x 32.51 µm in size	Unidentified
FEB169	White	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB170	Off white	Light cottony	Absent	Hyaline hyphae	Mycelia sterilia
FEB171	Off white	Velvety	Absent	Hyaline and branched hyphae	Mycelia sterilia
FEB172	Light brown	Light cottony	Absent	Brown, septate and branched hyphae	Mycelia sterilia
FEB173	Brown	Velvety	Present	Conidia are ellipsoidal, 8-15 µm in size	*Penicillium* sp.
FEB174	Off white	Light cottony	Absent	Hyaline, branched hyphae	Mycelia sterilia
FEB175	White	Cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB176	Light greyish white	Cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB177	Pink	Light cottony	Present	Macroconidia are hyaline, falcate shaped tapering towards the edge, formed in clusters from the tip of the conidiophore, 3-7 septation, 305.02 x 31.60 µm in size. Microconidia not formed. Chlamydospores were globose to subglobose, smooth or rough walled, hyaline, 41.50 µm in size	*Fusarium* sp.
FEB178	Dark grey	Cottony	Absent	Conidia were not observed. Conidiophores were long, straight or slightly flexous, olivaceous to brown	*Cladosporium tenuissimum*
FEB179	Off white	Light cottony	Present	Spindle shaped hyaline spores with 2-3 septations, 231.20 x 46.80 µm in size	Unidentified

FEB180	Light brown	Velvety	Present	Minute circular hyaline spores, 15-20 µm in size	Unidentified
FEB181	Yellow	Velvety	Present	Minute circular spores with 10-15 µm in size	*Penicillium* sp.
FEB182	Off whitish to grey	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB183	Brown	Light fluffy	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB184	Off white to light brown	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB185	Light grey	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB186	Light brown	Fluffy	Present	The sporangiophores are erect, simple or branched, have a larger multispore sporangia which are brown in color. They are globose in shape and called bobbing heads. They are about 160 – 320 µm in size. Sporangia are globose, hyaline, smooth walled and are about 60-80 µm in size	*Mucor circinelloides*
FEB187	Greyish green	Powdery	Present	Conidia are globose to subglobose, hyaline to greenish in color, smooth or sometimes rough, forms in chains on the phialides, 10 – 15 µm in size. Conidiophores are hyaline, smooth walled, septate, branched or unbranched, gives rise to the metulae. At the apex of the metuale, phialides are formed	*Penicillium citrinum*
FEB188	White	Fluffy	Present	Ovoid to cylindrical in shape, hyaline spore and about 48.88 µm in size	Unidentified
FEB189	Dark grey	Velvety	Present	Light brown, oval to ovoid shaped conidia and 42.84 µm in size	Unidentified
FEB190	Off white	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB191	Whitish green	Light cottony	Present	Conidia are globose, hyaline to green in color, formed in clusters, 12 - 20 µm in size	*Trichoderma* sp.
FEB192	Off white	Light cottony	Present	Hyaline ovoid shaped conidia, 20- 25 µm length in size	Unidentified
FEB193	Light brown	Fluffy	Present	Sporangia are globose and are about 60-80 µm in size	*Mucor* sp.

FEB194	White	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB195	White	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB196	Green	Light cottony	Present	Conidia are globose, hyaline to green in color, formed in clusters, 10 x 20 µm in size. Conidiophores are highly branched	*Trichoderma* sp.
FEB197	Off white	Velvety	Present	Rod shaped hyaline spores, 58.29 x 38.36 µm in size	Unidentified
FEB198	Whitish dark green	Light cottony	Present	Conidia are globose, hyaline to green in color, formed in clusters, 10 x 20 µm in size.	*Trichoderma* sp.
FEB199	Whitish to green	Light cottony	Present	Conidia are globose, hyaline to green in color, formed in clusters, 10 x 20 µm in size	*Trichoderma* sp.
FEB200	Dark green	Light cottony	Present	Conidia are globose, hyaline to green in color, formed in clusters, 12 x 20 µm in size	*Trichoderma* sp.
FEB201	Off white	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB202	White	Cottony	Present	Spores are hyaline, ovoid to ellipsoidal in shape, 52.76 x 16.44 µm in size	*Fusarium* sp.
FEB203	Greyish	Powdery	Present	Conidia are single-celled produced in chains from the phialide. Conida are hyaline, globose and are 10-20 µm in size. Conidiophores are hyaline.	*Penicillium* sp.
FEB204	Light brown	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB205	Off white with light brown conidial masses or pigments growing in concentric	Velvety	Present	Conidia are minute, globose to oval in shape, hyaline, 8.20 – 10.02 µm in size. Conidiophore are hyaline, aseptate, branched or unbranched and bears the conidia at the tip or lateral sides	Unidentified

	circles				
FEB206	White	Cottony	Absent	Hyaline, septate and branched	Mycelia sterilia
FEB207	Off white	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB208	Whitish pink	Light cottony	Present	Microconidia are single celled, ellipsoidal or cylindrical, straight or curved, hyaline, 48 x 16 µm in size. Macroconidia was not observed	*Fusarium* sp.
FEB209	Off white	Light cottony	Present	Conidia are single celled, Ovoid, tapering towards the end, straight or slightly curving, hyaline, 44.20 x 26.20 µm in size	Unidentified
FEB210	White	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB211	White	Cottony	Absent	Hyaline, septate and branched	Mycelia sterilia
FEB212	Brown	Cottony	Absent	Hyaline, septate, branched and thick hyphae	Mycelia sterilia
FEB213	Light brown	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB214	White	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB215	Light pinkish to white	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB216	Light brown to white	Light cottony	Absent	Hyaline, septate and branched hyphae	*Diaporthe* sp.
FEB217	Yellowish green	Velvety	Present	Conidia are minute, globose to subglobose, hyaline to bluish green in colour, 10 – 15 µm in size, forms in chains at the tip of the phialides in basipetal succession. The phialides were observed in branches, giving a flask like appearance. Phialides were long and slender. Metulae were observed to be branched. The conidiophores were thin, long, slender, septate, branched, hyaline, 350 – 640 µm in length	*Penicillium* sp.
FEB218	Off white	Light cottony	Absent	Hyaline, septate and branched hyphae	*Diaporthe* sp.
FEB219	White with	Light	Present	Spores are 5 celled with 4 septation. The spores are fusiform with	*Pestalotiopsis* sp.

	black coloracervuli	cottony		straight or curved cell edges and has flagellum at both ends. The median cells are yellow to brown and apical and basal cells are hyaline. It is about 127.92 x 28.02 µm in size	
FEB220	Offwhite	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB221	Light grey	Cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB222	Greyish	Powdery	Present	Conidia are minute, globose or ovoid in shape, 10 – 12 µm in diameter, formed in chains in basipetal succession, formed on a specialized conidiogenous cell called the phialide which were observed to be branched. Conidiophores were branched or unbranched, long, slender, hyaline	*Penicillium* sp.
FEB223	Yellowish green	Velvety	Present	Conidia are minute, globose to subglobose, hyaline to bluish green in colour, 10 – 15 µm in size, forms in chains at the tip of the phialides in basipetal succession. The phialides were observed in branches, giving a flask like appearance. Phialides were long and slender. Metulae were observed to be branched. The conidiophores were thin, long, slender, septate, branched, hyaline, 350 – 640 µm in length	*Penicillium* sp.
FEB224	Light brown	Light cottony	Absent	Hyaline, aseptate and branched	Mycelia sterilia
FEB225	Yellowish green	Velvety	Present	Conidia are minute, globose to subglobose, hyaline to bluish green in colour, 10 – 15 µm in size, forms in chains at the tip of the phialides in basipetal succession. The phialides were observed in branches, giving a flask like appearance. Phialides were long and slender. Metulae were observed to be branched. The conidiophores were thin, long, slender, septate, branched, hyaline, 350 – 640 µm in length	*Penicillium* sp.
FEB226	Greyish	Powdery	Present	Conidia are single-celled produced in chains from the phialide. Conida are hyaline, globose and are 10-20 µm in size.	*Penicillium* sp.
FEB227	White	Light cottony	Absent	Hyaline, septate and branched hyphae	*Diaporthe* sp.

FEB228	White	Cottony	Present	Conidia were single celled, oblong in shape, hyaline, 133.89 x 43.22 µm in size	Unidentified
FEB229	Black	Cottony	Present	Conidia are globose to subglobose, dark brown in color, rough texture, 16 – 20 µm in diameter. The conidiophore are protrusions from a septate and hyaline hypha. They are smooth and hyaline. The dark globose vesicles are formed at the tip of the conidiophore which is 120 – 300 µm in diameter. The vesicles produces the metulae which supports the phialides on the conidiophore. The phialides produces the conidia	*Aspergillus niger*
FEB230	Whitish growth initially to off white with blackish color at the back of the plate	Light cottony	Absent	Hyaline, septate and branched hyphae were observed	*Diaporthe* sp.
FEB231	Off white with black acervuli	Light cottony at the center and matted around the edge	Present	Conidia are 2 celled, hyaline, ovoid with tapering ends, 54.27 x 15.37 µm in size	Unidentified
FEB232	White	Dense cottony	Absent	Hyaline, septate, branched and thick hyphae	Mycelia sterilia
FEB233	Grey with white margin	Dense cottony	Absent	Brown, septate and brown hyphae	Mycelia sterilia
FEB234	White	Cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia

FEB235	White	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB236	Blackish white	Cottony	Absent	Light brown, septate and branched hyphae	Mycelia sterilia
FEB237	White	Light cottony	Absent	Light brown, septate and branched hyphae	Mycelia sterilia
FEB238	Light brownish to grey	Velvety	Absent	Hyaline, septate and branched hyphae	*Diaporthe* sp.
FEB239	Light greyish white	Cottony	Present	Conidia are one celled, ovoid to oblong in shape, hyaline, oil globule at the center, 90.48 x 36.42 µm in size	*Colletotrichum* sp.
FEB240	Olive green	Powdery	Present	Conidia are circular to oval in shape and are formed in chains in stalks called conidiophore. The conidia are around 10 – 15 µm in size	*Penicillium* sp.
FEB241	Off white with light greyish center and margin	Cottony	Absent	Hyaline, septate and branched hyphae	*Diaporthe* sp.
FEB242	Dark grey	Cottony	Present	Conidia are one celled, ovoid or oblong in shape, hyaline, 107.08 x 40.15 µm in size	*Colletotrichum* sp.
FEB243	Off white	Velvety	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB244	Bluish grey	Powdery	Present	Conidia are minute, globose or oval, olive green in color, 8 - 10 µm in diameter, formed in chains in basipetal succession, found singly or in clusters, formed on a specialized conidiogenous cell called the phialide which were also observed under the microscope. Branching of the phialides were also observed. Phialides were thick and short appearance. Conidiophores were hyaline and thick. Metulae were thick and branching was also observed	*Penicillium* sp.
FEB245	Off white with greyish black	Light cottony	Absent	Hyaline, septate and branched hyphae	*Diaporthe* sp.

	underneath				
FEB246	Whitish green	Velvety	Present	Conidia are minute, globose to subglobose, hyaline to bluish green in colour, 10 – 15 µm in size, forms in chains at the tip of the phialides in basipetal succession. The phialides were observed in branches, giving a flask like appearance. Phialides were long and slender. Metulae were observed to be branched. The conidiophores were thin, long, slender, septate, branched, hyaline, 350 – 640 µm in length	*Penicillium* sp.
FEB247	Light grey	Light cottony	Absent	Brown, septate and branched hyphae	Mycelia sterilia
FEB248	Brownish	Velvety	Absent	Brown, septate and branched	Mycelia sterilia
FEB249	White with black background	Light cottony	Absent	Hyaline, septate and branched hyphae	*Diaporthechromolaenae*
FEB250	Whitish green	Velvety	Present	Conidia are minute, globose to subglobose, hyaline to bluish green in colour, 10 – 15 µm in size, forms in chains at the tip of the phialides in basipetal succession. The phialides were observed in branches, giving a flask like appearance. Phialides were long and slender. Metulae were observed to be branched. The conidiophores were thin, long, slender, septate, branched, hyaline, 350 – 640 µm in length	*Penicillium* sp.
FEB251	Light brown	Fluffy	Present	The sporangiophores are erect, simple or branched, have a larger multispore sporangia which are brown in color. They are globose in shape and called bobbing heads. They are about 160 – 320 µm in size. Sporangia are globose, hyaline, smooth walled and are about 60-80 µm in size	*Mucor circinelloides*
FEB252	White with brownish black background	Light cottony	Absent	Hyaline, septate and branched hyphae	*Diaporthe* sp.
FEB253	Brownish	Powdery	Present	Light brown, septate and branched hyphae	Mycelia sterilia

FEB254	White with greyish brown background	Light cottony	Absent	Hyaline, septate and branched hyphae	*Phomopsis* sp.
FEB255	Yellowish green	Velvety	Present	Conidia are minute, globose to subglobose, hyaline to bluish green in colour, 10 – 15 µm in size, forms in chains at the tip of the phialides in basipetal succession. The phialides were observed in branches, giving a flask like appearance. Phialides were long and slender. Metulae were observed to be branched. The conidiophores were thin, long, slender, septate, branched, hyaline, 350 – 640 µm in length	*Penicillium* sp.
FEB256	Whitish yellow	Light cottony	Present	Conidia are oblong slightly tapering towards the ends, 3-4 septate, hyaline, 259.95 x 67.25 µm in size	*Fusarium* sp.
FEB257	Whitish grey	Velvety	Present	Conidia are minute, globose to subglobose, hyaline, found singly or in groups, 14.52 µm in size. Conidiophores were not observed	Unidentified
FEB258	Greyish	Powdery	Present	Conidia are minute, globose or ovoid in shape, 10.14 – 12.07 µm in diameter, they form in chains in basipetal succession. The conidia are formed on a specialized conidiogenous cell called the phialide. Conidiophores were branched or unbranched, long, slender, hyaline	*Penicillium* sp.
FEB259	White with greyish background	Light cottony	Absent	Hyaline, septate and branched hyphae	*Diaporthe* sp.
FEB260	Light yellowish center with white margin	Velvety	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB261	White	Light cottony	Absent	Hyaline, septate and branched hyphae	*Diaporthe* sp.
FEB262	Off white	Velvety	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB263	Greyish white	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia

FEB264	Yellowish green	Velvety	Present	Conidia are minute, globose to subglobose, hyaline to bluish green in colour, 10 – 15 µm in size, forms in chains at the tip of the phialides in basipetal succession. The phialides were observed in branches, giving a flask like appearance. Phialides were long and slender. Metulae were observed to be branched. The conidiophores were thin, long, slender, septate, branched, hyaline, 350 – 640 µm in length	*Penicillium* sp.
FEB265	Off white	Light cottony	Present	Conidia are 3-4 septate, falcate shape tapering towards the edge, hyaline, 202.14 x 37.34 µm in size	*Fusarium* sp.
FEB266	Off white	Velvety	Present	Conidia are globose, thick walled, smooth, light greenish in color, 28.24 µm in size	Unidentified
FEB267	Off white	Light cottony	Present	Conidia are single celled, ovoid shape slightly tapering towards the edge, hyaline, 64.48 x 28.25 µm in size	Unidentified
FEB268	Black	Cottony	Present	Conidia are single celled, ovoid to oblong in shape, light to dark brown in color, double walled, thick, singly or in clusters or chains, 114.29 x 71.31 µm in size. Conidiophore are brown in color and branched	Unidentified
FEB269	Greyish white	Light cottony	Present	Conidia are single celled, straight, cylindrical, hyaline, 32 – 96 x 12 – 18 µm in size. Conidiophore and setae were not observed	*Colletotrichum gloeosporioides*
FEB270	Off white with greyish black background	Light cottony	Absent	Hyaline, septate and branched hyphae	*Diaporthe* sp.
FEB271	Whitish grey	Cottony	Present	Conidia are oblong, light brown, 2 septate, 220.11 x 57.59 µm in size. Conidiophores are light brown and septate	Unidentified
FEB272	Greyish white	Light cottony	Absent	Hyaline, septate and branched hyphae	*Diaporthe* sp.
FEB273	Pink	Cottony	Present	Microconidia are single celled, ellipsoidal, slightly curved, hyaline, 44 x 14 µm in size. Macroconidia are septate with atleast 3-5 septation, sickle shape, curved, tapering towards the edge, hyaline, 170 x 20 µm in size	*Fusarium oxysporum*
FEB274	Yellow	Light	Present	Macroconidia are septate with 3-5 septations, sickle shape, slightly	*Fusarium* sp.

		cottony		curved, hyaline, observed in abundance, thick and smooth, 284.40 x 45.80 µm in size. Chlamydospores were globose to subglobose, smooth or rough walled, hyaline, 40.80 µm in size	
FEB275	Whitish yellow	Light cottony	Present	Macroconidia are septate with 3-5 septations, sickle shape, slightly curved, hyaline, observed in abundance, thick and smooth, 292.91 x 48.18 µm in size	*Fusarium* sp.
FEB276	White, greyish black underneath	Cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB277	Off white	Light cottony	Absent	Hyaline, septate and branched hyphae	*Diaporthe* sp.
FEB278	Off white	Light cottony	Absent	Hyaline, septate and branched hyphae	*Diaporthe* sp.
FEB279	Bluish	Powdery	Present	Conidia are minute, globose or oval, olive green in color, 8 - 10 µm in diameter, formed in chains in basipetal succession, found singly or in clusters, formed on a specialized conidiogenous cell called the phialide which were also observed under the microscope. Branching of the phialides were also observed. Phialides were thick and short appearance. Conidiophores were hyaline and thick. Metulae were thick and branching was also observed	*Penicillium* sp.
FEB280	Light grey	Light cottony	Absent	Hyaline, septate and branched hyphae	Mycelia sterilia
FEB281	White	Velvety	Present	Conidia are single celled, oval to subglobose in shape, light greenish in color, 10 – 15 µm in size	Unidentified

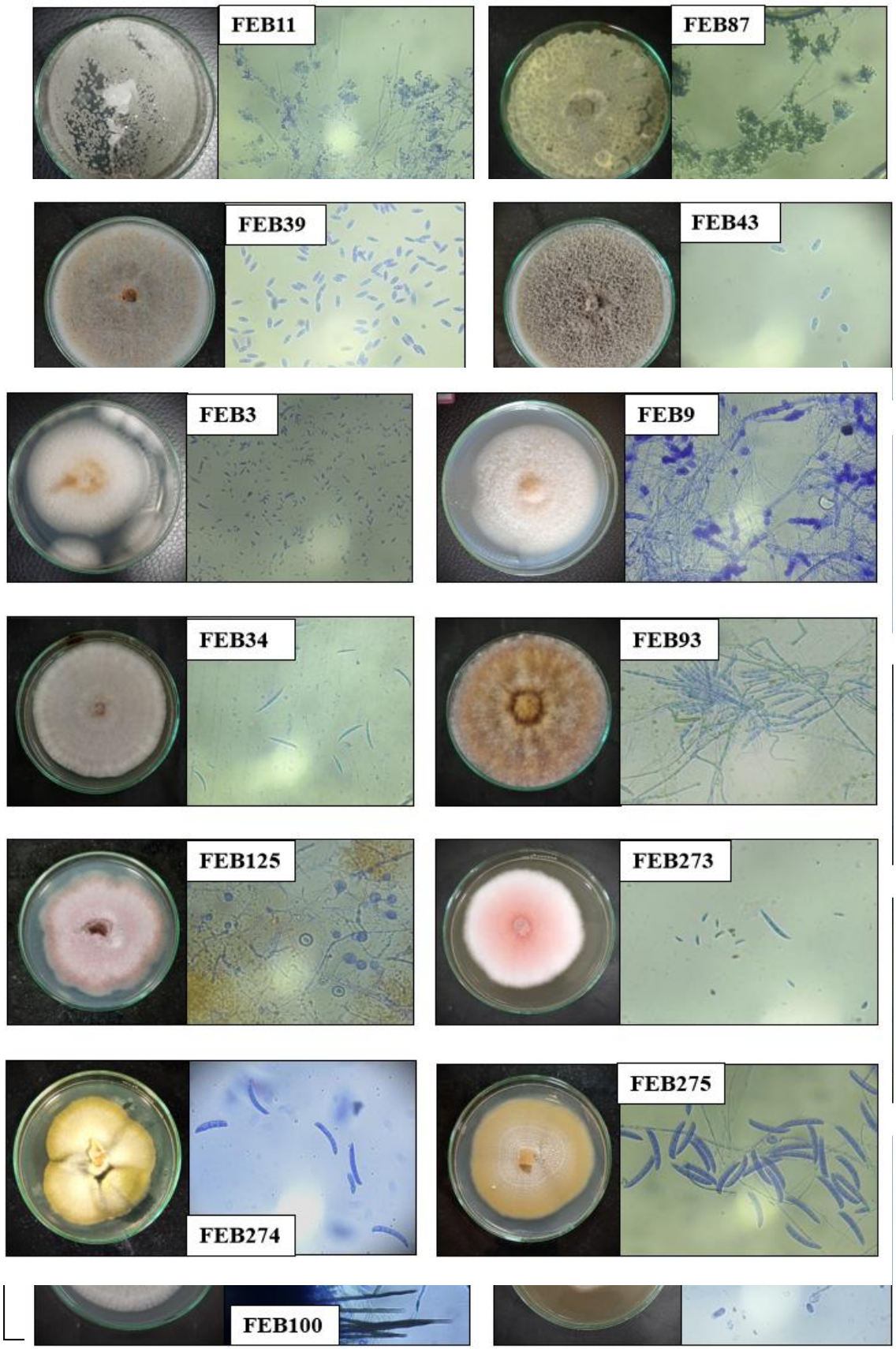


Plate 3b. *Colletotrichum* sp. in plates and as observed under microscope

Plate 3d. *Helminthosporium* sp.

Plate 3e. *Cladosporium tenuissimum*

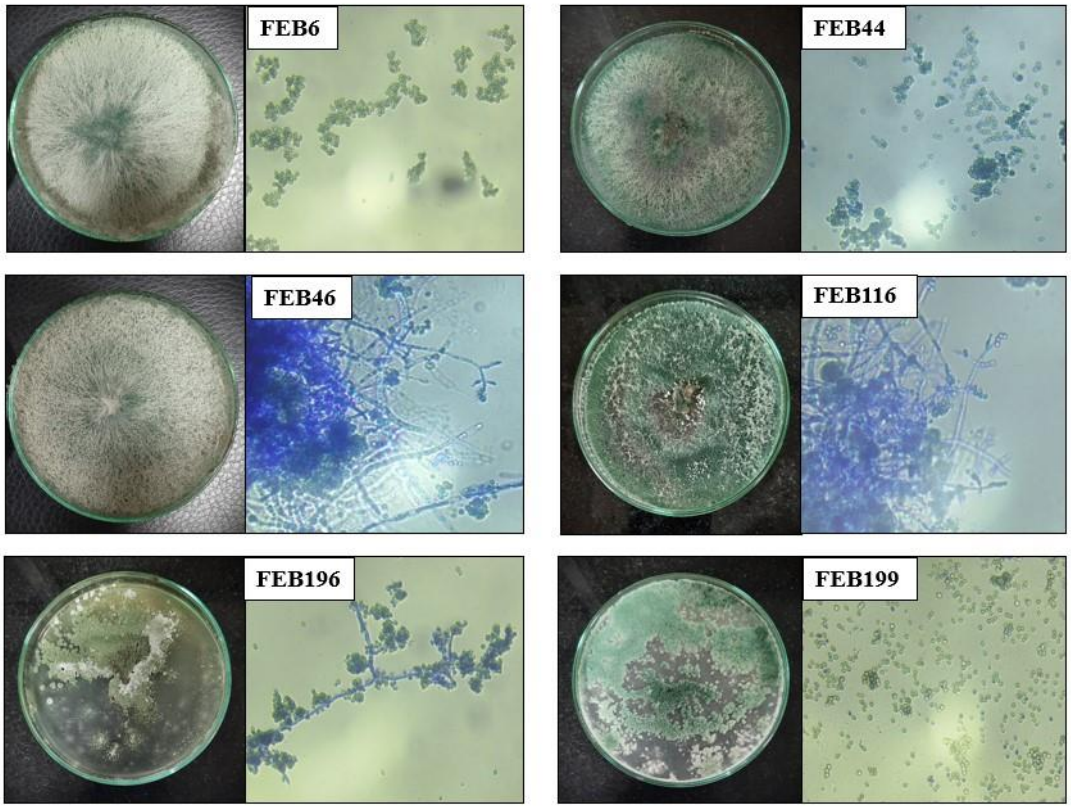


Plate 3f. *Trichoderma* sp. in plates and as observed under microscope (40x)

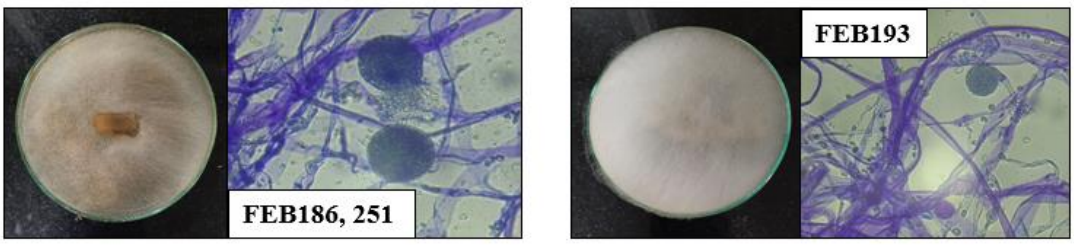
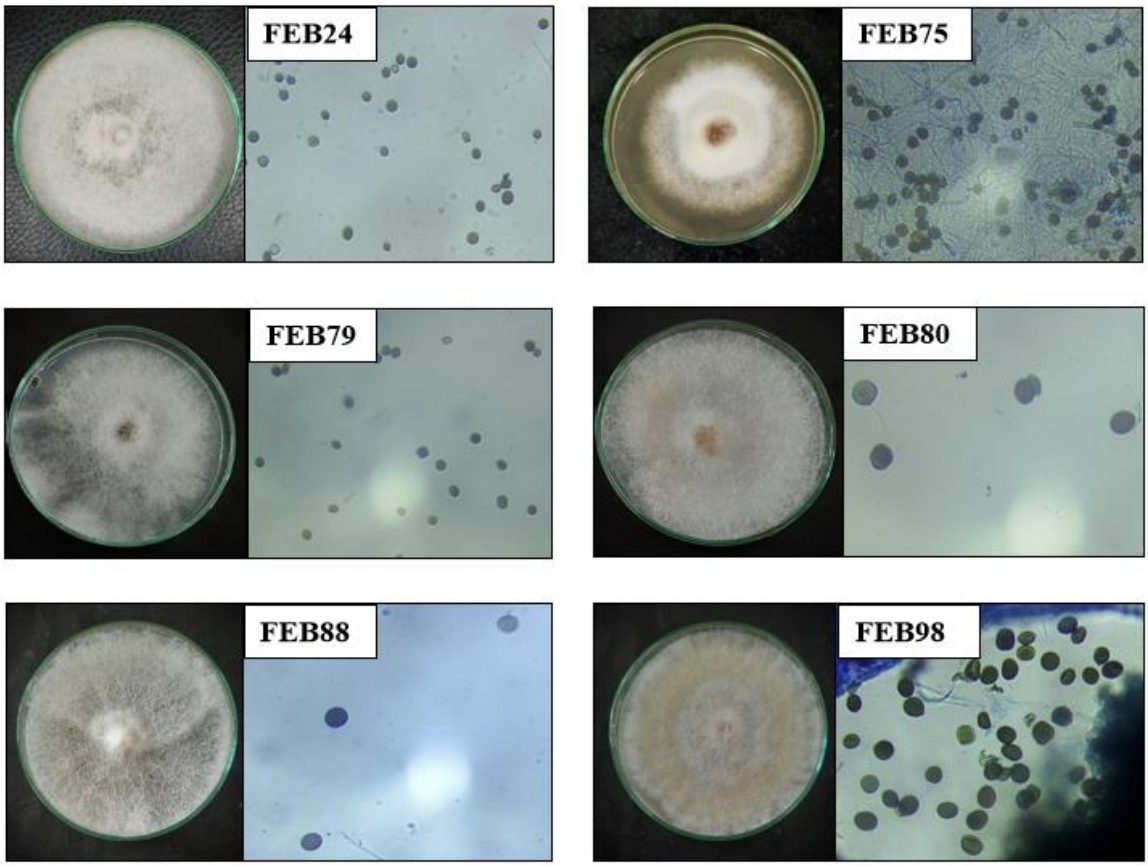


Plate 3g. *Mucor* sp. in plates and as observed under microscope (40x)



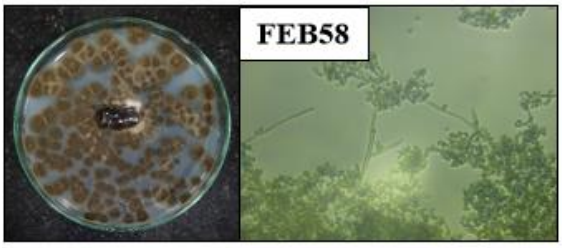


Plate 3j. *Pestalotiopsis* sp. in plates and as observed under microscope

Plate 3k. *Botrytis* sp. in plates and as observed under microscope (40x)

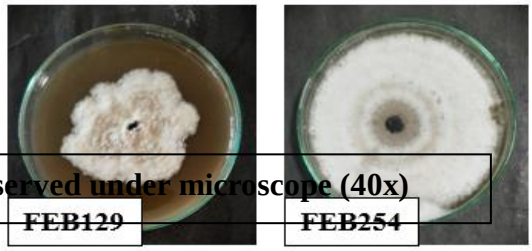
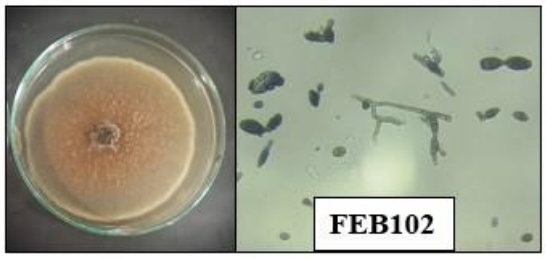


Plate 3i. *Alternaria* sp. in plates and as observed under microscope (40x)

Plate 3l. *Beauveria felina*

Plate 3m. *Phomopsis sp.*



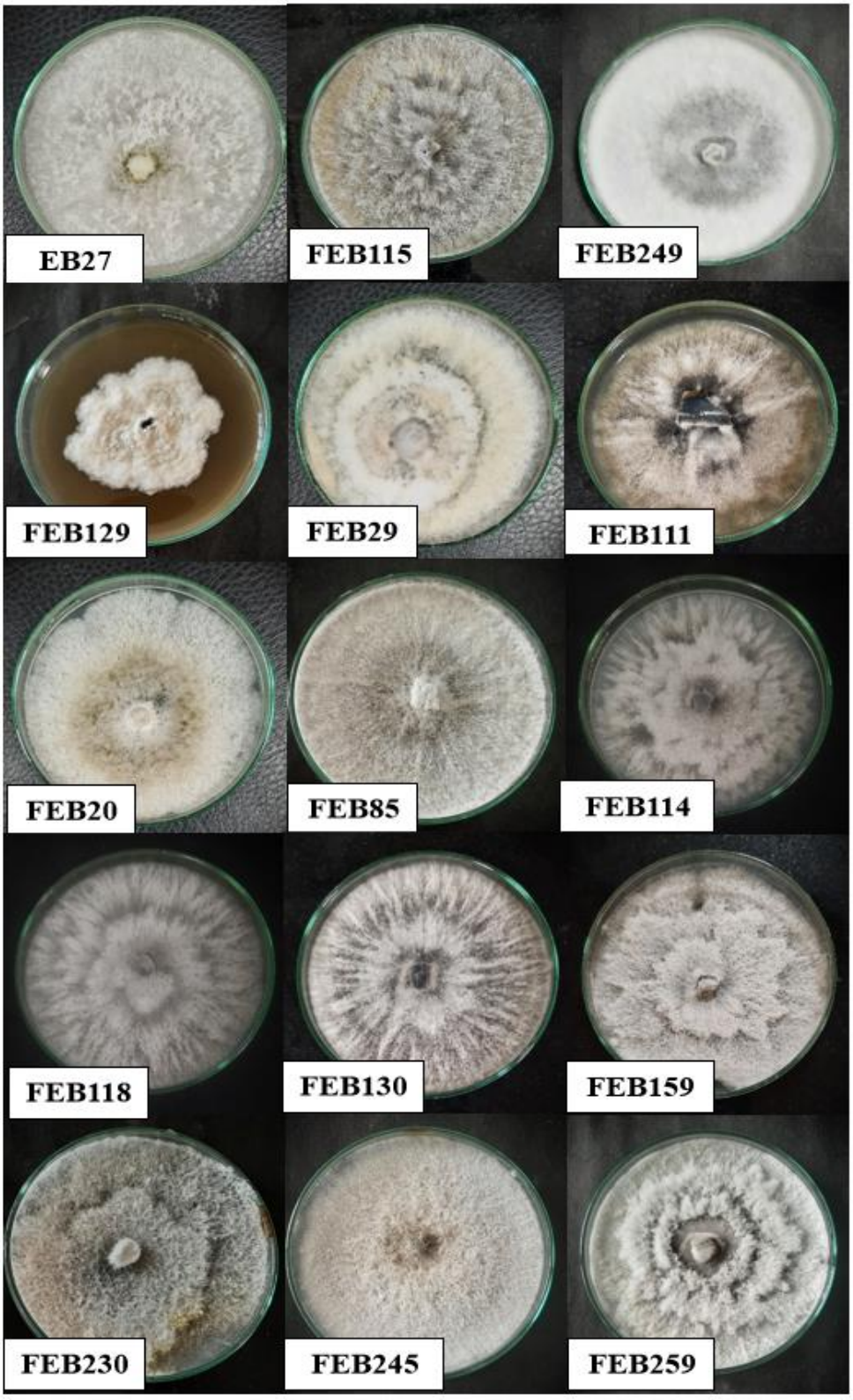


Plate 3o. *Diaporthe* sp.

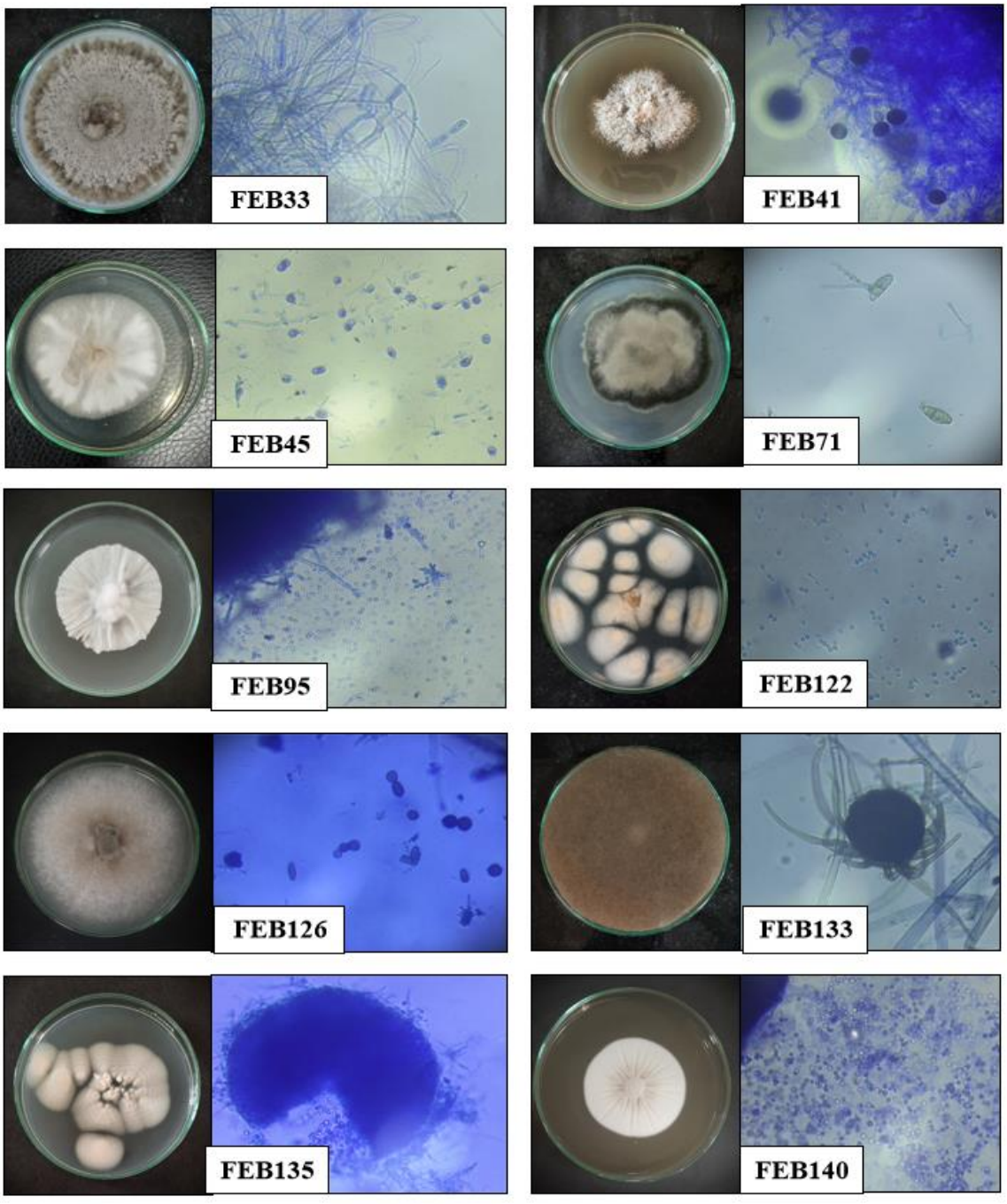


Plate 3p. Unidentified Fungal Endophyte Isolates

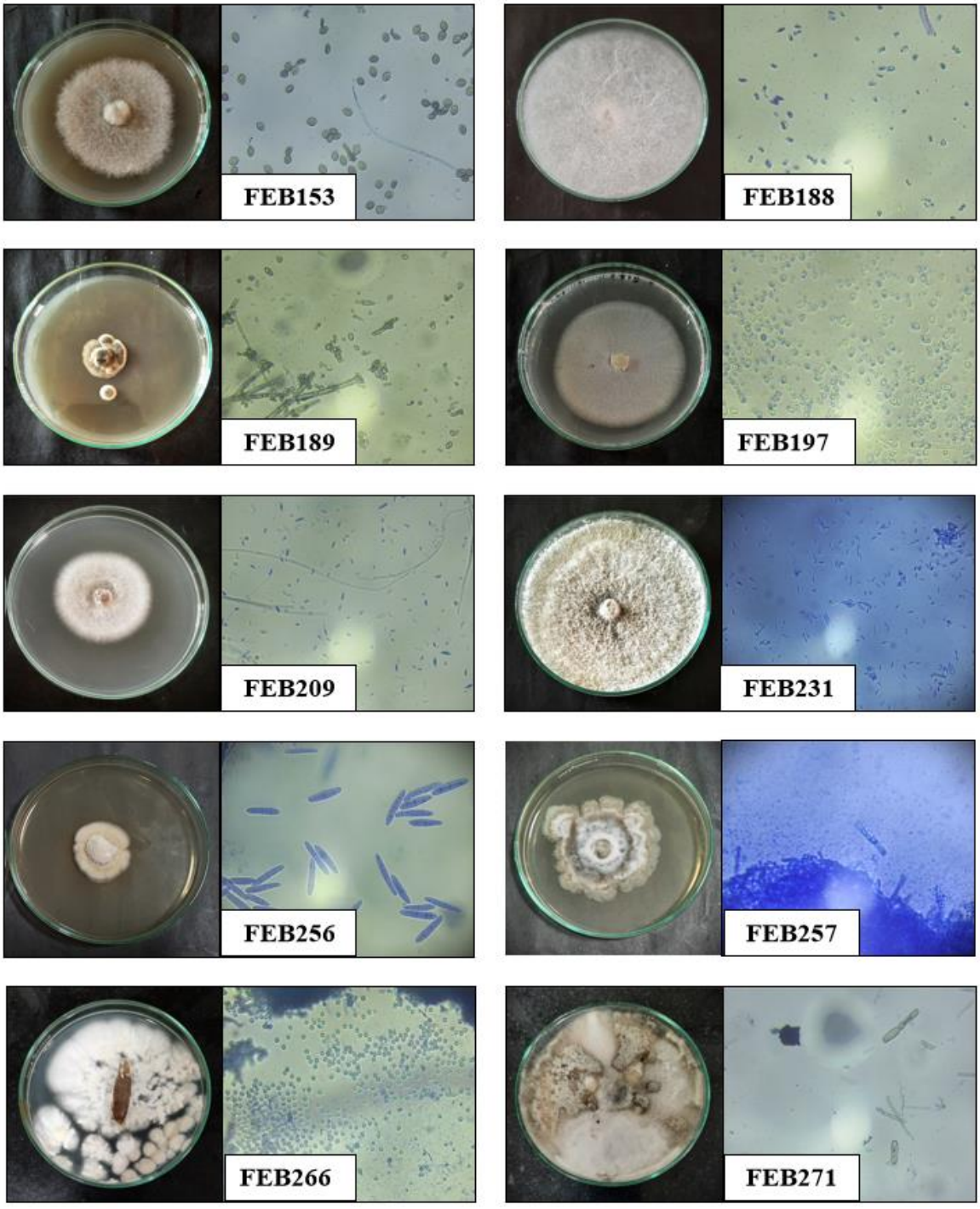


Plate 3q. Unidentified Fungal Endophyte Isolates

Plate 3. Fungal endophytes isolate in plates and as observed under the microscope (40x) (a to q)

Fungal endophytes are found in all the phyla of the kingdom Fungi. They mostly belong to the phylum Ascomycota, and are found to be often related to fungi that cause diseases, either in healthy tissue or as secondary invaders of tissues that are damaged (Schardl*et al*., 1997). Photita*et al*. (2001) isolated sixty-one fungal taxa from 7500 samples of *Musa acuminata* (wild) from five locations. They reported that *Colletotrichum gloeosporioides, Colletotrichum musae* and sterile mycelia were found to be common fungal endophytes isolated from wild *Musa acuminata* in all the collection sites in Thailand. However, the fungal endophytes were found to differ in all the five locations. They also reported species such as *Cladosporium* sp., *Fusarium* sp., *Helminthosporium* sp., *Pestalotiopsis* sp. and *Phomopsis* sp. and all of these belong to the phylum Ascomycota. Cao *et al*. (2002) isolated 163 endophytic fungi from 200 leaf samples of banana (*Musa acuminata*), some of which belonged to *Gloeosporiummusae*(45%),*Myxosporium* sp. (11%), *Deightoniellatorulosa* (8.5%), *Alternaria tenuis* (7.9%), *Uncinula* sp. (1.8%), *Penicillium* sp. (1.8%), *Aspergillus* sp. (1.2%), *Cladosporium* sp. (0.6%) and sterile mycelium (6.7%). Sixty-eight 68 fungal endophytes were isolated and identified from roots (100 samples) and some of which are *Aspergillus* sp. (31%), *Paecilomyces* sp. (16%), *Penicillium* sp. (15%), *Fusarium* sp. (10%) and sterile mycelium (10%).

Xia *et al*. (2011) explored the dispersal of diverse species of endophytic and epiphytic *Trichoderma* corresponding with the banana roots. One hundred and eighty-nine endophytic and epiphytic *Trichoderma* were isolated. Largest group comprised of *T. asperellum, T. virens* and *Hypocrealixii*, isolated from both the outside and inside of banana roots, followed by *T. atroviride* and *T. koningiopsis*, found only on the surface, lastly, *T. brevicompactum* was isolated from the inside of the roots. Zakaria and Rahman (2011) reported the isolation of endophytic *Fusarium* species from the roots of *Musa acuminata* (wild banana) that were randomly collected from different locations in Penang

Island, Malaysia. Fifty-four *Fusarium* species were isolated from 100 fragments of roots and the most commonly found species were *F. oxysporum* (41.5%), *F. solani* (32.1%) and *F. semitectum* (24.5%). *F. oxysporum* and *F. solani*from the tissues of the healthy roots of wild banana.

Potshangbam*et al*. (2017) carried out isolation of fungal endophytes from healthy rice and maize plants and stated from the 123 isolates, 99% belonged to the phylum Ascomycota and 1% belonged to Zygomycota. The highly repeated fungal endophyte related with both the crops were from the genus *Penicillium, Sarocladium, Fusarium* and *Aspergillus* and their development was not specific to tissues. Zakaria and Aziz (2018) isolated fungal endophytes from banana leaves and identified 17 species belonging to 10 genera, some of which are *Colletotrichum gloeosporioides, Colletotrichum siamense, Fusarium equiseti, Fusarium chlamydosporum, Pestalotiopsis oxyanthi, Pestalotiopsis theae, Pestalotiopsis eugeniae, Penicillium steckii, Penicillium purpurogenum* and *Aspergillus niger*.

Henao *et al*. (2019) studied fungal endophytes from the healthy roots, pseudostems, corms and leaves of banana plant cv. Manzano affected with Fusarium wilt and 143 isolates with 11 genera were isolated and some of which are *Fusarium* sp., *Colletotrichum* sp., *Phomopsis* sp., *Cladosporium* sp., *Trichoderma* sp., *Aspergillus* sp., *Penicillium* sp., mycelia sterilia etc. Out of these 143 fungal endophytes isolated, 45.46% were from leaf sample, 20.28% from root sample, 18.18% from pseudostem and 16.08% from corm. Mohanty and Gupta (2021) analysed fungal endophytes isolated from various banana varieties that are grown in Odisha. They isolated 36 fungi from different cultivated varieties and some of which are *Alternaria alternata, Aspergillus niger, Cladosporium cladosporioides, Colletotrichum gloeosporioides, Penicillium citrinum* and *Fusarium* sp.

Malubag*et al*. (2021) isolated and identified fungal endophytes from *Musa paradisiaca* (plantain banana) and 9 fungal endophytes were identified

belonging to genus *Cladosporium, Fusarium, Geotrichum, Nigrospora* and *Schizophyllum*. Panda *et al*. (2023) also reported on the fungal endophytic diversity in banana plants of Assam, India. They isolated 139 fungal endophytes belonging to forty fungal taxa and 14 genera from 15 varieties of banana and 10 different sites. Some of which are *Arthrinium, Aspergillus, Cladosporium, Fusarium, Mucor, Penicillium, Verticillium*, *Paecilomyces* mycelium sterile etc. All sites were found to differ in the diversity of fungal endophytes. The frequency of isolation was maximum for *Cladosporium cladosporioidies* (80%), *Paecilomyces* sp. (80%) followed by *Penicillium ruburm, Aspergillus* sp. 8 & 9 (70%). Thus, the observations recorded in the current investigation is supported by the works carried out by previous researchers, with regard to several endophytes.

4.3 Molecular Characterization and Identification of the Fungal Endophyte Isolates

In the proposed research plan (synopsis), it was envisaged to carry out molecular identification of three best performing fungal endophytes of banana. Accordingly, the performance of the fungal endophytes was studied in various experiments conducted under second and third objective of the research plan and the best performing isolates were selected. Hence, the results pertaining to molecular identification of the best performing fungal endophytes are given out in section 4.9

4.4 Plant Growth Promotion Activity Test

4.4.1 Indole acetic acid (IAA) production

Fungal endophytes form a symbiotic relationship with their host plant which is known and they also help in improving the plant growth and reduces the consequences of biotic and abiotic factors. An increased focus is being given to fungal endophytes now so that efforts can be made to find growth promoting fungal isolates that might be used to multiply the yield of crops and also the standard(Turbat*et al*., 2020).

The IAA production by the fungal endophytic isolates was quantitatively estimated according to Gordon and Weber (1951). The production of IAA was executed for all the 281 isolated fungal endophytes and the absorbance measured in a spectrophotometer. Standard solution of IAA (10% to 70%) was made and a normal probability plot was prepared. The concentration of IAA produced by all the isolates were estimated on the basis of regression equation (Table 4.3, Fig. 4.1 and Plate 4 and 5). All the isolates were found to produce IAA with a concentration range of 9.38 to 114.12 µg/ml with FEB75 (*Apiosporalongistroma*), FEB83 (*Colletotrichum horii*), FEB178 (*Cladosporium tenuissimum*), FEB192 (Unidentified), FEB194 (Unidentified) and FEB222 (*Penicillium* sp.) recording the maximum concentration of 114.12 µg/ml (Table 4.4 and Plate 4). The colour of the final concentration varied depending upon the quantity of IAA that was produced by each isolate and it ranged between light reddish or light pinkish to dark reddish to pinkish in colour. Out of these six best performing isolates, three were isolated from the roots of wild banana plant (2 from Peren and 1 from Mokokchung district), two from the leaves of wild banana plant (Kohima district) and one from the leaves of cultivated banana plant (Peren district).

Table 4.3. Simple regression between concentration and absorbance of the endophytic isolates for Indole Acetic Acid production

Variables	Regression equation	b	SE (b)	t value	Pr> /t	R^2
Intercept	y = 0.0274x - 0.1269	-0.12686	0.043969	-2.88516	0.034381	0.9936
X Variable	-	0.0274	0.000983	27.8689	< 0.01 (1.11E-06)	0.9936

The IAA produced by each isolate could be different and it may be affected by its ability to synthesize tryptophan as a precursor. It can also be influenced by the speed of growth of the isolates (Gusmaity*et al.*, 2019).

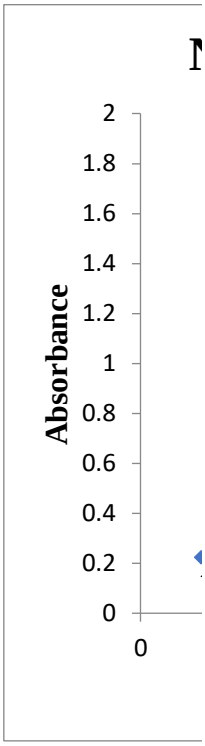


Fig. 4.1. Normal probability plot for IAA

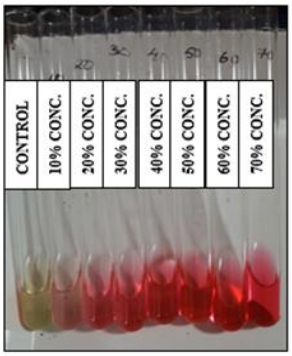
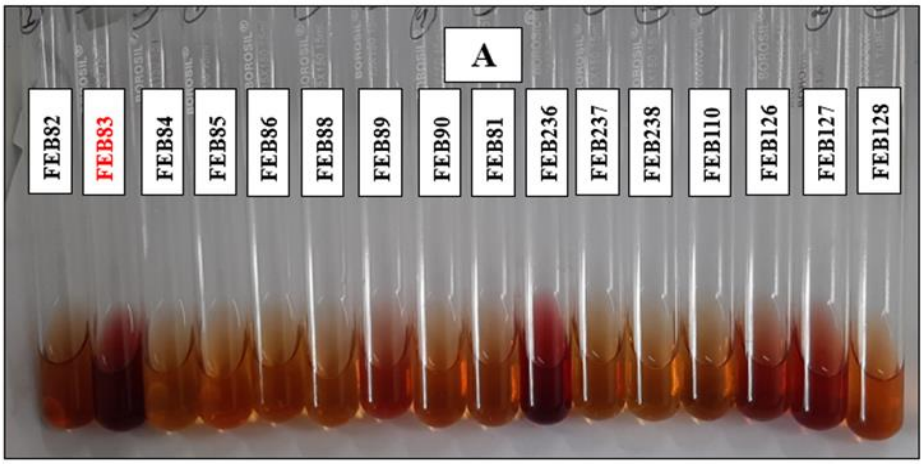
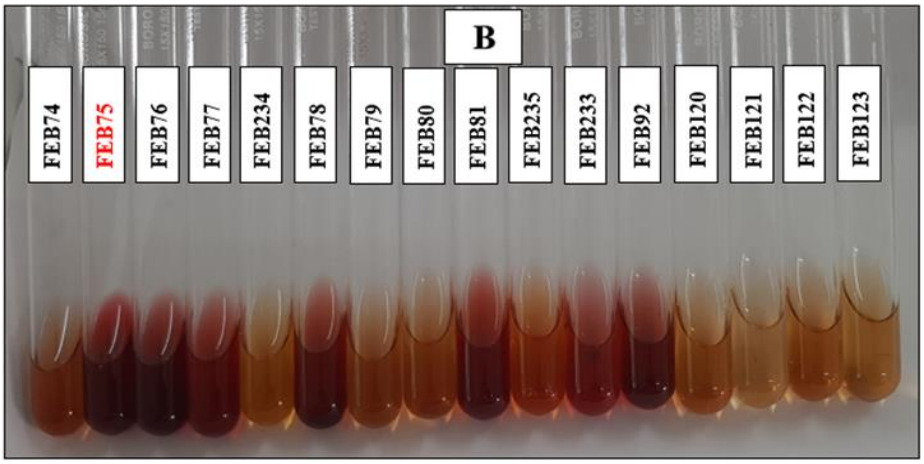
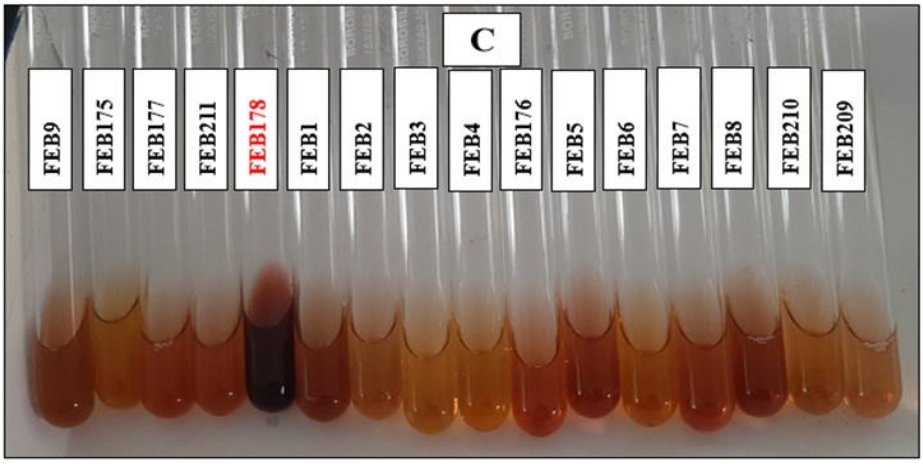


Plate 4. Standard concentration of IAA







Contd.

Junaidi and Bolhassan (2017) described the isolation of 10 fungal endophytes, all identified as *Fusarium oxysporum* from *Phyllathus niruri* Linn. Of these, only two endophytic fungal isolates (FO9 and FO10) recorded the production of high amount of IAA with a concentration of 23.52 µg/ml and 5.95 µg/ml, respectively.

Mehmood *et al*. (2018) studied the role of IAA from endophytic fungi that were isolated from the leaves of drought stressed *Withaniasomnifera* and found that the endophyte *Aspergillus awamori* Wl1 was capable of colonizing the maize roots and enhancing the growth of the host plant.

Gusmiaty*et al*. (2019) detailed on the analysis of IAA production from the rhizospheric fungi isolated from Suren community forest and they found that *Fusarium* had the highest IAA concentration out of the 5 genera that was isolated.

Turbat*et al*. (2020) reported on the plant growth promotion role of endophytic fungi,where the isolation was done from various parts of a medicinal plant (*Sophora flavescens*) important in Mongolia and China. Fifteen isolates associated with the genera *Alternaria, Didymella, Fusarium* and *Xylogone* were isolated and it was observed that all the isolates could produce IAA.

Khalil *et al*. (2021) isolated 15 fungal endophytes, belonging to 3 genera, *Penicillium, Alternaria* and *Aspergillus* that were obtained from leaves of *Ephedra pachyclada* to explore their plant growth promotion activity. *Penicillium commune* was observed to produce maximum IAA.

Savani *et al*. (2021) reported on the endophyte potential in the growth promotion activity against the Panama wilt disease of banana and only 3 endophytes could produce IAA with the highest concentration and out of these, only 1 fungal endophyte gave the highest production, which was *Trichoderma reesei* UH EF.

Fungal endophytes are known to grow inside the plant without causing any host plant damage (Rodriguez *et al*., 2009). They are microorganisms that does not produce symptoms, live inside the host plant, enhances the growth of the host plant, helps in uptake of the nutrients, reduces the severity of the disease and improves the tolerance of host to stresses (Schulz and Boyle, 2005; Rodriguez *et al*., 2012). Apart from these, endophytes are also established as a source of producing secondary metabolites. Among the metabolites, IAA production by endophytes plays a key part in growth of plants (Rodriguez *et al*., 2012). IAA is the most common plant hormone, that occurs naturally in plants and belongs to the class auxin (Jain*et al*., 2016). L-tryptophan is regarded to be the key predecessor for IAA formation in plants (Monteiro *et al*., 1988). IAA produced by endophytes assist in various process of development in plants, like development of roots, formation of axillary buds and flowers. IAA is crucial for growth of plants and their development (Reinhardt *et al*., 2000). Microbes IAA role in plant microbe relation has acquired a growth in recognition in the present day. Furthermore, several investigations have exhibited that IAA can function in microorganisms as a signalling molecule since it affects gene expression in these microorganisms (Yuan *et al*., 2008). The IAA production by endophytic fungal species can conquer pathogens and progression of disease by augmenting the immune response of plants ((Ludwig-Muller, 2015). IAA produced by fungal endophytes in various plant-fungi interplay can help in changing the basic defence mechanisms in plants (Fu *et al*., 2015).

4.4.2 Gibberellic acid (GA3) production

The GA3 production assessment was done for all the isolated 281 endophytic fungi and the absorbance was measured in a spectrophotometer. Standard solutions of GA3 (10% to 70%) were made and a normal probability plot was prepared. The concentration of GA3 produced by all the isolates was calculated using the regression equation (Table 4.5, Fig. 4.2 and Plate 6 and 7).

All the isolates were found to produce GA3 with a concentration range of 7.95 to 113.36 µg/ml with FEB186 (*Mucor circinelloides*) recorded as the best performing isolate producing 113.36 µg/mlfollowed by FEB251(*Mucor*

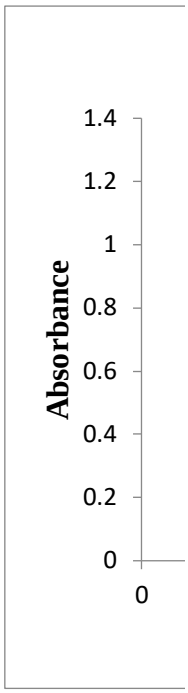


Fig. 4.2. Normal probability plot for GA3

Table 4.4. IAA and GA3 Production by the Fungal Endophytes of banana (µg/ml)

Fungal endophytes of banana	IAA (µg/ml)	GA3 (µg/ml)
FEB1	14.63	15.40
FEB2	16.16	12.85
FEB3	20.58	14.33
FEB4	24.34	12.19
FEB5	16.57	20.66
FEB6	23.46	14.18
FEB7	17.30	16.78
FEB8	17.22	16.12
FEB9	14.01	13.31
FEB10	27.04	12.24
FEB11	16.02	13.16
FEB12	14.92	12.49
FEB13	9.81	14.08
FEB14	11.13	14.79
FEB15	22.81	14.69
FEB16	97.59	28.82
FEB17	16.31	33.57
FEB18	22.08	14.84
FEB19	14.78	12.29
FEB20	104.38	17.34
FEB21	32.22	10.71
FEB22	48.46	10.86
FEB23	18.03	11.78
FEB24	16.97	14.48
FEB25	35.47	12.80
FEB26	13.43	12.90
FEB27	86.27	44.94
FEB28	93.17	8.52
FEB29	10.03	18.72
FEB30	77.62	48.36
FEB31	11.64	16.22
FEB32	17.26	13.11
FEB33	17.88	11.42
FEB34	25.58	12.14
FEB35	18.5	8.67
FEB36	24.19	9.43
FEB37	35.95	9.69
FEB38	15.69	9.28
FEB39	25.76	8.87
FEB40	17.88	8.92
FEB41	18.57	7.95
FEB42	17.66	8.52
FEB43	15.58	9.03
FEB44	9.96	13.97

FEB45	10.98	29.89
FEB46	15.00	18.01
FEB47	14.52	16.17
FEB48	19.59	13.16
FEB49	9.81	13.77
FEB50	11.75	12.95
FEB51	38.76	10.4
FEB52	15.07	11.58
FEB53	20.95	9.59
FEB54	14.85	9.33
FEB55	14.34	12.14
FEB56	18.86	11.93
FEB57	13.03	10.15
FEB58	15.76	9.43
FEB59	15.65	15.86
FEB60	17.66	85.25
FEB61	18.35	11.37
FEB62	26.57	15.25
FEB63	75.14	11.98
FEB64	17.22	14.28
FEB65	87.62	12.29
FEB66	21.02	14.59
FEB67	87.26	13.97
FEB68	33.76	12.19
FEB69	25.18	11.42
FEB70	88.86	15.71
FEB71	27.62	13.87
FEB72	38.21	13.87
FEB73	27.51	13.82
FEB74	23.28	12.75
FEB75 (*Apiosporalongistroma*)	**114.12**	11.47
FEB76	105.22	14.43
FEB77	84.52	18.26
FEB78	100.11	12.70
FEB79	19.08	13.01
FEB80	19.67	13.57
FEB81	97.92	14.48
FEB82	28.79	19.28
FEB83(*Colletotrichum horii*)	**114.12**	89.79
FEB84	18.54	17.60
FEB85	22.30	96.07
FEB86	22.22	34.79
FEB87	16.31	16.83
FEB88	19.08	22.55
FEB89	32.55	16.02
FEB90	20.36	16.22
FEB91	20.22	27.55
FEB92	56.75	12.60

FEB93	19.38	17.90
FEB94	18.50	29.64
FEB95	16.86	17.55
FEB96	17.04	23.87
FEB97	25.51	20.40
FEB98	16.38	21.83
FEB99	30.54	18.01
FEB100	20.62	14.99
FEB101	16.53	18.31
FEB102	21.20	11.37
FEB103	31.16	11.17
FEB104	23.24	11.12
FEB105	18.17	11.27
FEB106	17.11	11.47
FEB107	19.38	11.17
FEB108	16.13	11.37
FEB109	78.35	11.27
FEB110	19.49	16.73
FEB111	14.96	13.21
FEB112	20.22	14.13
FEB113	16.20	16.17
FEB114	104.08	26.32
FEB115	109.74	16.32
FEB116	42.15	14.28
FEB117	17.99	14.74
FEB118	14.49	13.57
FEB119	91.24	16.73
FEB120	18.21	12.19
FEB121	12.48	18.92
FEB122	17.59	13.97
FEB123	16.49	14.79
FEB124	99.70	13.87
FEB125	21.13	15.56
FEB126	40.18	16.32
FEB127	64.34	30.35
FEB128	29.12	12.49
FEB129	22.95	14.69
FEB130	64.05	15.56
FEB131	85.32	35.51
FEB132	55.00	20.86
FEB133	14.49	14.13
FEB134	15.18	13.46
FEB135	26.38	13.62
FEB136	62.62	14.38
FEB137	27.48	12.70
FEB138	35.29	13.01
FEB139	20.32	11.78
FEB140	22.04	11.88

FEB141	17.48	10.51
FEB142	15.80	10.20
FEB143	44.23	12.19
FEB144	17.48	13.06
FEB145	12.92	10.15
FEB146	38.17	14.23
FEB147	18.43	12.44
FEB148	19.05	12.70
FEB149	22.70	8.92
FEB150	20.58	16.93
FEB151	19.01	8.72
FEB152	12.30	13.16
FEB153	13.68	13.06
FEB154	12.55	11.47
FEB155	26.35	9.08
FEB156	46.35	18.46
FEB157	74.16	9.38
FEB158	17.59	10.51
FEB159	15.65	23.16
FEB160	97.33	12.24
FEB161	17.99	12.60
FEB162	13.39	13.01
FEB163	23.46	15.76
FEB164	13.03	14.59
FEB165	15.51	12.19
FEB166	16.57	13.16
FEB167	28.86	12.29
FEB168	17.84	17.04
FEB169	13.76	8.72
FEB170	20.80	10.71
FEB171	83.17	16.78
FEB172	17.04	12.55
FEB173	51.53	11.17
FEB174	9.38	12.24
FEB175	19.67	13.06
FEB176	34.01	11.88
FEB177	19.41	13.31
FEB178(*Cladosporium tenuissimum*)	**114.12**	13.97
FEB179	63.54	8.92
FEB180	26.82	9.38
FEB181	50.32	13.92
FEB182	10.29	8.52
FEB183	21.68	8.97
FEB184	12.62	9.13
FEB185	13.32	12.19
FEB186(*Mucor circinelloides*)	48.10	**113.36**
FEB187	20.80	13.21
FEB188	35.00	11.88

FEB189	21.89	9.03
FEB190	15.47	14.13
FEB191	24.34	12.19
FEB192 (Unidentified)	**114.12**	13.31
FEB193	24.05	53.72
FEB194(Unidentified)	**114.12**	81.83
FEB195	17.84	11.12
FEB196	12.26	11.63
FEB197	33.03	10.96
FEB198	26.02	17.04
FEB199	21.57	12.70
FEB200	43.24	11.17
FEB201	13.57	9.38
FEB202	35.73	29.64
FEB203	21.16	9.08
FEB204	17.77	13.01
FEB205	17.77	10.56
FEB206	27.19	12.70
FEB207	13.57	15.56
FEB208	14.67	12.19
FEB209	21.97	14.28
FEB210	19.38	11.58
FEB211	17.22	12.39
FEB212	16.16	10.91
FEB213	16.82	12.80
FEB214	85.80	10.56
FEB215	17.92	23.57
FEB216	97.81	11.73
FEB217	75.36	13.36
FEB218	26.09	10.61
FEB219	19.05	11.12
FEB220	100.98	22.70
FEB221	52.33	10.81
FEB222(*Penicillium* sp.)	**114.12**	11.42
FEB223	15.29	21.22
FEB224	20.25	12.8
FEB225	22.37	13.01
FEB226	17.11	12.09
FEB227	36.38	78.72
FEB228	86.86	9.74
FEB229	11.75	16.88
FEB230	14.49	12.65
FEB231	16.82	13.16
FEB232	19.89	10.71
FEB233	36.68	12.19
FEB234	13.50	18.31
FEB235	28.06	11.83
FEB236	106.60	12.60

FEB237	18.61	13.41
FEB238	16.46	22.44
FEB239	56.82	12.95
FEB240	15.95	13.41
FEB241	14.78	14.69
FEB242	65.58	11.42
FEB243	11.64	12.19
FEB244	20.76	13.16
FEB245	29.12	12.39
FEB246	14.49	13.16
FEB247	60.07	13.67
FEB248	15.84	18.41
FEB249	21.46	19.38
FEB250	66.38	10.91
FEB251(*Mucor circinelloides*)	12.30	**109.64**
FEB252	12.92	14.03
FEB253	14.81	16.27
FEB254	96.13	11.42
FEB255	13.32	12.85
FEB256	44.67	15.91
FEB257	80.36	18.72
FEB258	18.50	30.76
FEB259	15.73	17.29
FEB260	14.38	12.75
FEB261	21.60	18.11
FEB262	17.08	10.76
FEB263	35.98	10.86
FEB264	15.11	10.51
FEB265	26.57	18.21
FEB266	96.6	12.14
FEB267	16.27	23.46
FEB268	31.09	11.42
FEB269(*Colletotrichum gloeosporioides*)	105.40	**99.94**
FEB270	15.65	11.02
FEB271	20.80	23.01
FEB272	15.91	12.09
FEB273	18.06	12.09
FEB274	25.32	10.25
FEB275	13.65	12.49
FEB276	16.02	10.45
FEB277	98.54	29.64
FEB278	41.57	21.22
FEB279	15.65	12.49
FEB280	19.05	13.16
FEB281	20.76	14.23

FEB: Fungal Endophytes of Banana

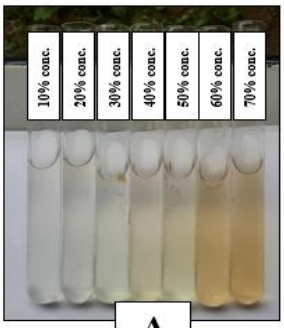


Plate 6. Standard concentration of GA3

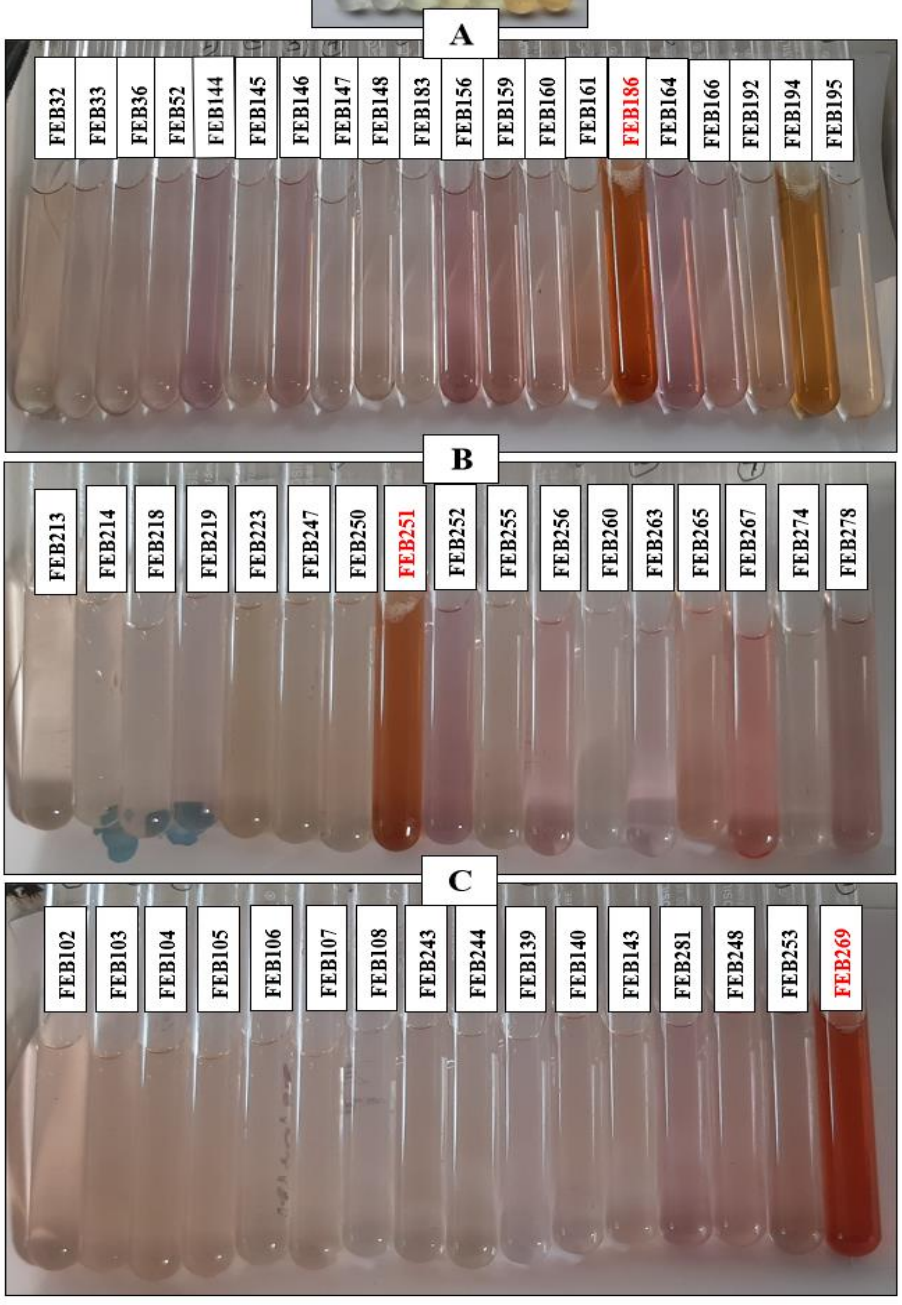


Plate 7. Isolates showing GA3 production (A, B and C)

circinelloides) with 109.64 µg/ml and FEB269 (*Colletotrichum gloeosporioides*) with 99.94 µg/ml concentration (Table 4.4 and Plate 6). The colour of the final concentration varied depending on the quantity of GA3 produced by each isolate and the best performing isolates produced reddish brown to brown colour.

Table 4.5. Simple regression between concentration and absorbance of the endophytic isolates for Gibberellic Acid production

Variables	Regression equation	b	SE (b)	t value	Pr> /t	R^2
Intercept	y = 0.0196x - 0.1369	-0.13686	0.037644	-3.63558	0.014973	0.9909
X Variable	-	0.019611	0.000842	23.2978	< 0.01 (2.71E-06)	0.9909

All the three isolates were isolated from the roots of wild banana plant. FEB186 (*Mucor circinelloides*) was isolated from Peren district and FEB251 (*Mucor circinelloides*) and FEB269 (*Colletotrichum gloeosporioides*) were isolated from Mokokchung district.

At a commercial level, the highly demanded among the family of gibberellin A is gibberellin A3 (GA3), for agronomical as well as for researches in scientific community (Resende *et al*., 2000; Gupta and Chakrabarty, 2013). Jae-Han *et al*. (2002) reported that the fungal endophyte *Penicillium citrinum* stands out among other endophytes in producing GA3 and in plant growth promotion. Hamayun*et al*. (2010) reported on the isolation and analysis of growth promoting capacity of novel strains that were isolated from roots of soil grown cucumber and all 19 fungal endophyte isolates were found to produce growth promoting substances and *Cladosporium* sp. MH-6 was found to be produce the highest amount of GA3.

Many fungal endophytes such as *Aspergillus niger, A. flavus, Penicillium funiculosum, P. corylophilum, Fusarium oxysporum, Paecilomycesformosus* and *Rhizopus stolonifer* have been documented to produce plant hormones that includes GA3s (Khan *et al*., 2015; Deng and Cao,

2017). Zhang *et al*. (2016) and Hamayun*et al*. (2017) also detailed that various species of fungi like *A. niger, A. flavus, F. oxysporum, P. funiculosum, P. corylophilum* and *P. cyclopium* produces high quantity of gibberellic acids.

Most species of fungi having the ability to produce GA belongs to ascomycota, a category of fungi that can form ascus. Endophytes treated plants are usually observed to be healthier than those that lack such relationship (Khan *et al*., 2008; Larriba*et al*., 2015), which in nearly all instances is credited to the production of plant hormones like GA3s by endophyte (Waqas *et al*., 2012).

Apart from IAA, endophytic fungi are also recognized to produce phytohormones, called gibberellins, that increases growth of plants and reduces the detrimental effects of abiotic stresses (Khan *et al*. 2011). Gibberellins are tetracyclic diterpenoic acids which has the potential to control several plant physiological and developmental process, that includes germination of seeds, development of seedlings, growth of stems and leaf, initiation of flower and growth of fruit and flower (Pharis and King, 1985; Crozier, 2000; King and Evans, 2003; Davies 2010). Growth of roots, development of root hair, suppression of floral bud differentiation, reproductive and vegetative bud dormancy and senescence delay of several organs in varieties of species of plants are also managed by gibberellins (Tanimoto, 1987; Bottini and Luna, 1993; Fulchieri*et al*., 1993; Reinoso *et al*., 2002). Gibberellic acid (GA3) is a terpenoid hormone, which is the major product of gibberellins in bacteria and fungi, a key hormone of plant that controls growth of plants and their development (Desai, 2017).

4.4.3 Phosphate solubilization

The analysis of phosphate solubilization by all the fungal endophytes isolatesare depicted in Table 4.6 and Plate 8. The phosphate solubilization test for the isolates was carried out qualitatively on Pikovskya's agar supplemented

with tri-calcium phosphate as an inorganic phosphate. The results showthat out of the 281 isolates, a total of 44 isolates were found to bepositive for

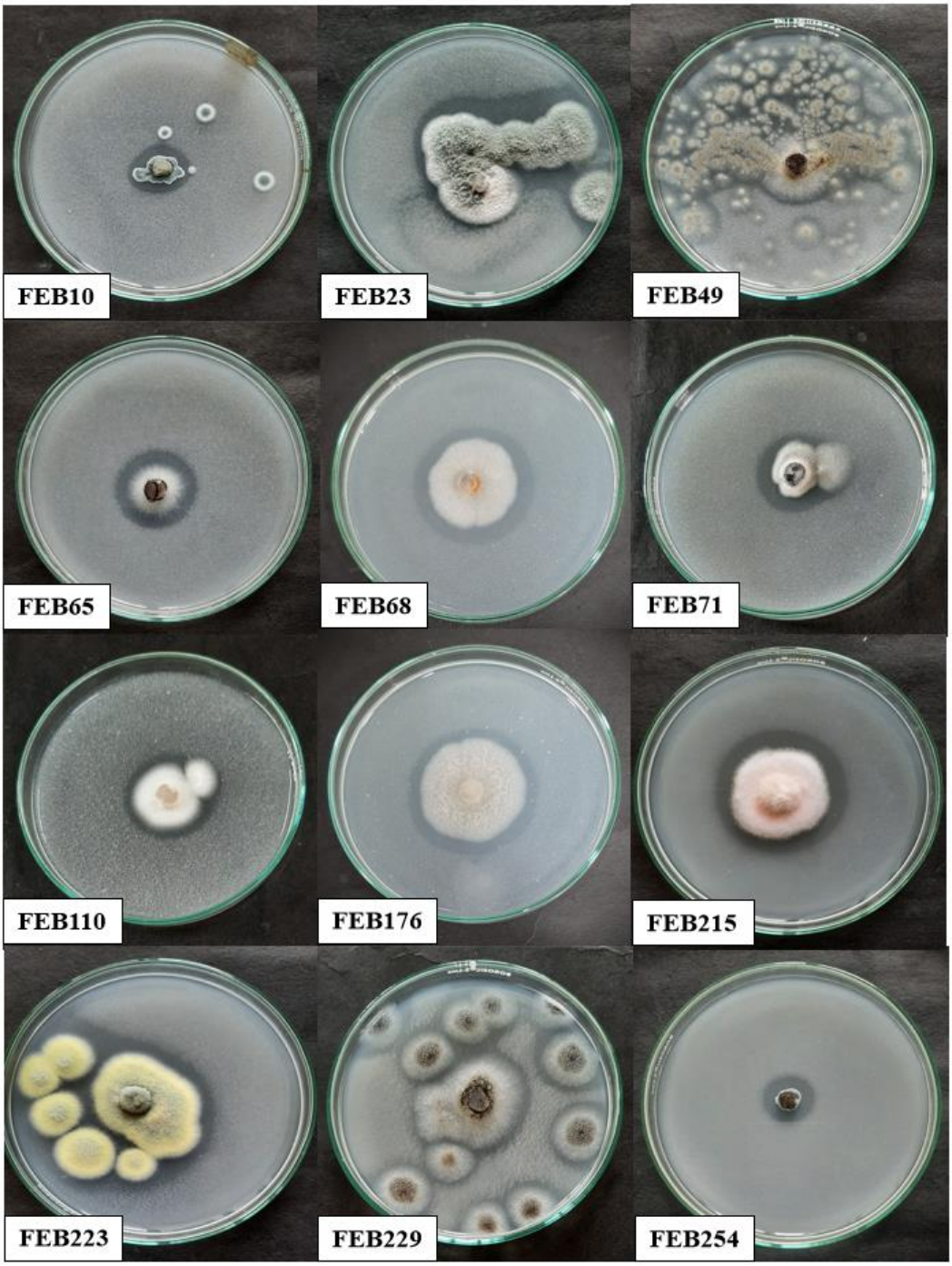


Plate 8A. Isolates showing positive reaction for phosphorous solubilization test

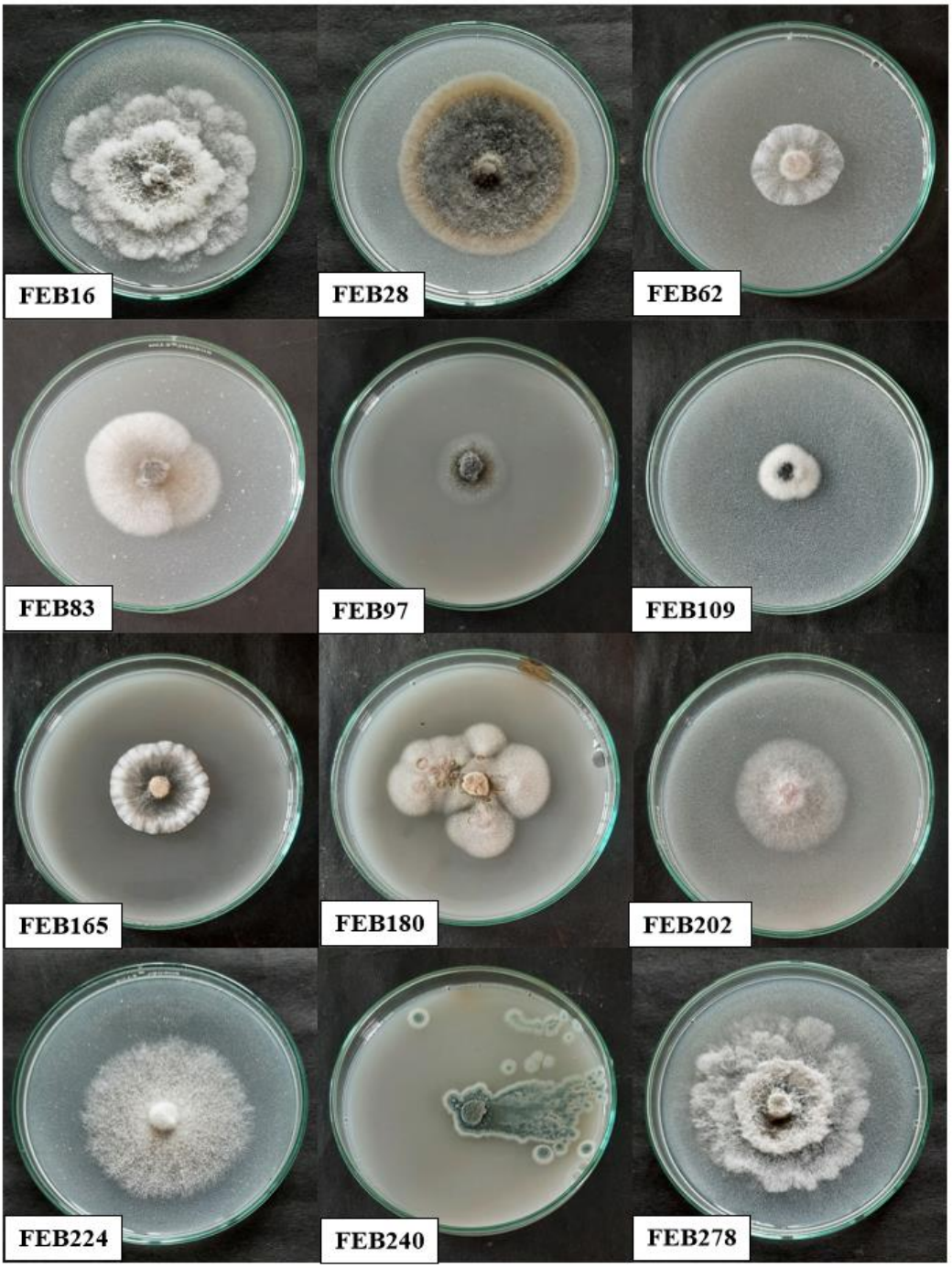


Plate 8B. Isolates showing negative reactionfor phosphorous solubilization test

Plate 8. Phosphorous solubilization test (A & B)

phosphate solubilization test. FEB10 (*Penicillium* sp.), FEB23 (*Aspergillus versicolor*), FEB49 (*Aspergillus niger*), FEB65 (*Colletotrichum fructicola*), FEB68 (*Colletotrichum gloeosporioides*), FEB71 (*Alternaria* sp.), FEB110 (Unidentified), FEB176 (Unidentified), FEB215 (Unidentified), FEB223 (*Penicillium* sp.), FEB229 (*Aspergillus niger*) and FEB254 (*Phomopsis* sp.) showed the strongest solubilisation activity of phosphorous. Formation of clear zone around the growth of the isolates after 72 hours showed positive reaction for phosphate solubilisation. This finding shows that the isolates have great impact on the growth of plant.

Out of these 12 isolates that showed strongest solubilization activity, eight were isolated from leaves of wild banana (FEB10 and FEB23 from Chumoukedima, FEB65, FEB68 and FEB71 from Kohima and FEB215, FEB223 and FEB229 from Mokokchung district), 3 from roots of wild banana (FEB49 from Chumoukedima, FEB110 from Kohima and FEB254 from Mokokchung district) and 1 (FEB176) from the leaves of cultivated banana isolated from Kohima district.

The current investigation is established by the research done by previous researchers such as Nath *et al*. (2015) reported that all the 9 fungal endophytes isolated from tea plant could solubilize phosphate with the highest being *Penicillium sclerotiorum* followed by *Penicillium* sp., *A. niger* and *A. fumigatus*. Savani *et al*. (2021) conducted phosphate test for 3 endophytes isolated from banana plant *Trichoderma reesei, Rigidiporusvinctus* and *Sphingobacteriumtabacisoli* and only *S. tabacisoli* was found to solubilize phosphate. Kumar and Prasher (2023) reported that *Colletotrichum gloeosporioides* and *Aspergillus fumigatus* isolated from *Dillenia indica* rhizosphere could solubilize phosphate.

The current investigation has also been confirmed by other workers like Wakelin *et al*. (2004), Adhikari and Pandey (2018), Khalil *et al*. (2021) who reported that *Penicillium* and *Aspergillus* can solubilize phosphate.

*Aspergillus*and *Penicillium* have been generally found to solubilize several forms of inorganic phosphate (Whitelaw, 2000).

One of the vital nutrients for general plant growth and productiveness after nitrogen is phosphorous (P). Its chemical and structural properties minimize its free accessibility and limits the nutrient for growth of plants (Mehta *et al*. 2013). Various fixation reactions that occur during the biogeochemical cycling significantly diminish the availability of phosphorus in soil, despite the vast reserve of this element (Kumar*et al*., 2015). Given that phosphorus is the primary nutrient element needed by plants, chemicals-based phosphate fertilizers are globally used by farmers to maximize crop yields (Sharma *et al*., 2015). But this has raised trouble related to degradation of environment and health of human. Using native microflora, especially those that can solubilize insoluble mineral components in soil, is an alternative strategy to reduce the total usage and demand of chemical fertilizers (Mehta *et al*., 2019). In the last three decades, a broad range of phosphate-solubilizing microorganisms have been identified, and a substantial number of fungi and bacteria have had their potential for P-solubilization assessed (Sharma *et al*., 2017). Endophytes are able to solubilize forms of phosphorous that are not soluble. Majority of microorganisms that are soil related can effectively solubilize insoluble phosphate to increase the production of phosphorous, hence making it accessible for plants (Alori *et al*., 2017). In addition, organic acids are added to the soil by endophytes, which help in the solubilization of phosphate complexes and their conversion into ortho-phosphates for uptake by plants and its utilization (Yadav, 2018).

4.4.4 Amylase activity test

The fungal endophytes isolates were assayed for their potential to secrete amylase enzyme. The isolates exhibited amylase activity ranging from no enzyme activity to low, medium and strong enzyme activity. The results are depicted in Table 4.6.

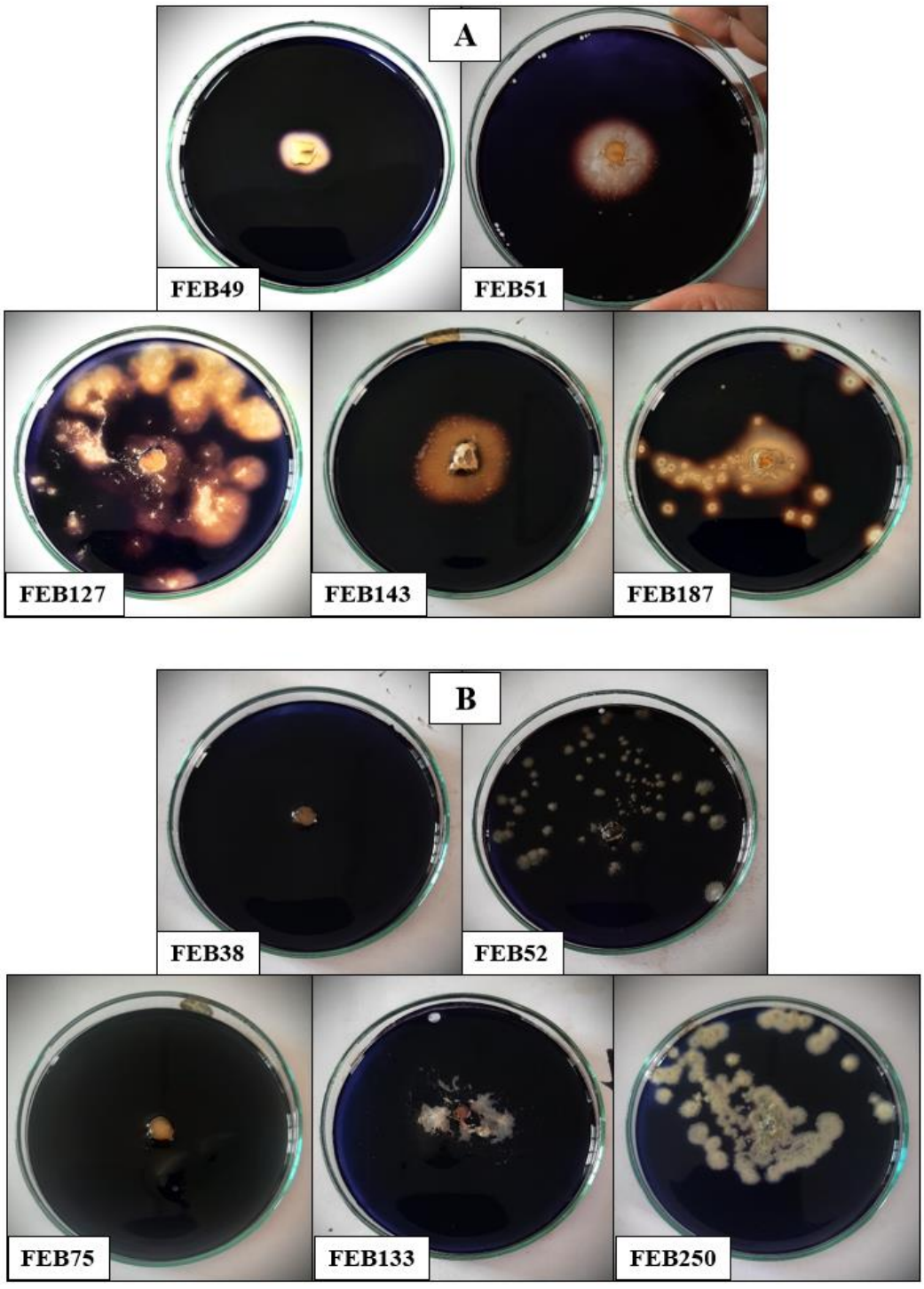


Plate 9. Amylase activity by the isolated fungal endophytes (A and B)

A: Isolates showing positive reaction

B. Isolates showing negative reaction

The findings showed that 73 isolates showed positive reaction to amylase activity test with FEB49 (*Aspergillus niger*), FEB51 (*Aspergillus clavatonanicus*), FEB127 (*Penicillium* sp.), FEB143 (*Beauveria felina*) and FEB187 (*Penicillium citrinum*) giving strong production of amylase (Plate 9). This was proved by the formation of clear zone around the colony plates when the plates were flooded with 1% iodine solution after five days of incubation.

Of the five isolates showing strong amylase production, isolation of three endophytes were from the roots of wild banana plant (FEB49 from Chumoukedima, FEB127 from Kohima and FEB187 from Peren district) and two from the roots of cultivated banana plant (FEB51 from Chumoukedima and FEB143 from Kohima district).

The current outcome are in conformation with Sunitha *et al*. (2012) who reported that 11 fungal endophytes exhibited positive results for amylase activity test, some of which includes *Fusarium* sp., *Colletotrichum* sp., *Alternaria* sp., *Cladosporium* sp.Mahfooz*et al*. (2017) also reported on the positive amylase activity of fungal endophytes such as *Penicillium oxalicum, Alternaria alternata, Fusarium circinatum, Pestalotiopsis versicolor* and *Penicillium megasporum* that were isolated from *Cupressus torulosa* D. Don. Malubag*et al*. (2021) reported that fungal endophytes *F. chlamydosporium, F. keratoplasticum* and the three different strains of *F. solani* could produce amylase. Reyes *et al*. (2021) reported that fungal endophytes such as *Fusarium oxysporum, Colletotrichum fructicola, Colletotrichum gloeosporioides* could produce amylase. Hawar (2022) reported that *Aspergillus niger, A. flavus, Cladosporium* sp., and *Mucor* sp. could produce amylase with *Aspergillus niger* showing strong enzyme activity.

Other workers such as Joel and Bhimba (2012) and Fouda *et al*. (2015) reported that fungal endophytes like *Pestalotiopsis microspore, Aspergillus oryzae, Penicillium chrysogenum* showed amylase production that corroborates the findings of this present study.

Countless microorganisms have been scanned for their enzyme production. Endophytic fungus release proteins alongside their plant host that are thought to support development, nutrition, and defense. One of the enzymes that is produce by endophytic fungal species is amylase that serves as a mechanism to resistance that is antagonistic to plant pathogens and for acquiring nutrients from the host (Vasundhara *et al*. 2019). Rao *et al*. (1998) reported that amylase enzymes can perform as antimicrobials or can generate compounds with antimicrobial activity.

4.5 Chitinase activity

The endophytic fungi isolates were examined to check their capacity to produce chitinase enzyme using colloidal chitin agar medium. In the present study no fungal endophyte was observed to show positive for chitinase enzyme production (Table 4.6 and Plate 10).

Other workers like Dolatabad*et al*. (2017) reported that *T. harzianum* TH 5-1-2, *T. atroviride* TA 2-2-1 and *T. harzianum* TH 10-2-2 could produce chitinase enzyme, however, *Byssochlamys nivea, Chaetomium interruptum, Fusarium incarnatum-equiseti, F. acuminatum, F. tricinctum* etc could not produce chitinase.Mahfooz*et al*. (2017) reported on the chitinase activity of some fungal endophyte, out of which *Penicillium oxalicum, A. alternata, Daldinia sp., Fusarium circinatum* and *Penicillium megasporum* were found to be positive for chitinase test and only *Pestalotiopsis versicolor* was found to be negative. Sharma *et al*. (2018) accounted that all 30 native *Pseudomonas* isolates were able to produce chitinase enzyme. Puig and Cumagun (2019) also reported that *Pestalotiopsis* CGP117, an endophyte did not produce chitinase enzyme.

Meenavalli*et al*. (2011) studied the chitinase enzyme produced by fungal endophytes isolated from numerous host species where, out of the 162 isolates, only 31 isolates showed chitinase production. Genera like *Alternaria,*

Nigrospora, Cladosporium, Pestalotiopsis and *Phyllosticta* and some species of

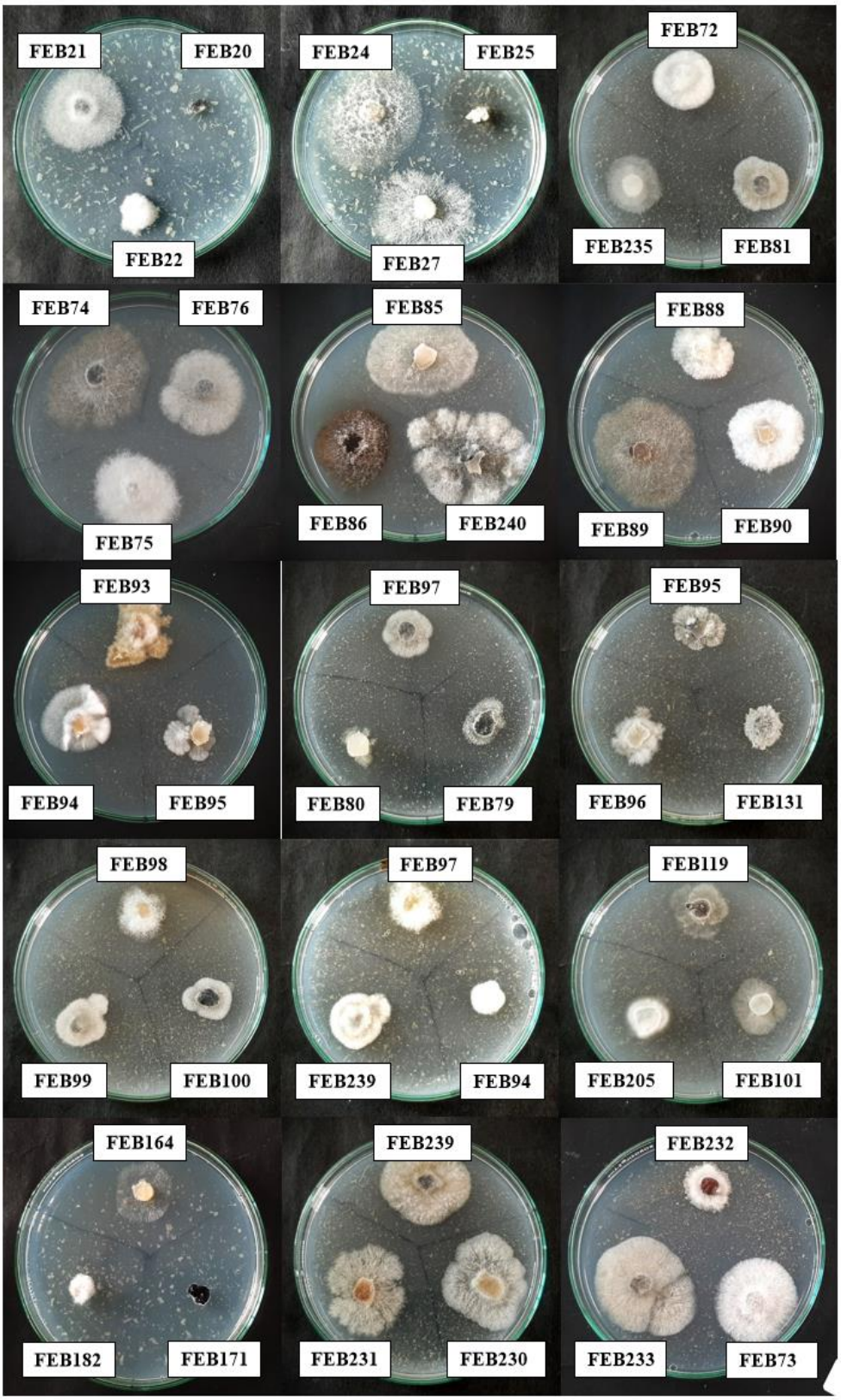


Plate 10. Chitinase activity test for the isolated fungal endophytes showing negative reaction

Colletotrichum, *Fusarium*, *Cladosporium* and *Phomopsis* did not produce chitinase. They found that because of high level genetic diversity in fungal endophytes with association of chitinase enzyme, as the same species or isolates of endophytes were isolated from distinct host, they differed in their ability to produce chitinase enzyme. This may explain why the fungal endophytes isolates in this current investigation showed negative response to chitinase test.

The fundamental composition of the cell wall of a fungus is made up of chitin. This insoluble chitin polymer is degraded with the assistance of an enzyme known as chitinolytic or chitinase enzyme (Seidl, 2008). Fungi producing chitinase enzyme have been deliberated for their possibility in managing fungi that are pathogenic to plants (Klemsdal*et al*. 2006). Endophytic fungi are a vital source of various kinds of chitin modifying enzymes (Meenavalli*et al*., 2011). Fungal endophytes producing chitinase enzymes helps in termination of the cell wall of plant pathogens in the process of antagonism by these endophytes (Nugroho *et al*., 2003).

4.6 Siderophore production

Chrome Azurol S (CAS) assay was used to detect the production of siderophore by the isolated 281 endophytic fungi from the banana species. The findings of the qualitative assay are presented in Table 4.6. In the current investigation, 92 isolates were found to show positive reaction. FEB27 (*Diaporthephaseolorum*), FEB38 (Unidentified), FEB46 (*Trichoderma asperellum*), FEB49 (*Aspergillus niger*), FEB120 (Unidentified), FEB121 (*Aspergillus* sp.), FEB129 (*Diaporthe*sp.), FEB217 (*Penicillium* sp.), FEB222 (*Penicillium* sp.), FEB223 (*Penicillium* sp.) and FEB262 (Unidentified) were the best performing isolates (Plate 11). The colonies with orange color zones after incubation period of 5 days were regarded positive for siderophore production.

Out of the 11 isolates showing strong siderophore production, six were isolated from the roots of wild banana plant (FEB46 and FEB49 from Chumoukedima, FEB120, FEB121 and FEB129 from Kohima and FEB262 from Mokokchung district), four from leaves of wild banana plant (FEB27 from Chumoukedima and FEB217, FEB222 and FEB223 from Mokokchung district) and one (FEB38) from the leaves of cultivated banana plant from Chumoukedima district.

Related works were done by other workers such as Suebrasri*et al*. (2020) and Savani *et al*. (2021) who reported that the endophytic strains of *Trichoderma koningii* ST-KKU and *Trichoderma reesei* UH EF, respectively, could produce siderophores. The siderophore production is also reported for endophytic strains of *Trichoderma harzianum* that colonizes beans (*P. vulgaris*) (Eslahi*et al*., 2020). Gateta*et al*. (2023) reported that that highest production of siderophore was observed in *Trichoderma pinophilus* PBMP28 and *Aspergillus flavus* KKMP34.

Penicillium endophyte SLS12 (Shi *et al*., 2017), *P. peniophoroides* SLS13 (Ye *et al*., 2019) have been reported to produce siderophore. Toghueo*et al*. (2023) reported that out of 22 *Diaporthe* sp. isolated from the roots of *Festuca rubra* subsp. *pruinosa*, 20 strainscould produce siderophore. Thus, the findings in the present work are in agreement with the works done by earlier workers.

The production of siderophores by these isolates could be due to considerably higher collection of Fe in the leaves and roots of plants (Toghueo*et al*., 2023). Since siderophores are a major element that regulates iron possession by plant pathogens (Yadav *et al*., 2018), these isolates are expected to aid growth of plant in terms of acting as a bio-control agent.

Siderophores are low molecular weight mixtures that have the capacity for chelating iron and make it available to the plants. Endophytes are known to produce siderophore compounds (Yadav, 2018). Endophytes producing

siderophores can provide activities of biocontrol against pathogens through the integration of phenolate, catecholate and hydroxymate (Rajkumar *et al*., 2010). Plants that have deficiency of iron can be increased with the help of siderophores that assist in nitrogen fixation (Kraepiel*et al*., 2009). Endophytes also provides

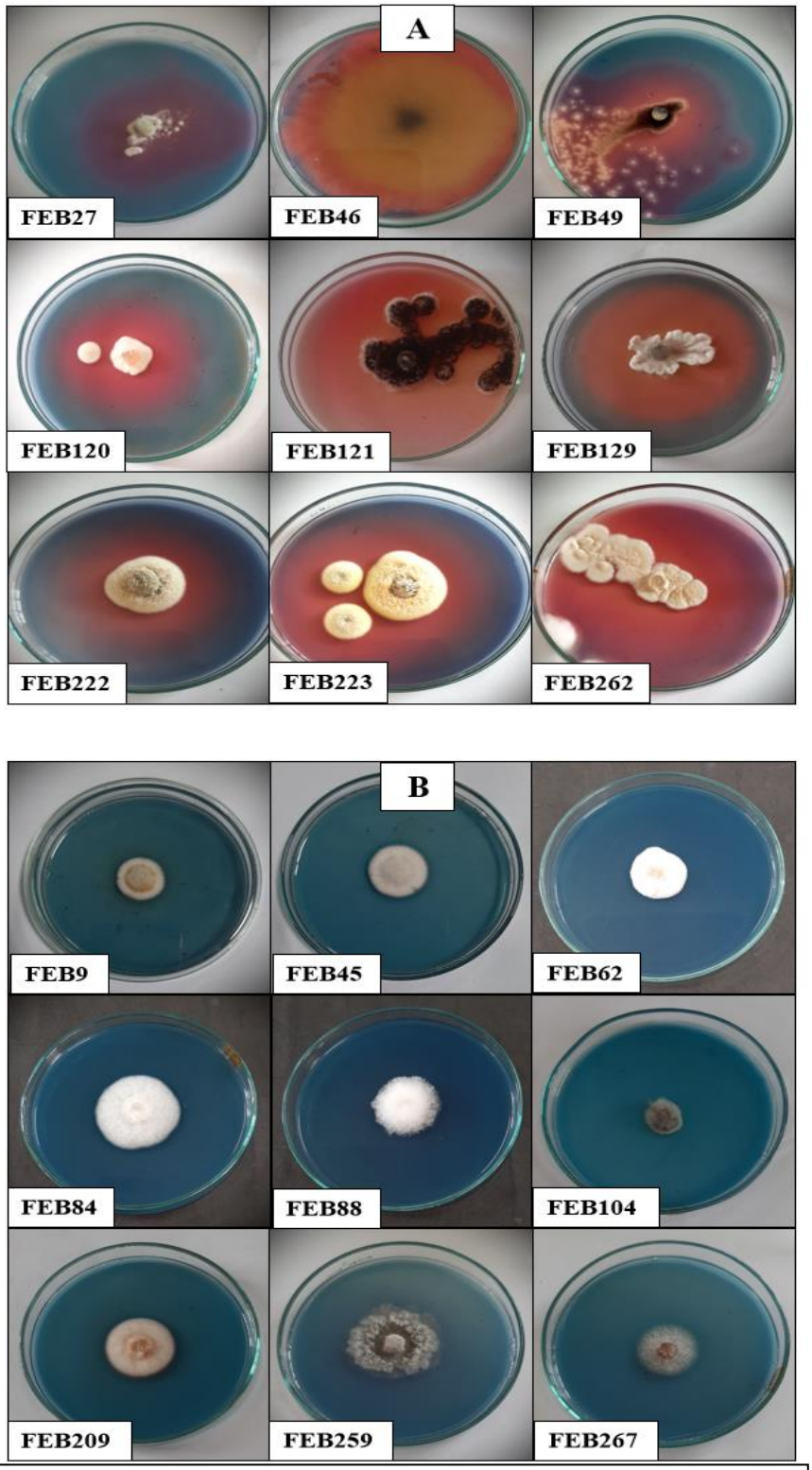


Plate 11. Siderophore production test for the isolated fungal endophytes

A. Isolates showing positive reaction
B. Isolates showing negative reaction

Table 4.6. Phosphate solubilization, amylase activity, chitinase activity and siderophore production test for the isolated fungal endophytes of banana

Fungal endophytes of banana	Phosphate Solubilization Test	Amylase Activity Test	Chitinase Activity	Siderophore Production
FEB1	+	++	-	+
FEB2	+	+	-	-
FEB3	-	+	-	-
FEB4	-	-	-	-
FEB5	-	-	-	-
FEB6	-	-	-	+
FEB7	-	+	-	-
FEB8	+	+	-	-
FEB9	-	-	-	-
FEB10 (*Penicillium* sp.)	**+++**	++	-	-
FEB11	+	+	-	+
FEB12	-	+	-	+
FEB13	-	-	-	++
FEB14	-	+	-	+
FEB15	-	+	-	-
FEB16	-	-	-	+
FEB17	-	-	-	-
FEB18	-	+	-	-
FEB19	-	++	-	-
FEB20	-	+	-	+
FEB21	-	++	-	-
FEB22	-	-	-	-
FEB23(*Aspergillus versicolor*)	**+++**	++	-	++
FEB24	-	-	-	+
FEB25	-	-	-	-
FEB26	-	++	-	-
FEB27(*Diaporthephaseolorum*)	-	-	-	**+++**
FEB28	-	-	-	-
FEB29	-	++	-	-
FEB30	-	-	-	+
FEB31	-	-	-	-
FEB32	-	+	-	-
FEB33	-	-	-	-
FEB34	-	++	-	-
FEB35	-	-	-	+
FEB36	-	-	-	-
FEB37	-	-	-	-
FEB38(Unidentified)	-	-	-	**+++**
FEB39	-	-	-	-
FEB40	-	-	-	-
FEB41	-	-	-	-
FEB42	-	-	-	-
FEB43	-	-	-	-
FEB44	-	-	-	-

FEB45	-	-	-	-
FEB46(*T. asperellum*)	-	-	-	+++
FEB47	-	-	-	+
FEB48	-	-	-	++
FEB49(*Aspergillus niger*)	+++	+++	-	+++
FEB50	-	-	-	-
FEB51(*Aspergillus clavatonanicus*)	-	+++	-	-
FEB52	-	-	-	-
FEB53	-	-	-	++
FEB54	-	-	-	-
FEB55	-	-	-	-
FEB56	-	-	-	-
FEB57	-	-	-	-
FEB58	-	-	-	-
FEB59	-	-	-	-
FEB60	-	-	-	-
FEB61	-	-	-	-
FEB62	-	-	-	-
FEB63	-	-	-	-
FEB64	-	-	-	-
FEB65(*C. fructicola*)	+++	-	-	-
FEB66	-	++	-	-
FEB67	-	-	-	-
FEB68(*C. gloeosporioides*)	+++	-	-	-
FEB69	-	-	-	-
FEB70	-	-	-	-
FEB71(*Alternaria* sp.)	+++	-	-	+
FEB72	-	-	-	-
FEB73	+	+	-	-
FEB74	-	-	-	-
FEB75	-	-	-	+
FEB76	-	-	-	-
FEB77	-	-	-	+
FEB78	-	-	-	-
FEB79	-	-	-	+
FEB80	-	-	-	+
FEB81	-	-	-	-
FEB82	-	-	-	-
FEB83	-	-	-	-
FEB84	-	-	-	-
FEB85	-	-	-	+
FEB86	-	-	-	-
FEB87	++	+	-	+
FEB88	-	-	-	-
FEB89	-	-	-	-
FEB90	-	-	-	-
FEB91	-	-	-	-
FEB92	-	-	-	-
FEB93	-	-	-	-
FEB94	-	-	-	-

FEB95	-	-	-	-
FEB96	-	-	-	-
FEB97	-	-	-	-
FEB98	-	+	-	-
FEB99	-	-	-	-
FEB100	-	-	-	-
FEB101	-	-	-	-
FEB102	-	+	-	-
FEB103	-	+	-	-
FEB104	-	+	-	-
FEB105	-	+	-	-
FEB106	+	-	-	+
FEB107	-	+	-	-
FEB108	-	-	-	-
FEB109	-	-	-	-
FEB110(Unidentified)	**+++**	-	-	++
FEB111	-	-	-	-
FEB112	-	-	-	-
FEB113	-	-	-	-
FEB114	-	-	-	-
FEB115	-	-	-	+
FEB116	-	-	-	-
FEB117	-	-	-	+
FEB118	-	-	-	-
FEB119	-	-	-	-
FEB120(Unidentified)	+	-	-	**+++**
FEB121(*Aspergillus* sp.)	-	++	-	**+++**
FEB122	-	-	-	+
FEB123	+	++	-	+
FEB124	-	-	-	-
FEB125	-	-	-	-
FEB126	-	+	-	-
FEB127(*Penicillium* sp.)	-	**+++**	-	++
FEB128	-	-	-	-
FEB129(*Diaporthe*sp.)	-	-	-	**+++**
FEB130	-	+	-	-
FEB131	-	-	-	-
FEB132	-	-	-	-
FEB133	-	-	-	++
FEB134	-	+	-	-
FEB135	-	-	-	-
FEB136	-	-	-	-
FEB137	-	-	-	-
FEB138	-	-	-	-
FEB139	-	+	-	-
FEB140	-	+	-	-
FEB141	++	-	-	+
FEB142	+	-	-	-
FEB143(*Beauveria felina*)	-	**+++**	-	+
FEB144	-	-	-	+

FEB145	-	-	-	-
FEB146	-	++	-	+
FEB147	-	++	-	++
FEB148	-	-	-	-
FEB149	-	-	-	-
FEB150	-	-	-	-
FEB151	-	+	-	-
FEB152	-	-	-	-
FEB153	-	-	-	-
FEB154	-	-	-	-
FEB155	-	-	-	-
FEB156	-	-	-	-
FEB157	-	++	-	-
FEB158	-	-	-	-
FEB159	-	-	-	-
FEB160	-	-	-	-
FEB161	-	-	-	-
FEB162	-	-	-	-
FEB163	-	+	-	+
FEB164	-	+	-	-
FEB165	-	+	-	+
FEB166	-	+	-	-
FEB167	-	-	-	-
FEB168	-	++	-	-
FEB169	-	+	-	+
FEB170	-	+	-	-
FEB171	-	+	-	-
FEB172	+	-	-	-
FEB173	-	-	-	+
FEB174	-	-	-	-
FEB175	-	+	-	-
FEB176(Unidentified)	**+++**	-	-	+
FEB177	-	-	-	-
FEB178	+	-	-	-
FEB179	-	-	-	-
FEB180	-	-	-	+
FEB181	-	-	-	+
FEB182	-	-	-	-
FEB183	-	-	-	-
FEB184	-	-	-	+
FEB185	-	-	-	-
FEB186	-	-	-	+
FEB187(*Penicillium citrinum*)	-	**+++**	-	-
FEB188	-	-	-	+
FEB189	-	-	-	-
FEB190	-	-	-	-
FEB191	-	-	-	-
FEB192	-	-	-	-
FEB193	-	-	-	-
FEB194	-	-	-	-

FEB195	-	-	-	-
FEB196	-	-	-	-
FEB197	-	-	-	+
FEB198	-	-	-	-
FEB199	-	-	-	-
FEB200	+	-	-	+
FEB201	-	+	-	-
FEB202	-	-	-	-
FEB203	-	++	-	+
FEB204	-	-	-	-
FEB205	-	++	-	+
FEB206	-	-	-	-
FEB207	-	-	-	+
FEB208	-	-	-	-
FEB209	-	-	-	-
FEB210	+	-	-	++
FEB211	-	+	-	-
FEB212	-	-	-	-
FEB213	-	-	-	-
FEB214	++	+	-	+
FEB215(Unidentified)	**+++**	+	-	++
FEB216	-	+	-	+
FEB217(*Penicillium* sp.)	-	-	-	**+++**
FEB218	-	-	-	-
FEB219	-	-	-	+
FEB220	+	-	-	-
FEB221	-	-	-	-
FEB222(*Penicillium* sp.)	-	++	-	**+++**
FEB223(*Penicillium* sp.)	**+++**	-	-	**+++**
FEB224	-	++	-	+
FEB225	-	-	-	-
FEB226	-	++	-	-
FEB227	-	-	-	+
FEB228	-	-	-	-
FEB229(*Aspergillus niger*)	**+++**	+	-	++
FEB230	+	-	-	+
FEB231	-	+	-	+
FEB232	-	-	-	-
FEB233	-	-	-	-
FEB234	-	-	-	-
FEB235	-	-	-	-
FEB236	-	-	-	-
FEB237	-	+	-	+
FEB238	-	+	-	-
FEB239	-	-	-	-
FEB240	-	-	-	++
FEB241	-	-	-	-
FEB242	-	-	-	-
FEB243	-	-	-	-
FEB244	+	+	-	-

FEB245	+	+	-	+
FEB246	-	-	-	+
FEB247	+	-	-	-
FEB248	++	-	-	-
FEB249	-	-	-	-
FEB250	+	-	-	+
FEB251	-	-	-	-
FEB252	-	-	-	-
FEB253	-	-	-	+
FEB254(*Phomopsis* sp.)	**+++**	-	-	+
FEB255	-	-	-	++
FEB256	-	-	-	+
FEB257	+	-	-	+
FEB258	-	++	-	++
FEB259	-	-	-	-
FEB260	+	+	-	+
FEB261	-	-	-	+
FEB262(Unidentified)	+	-	-	**+++**
FEB263	-	-	-	-
FEB264	-	-	-	-
FEB265	-	++	-	-
FEB266	+	-	-	+
FEB267	-	-	-	-
FEB268	-	-	-	++
FEB269	-	-	-	+
FEB270	+	-	-	+
FEB271	++	-	-	+
FEB272	-	-	-	-
FEB273	-	-	-	-
FEB274	+	-	-	-
FEB275	-	-	-	-
FEB276	-	-	-	-
FEB277	+	-	-	+
FEB278	-	-	-	++
FEB279	-	-	-	-
FEB280	-	-	-	-
FEB281	+	+	-	++

- = Negative, + = Low Production, ++ = Medium Production, +++ = Strong Production

FEB: Fungal Endophytes of Banana

iron to iron deficient plants and helps in plant growth and crop yield (Rajkumar *et al*., 2010). As compared to rhizospheric microbes, endophytes provide better mobilization of nutrients. Since they originate from the internal microbiome, they are more suited to the activities of the internal tissues of the plants (Verma *et al*., 2021).

4.7 Collection, isolation, pathogenicity test, characterization and identification of the Fusarium wilt pathogen of banana

4.7.1 Collection of the disease specimen

The disease specimen (infected banana pseudostem) exhibiting typical symptoms of Fusarium wilt from banana plant was collected from the farmer's field, Dimapur, Nagaland.

The symptoms observed in the field were yellowing or wilting of the whole leaves, collapsing and drying of the leaves. When the pseudostems were cut open, reddish colour discoloration of the vascular tissues was observed, which is the major characteristic symptoms of Fusarium wilt disease of banana (Plate 12 and 13). The result is in accordance with related examinations accounted by earlier workers such as Ploetz (2006), Leong *et al*. (2009), Li *et al*. (2011), Kai-li *et al*. (2019).

4.7.2 Isolation and purification of the pathogen

Isolation of pathogen from the collected banana sample was done in the laboratory as per the method given by Saravanan *et al*. (2004). The colony developed after 4-5 days of incubation producing whitish pink cottony colony on the PDA medium. The pure cultures hence procured were stored in PDA slants for further examination (Plate 14).

4.7.3 Morphological identification of the pathogen

The isolated culture of Fusarium wilt pathogen was critically examined visually as well as microscopically for cultural and morphological characters. The isolated pathogen produced whitish pink colony on the PDA medium which

Plate. 12. Wilting/drying of the whole plant

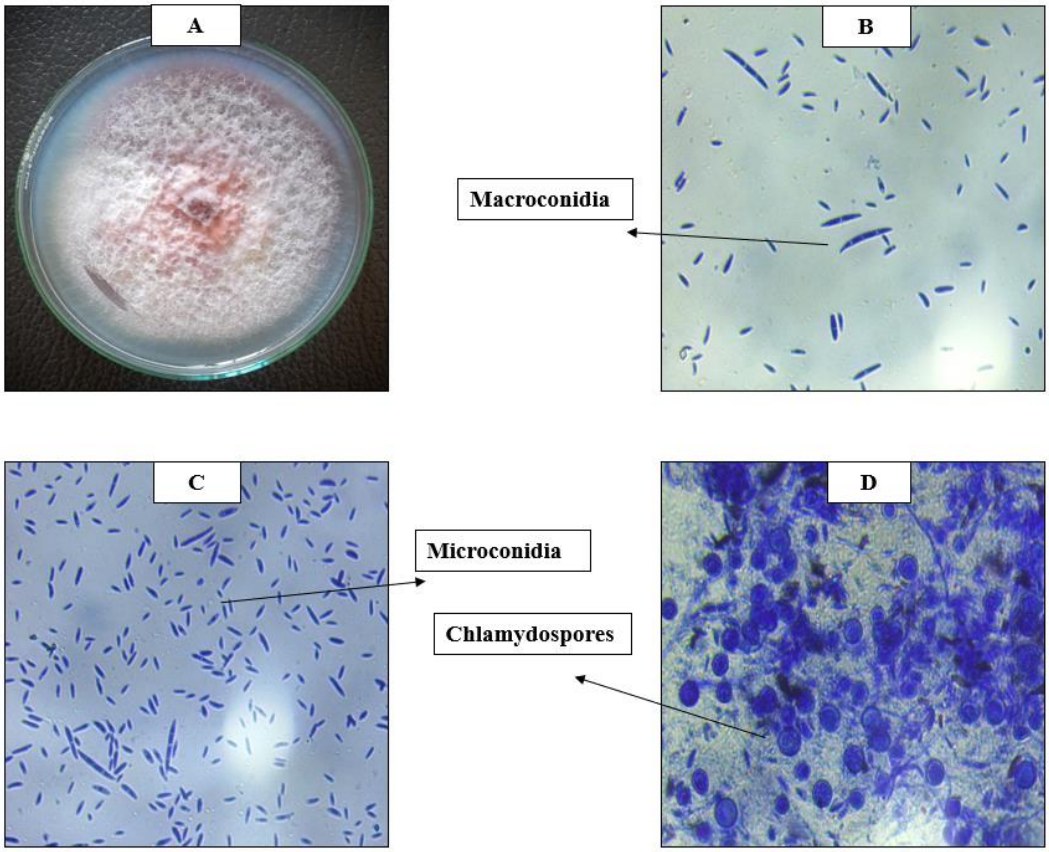


Plate 14. Pure culture of the pathogen (A) and microscopic view of the pathogen under 40x (B, C and D)

Table 4.7. Morphological Characterization of the isolated Fusarium wilt pathogen of banana

Characteristics				
Colony Color	**Colony Aspects or Texture**	**Spore**	**Microscopic Characteristics**	**Identification**
Whitish pink	Cottony	Present	Microconidia were found in abundance, non-septate or one celled, oval to kidney shaped, hyaline, 44.20 x 16 µm in size. Macroconidia were produced sparsely and were 4-6 celled, slightly sickle shaped with tapered ends, 160.12 x 20.80 µm in size. Chlamydospores are globose, were produced singly or in pairs, found in abundance, smooth or rough walled, 40 – 44 µm in size.	*Fusarium oxysporum*

had cottony growth. When observed under the microscope, microconidia were found in abundance. They were non septate or one celled, oval to kidney shaped, hyaline, 44.20 x 16 µm in size. Macroconidia were produced sparsely and were 4-6 celled, slightly sickle shaped with tapered ends, 160.12 x 20.80 µm in size. Chlamydospores were globose, produced singly or in pairs, found in abundance, smooth or rough walled, 40 – 44 µm in size (Table 4.7 and Plate 14). Measurements were all done under 40x objective lens. Based on the morphological characters, it was identified as *Fusarium oxysporum*.

The present findings are in consistent with the works done by earlier workers like Leslie and Summerell (2006) and Ploetz (2006), Thangavelu *et al*. (2019) described that the microconidia were found in abundance, one or two celled conidia, oval to kidney shape. Macroconidia produced sparsely with four to six celled and sickle shaped and chlamydospores as globose, produced singly or in pairs.

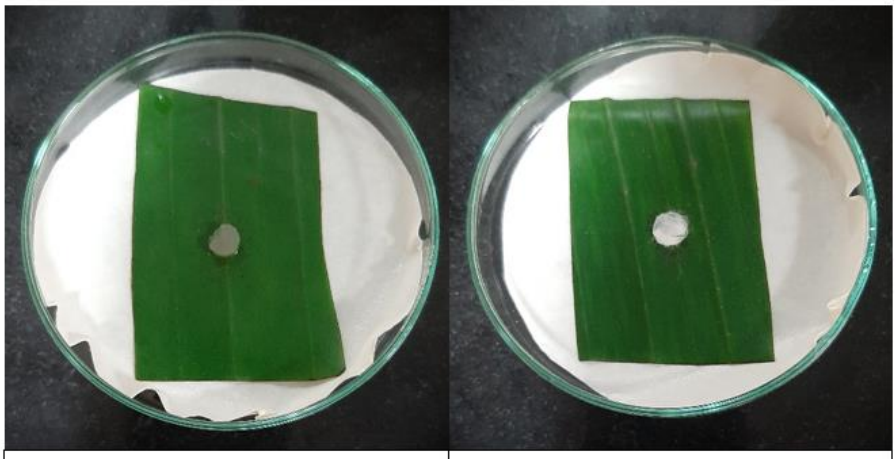
4.7.4 Pathogenicity test using detached leaf assay

To confirm pathogenicity, detached banana leaf assay was carried out as method given by Udompongsuk and Soytong (2016). Non-inoculated negative controls were inoculated with an agar plug without the fungus and with *Fusarium oxysporum* f. sp. *lycopersici* (fol). Comparison was also made with the*Fusarium oxysporum* f. sp. *cubense* (foc) treated leaves as a positive control, that was procured from ITCC (Indian Type Culture Collection), IARI, New Delhi. The results were compared after 10 days of incubation and typical yellowing of the leaves were observed on the detached leaves that were inoculated with the test pathogen. Symptoms similar to the test pathogen were also noticed on the *Fusarium oxysporum* f. sp. *cubense* (foc) treated leaves. Howeversymptoms were not observed on the negative controls (Plate 15). This proves that the isolated tested pathogen is the causal agent for Fusarium wilt in banana.

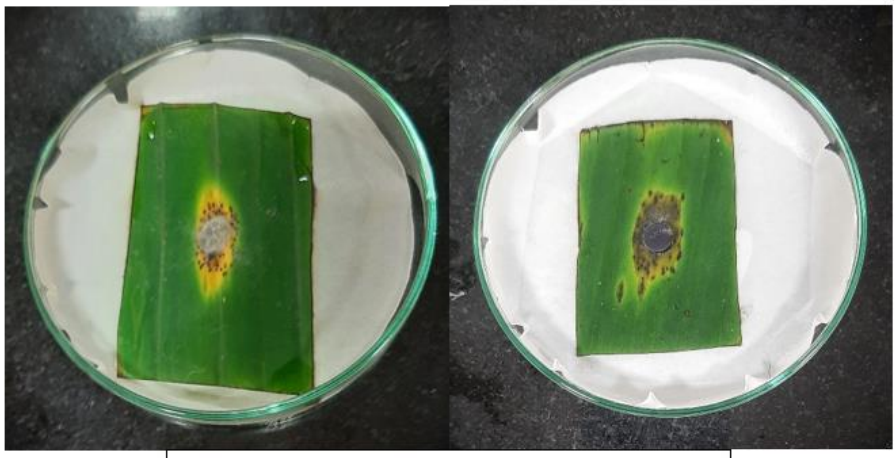
Observations akin were also documented by Udompongsuk and Soytong (2016) who conducted detached leaf pathogenicity test for foc causing banana wilt. The detached leaves inoculated with the tested pathogen produced yellowing symptoms after 7-10 days of incubation, whereas no symptoms were observed in the control plates. Patel and Jampala (2018) also conducted detached leaf pathogenicity test for the isolated pathogen of Fusarium wilt disease of banana and after 10 days of incubation, they observed the development of yellowing symptoms on the tested detached leaves.

4.5.5 Molecular identification and phylogenetic analysis of the isolated Fusarium wilt pathogen of banana

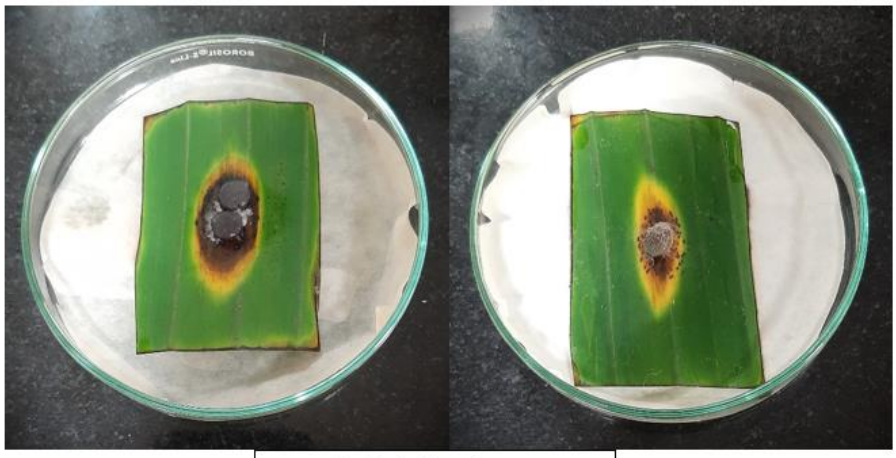
Molecular identification of the isolated pathogen was done using Internal Transcribed Spacer (ITS) region of the 18S rRNA. Sequence analysis was done to confirm the identity of the pathogen. The result was compared from NCBI database and the isolated pathogen was identified as *Fusarium oxysporum*.



A. Negative control with agar | B. Negative control with Fol



C. Positive control with Foc isolate from ITCC



D. Tested pathogen

Plate 15. Pathogenicity test for the isolated pathogen through detached leaf assay (A, B, C and D)

Table 4.8. Molecular Identification of the Isolated Fusarium wilt Pathogen of Banana using ITS primers

Fungal Isolates	Sequence	Base pairs	Homolog Sequence	Sequence Identity %	Closest Accession Number	GenBank Accession No.
Banana Fusarium wilt pathogen	TCCGTAGGTGAACCTGCGGAGGGATCATTACCGAG TTTACAACTCCCAAACCCCTGTGAACATACCACTTG TTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACG GGACGGCCCGCCAGAGGACCCCTAAACTCTGTTTC TATATGTAACTTCTGAGTAAAACCATAAATAAATCA AAACTTTCAACAACGGATCTCTTGGTTCTGGCATCG ATGAAGAACGCAGCAAAATGCGATAAGTAATGTGA ATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG CACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCT GTTCGAGCGTCATTTCAACCCTCAAGCACAGCTTGG TGTTGGGACTCGCGTTAATTCGCGTTCCTCAAATTG ATTGGCGGTCACGTCGAGCTTCCATAGCGTAGTAGT AAAACCCTCGTTACTGGTAATCGTCGCGGCCACGC CGTTAAACCCCAAACTTCTGAAATGTTGACCTCGGA TCAGGTAGGAATACCCGCTGAACTTAAGCATATCA ATAAGCGGAGGAAAAA	550	*Fusarium oxysporum*	99.64%	FJ605247	PP587552

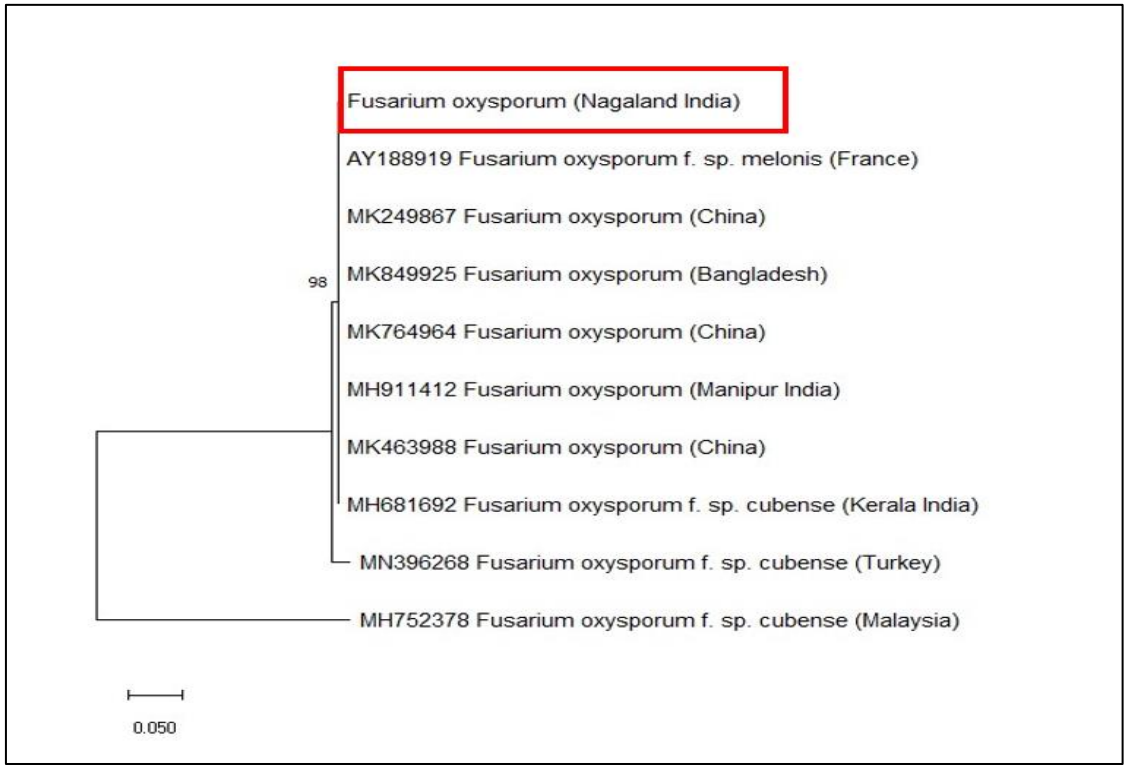


Fig.4.3. Phylogenetic analysis of ITS sequences of Fusarium wilt pathogen with reference sequences retrieved from NCBI (National Center for Biotechnology Information). The analysis was implemented in MEGA 11 using the neighbor-joining method. The number given over branches indicate bootstrap coefficient.

The ITS forward (ITS1; 5’ -TCC GTA GGT GAA CCT GCG G- 3’) and ITS reverse (ITS4; 5’ -TCC TCC GCT TAT TGA TAT GC- 3’) oligonucleotide pairs amplified a DNA fragment of approximately 552 bp amplicon size of the isolated pathogen. The ITS sequence was submitted to NCBI Genbank (Accession no. PP587552). The BLAST analysis result was utilized to construct the phylogeny tree using Mega11 software (Table 4.8 and Fig 4.3).

Akin evaluation was also done by other workers. Kai-li *et al*. (2019) studied the distribution of *Fusarium oxysporum* f. sp. *cubense* (foc) races in Fuijan Province, China. They isolated 33 putative Fusarium strains and identified 19 strains as *Fusarium oxysporum* based on Internal Transcribed Spacer (ITS) using ITS1 and ITS4 primers. Prakash *et al*. (2023) isolated 8 infected samples of Fusarium wilt disease of banana from eight different fields. These isolates were identified based on 18s rRNA sequencing using ITS1 and ITS4 primers. They were identified and confirmed as *Fusarium oxysporum*. Thus, the current investigation is in support by the works done by earlier workers in respect to identification based on 18s rRNA sequencing.

The isolated pathogen though found pathogenic to the banana plant, could not be identified up to sub-species level as Fusarium or *Fusarium oxysporum* f. sp. *cubense* (foc) specific primers were not used in the present study.

4.8 To assess biocontrol activities of the endophytes against Fusarium wilt pathogen of banana

4.8.1*In vitro* screening through dual plate culture of the fungal endophytes against the Fusarium wilt pathogen of banana

All the isolated fungal endophytes of banana were analysed for their inhibitory effect on the radial growth of the Fusarium wilt pathogen of bananaby dual culture technique. The data obtained are presented in Table 4.9 and Plate 16. The fungal endophytes exhibited varied level of inhibitory traits

against the pathogen.The growth of the pathogen was observed to progressuntil they came

in contact with the leading edges of the colonies of the fungal endophytes.

The per cent inhibition over control was calculated five days after inoculation. The highest inhibitionper cent of 61.90% of the pathogen mycelial growth was documented by FEB116 (*Trichoderma asperellum*) which was found to be the most promising endophyte and statistically significant from the other fungal endophytes. It was followed by FEB249 (*Diaporthechromolaenae*) with 57.14% and FEB23 (*Aspergillus versicolor*) with 55.24% inhibition which were found to be statistically at par with each other. The per cent inhibition by the rest of the fungal endophytes ranged from 6.70% in case of FEB170 (Unidentified), FEB172 (Unidentified), FEB189 (Unidentified) to 52.38% in case of FEB5 (*Trichoderma hamatum*) and FEB27 (*Diaporthephaseolorum*).

Of the best three isolates, isolation of two isolates were from roots of wild banana plant (FEB116 from Kohima and FEB249 from Mokokchung district) and one (FEB23) from the leaves of wild banana plant from Chumoukedima district.

Several workers have detailed the inhibitory effect of fungal endophytes against the wilt pathogen of banana. Dagamac*et al*. (2008) reported the antagonistic effect of fungal endophytes isolated from the roots of banana and found that all three *Aspergillus* species tested against *Fusarium oxysporum* f. sp. *cubense* (foc) could inhibit the growth of the pathogen. Garoe*et al*. (2013) also reported the antagonistic effects of fungal endophytes against Foc isolated from the banana corm and two *Aspergillus* sp. were found to inhibit the mycelial growth of the pathogen *in vitro*. Similarly, Hidayat *et al*. (2019) also reported on the inhibitory effect of endophytic *Aspergillus* sp. against Foc.

Many have described that *Aspergillus* strains isolated as fungal endophytes from rice, mangrove, soybean and maize, have antagonistic traits (Kandhari *et al*., 2000; Maria and Sridhar, 2004; Pimentel *et al*., 2006; Kiewnick and Sikora, 2006).

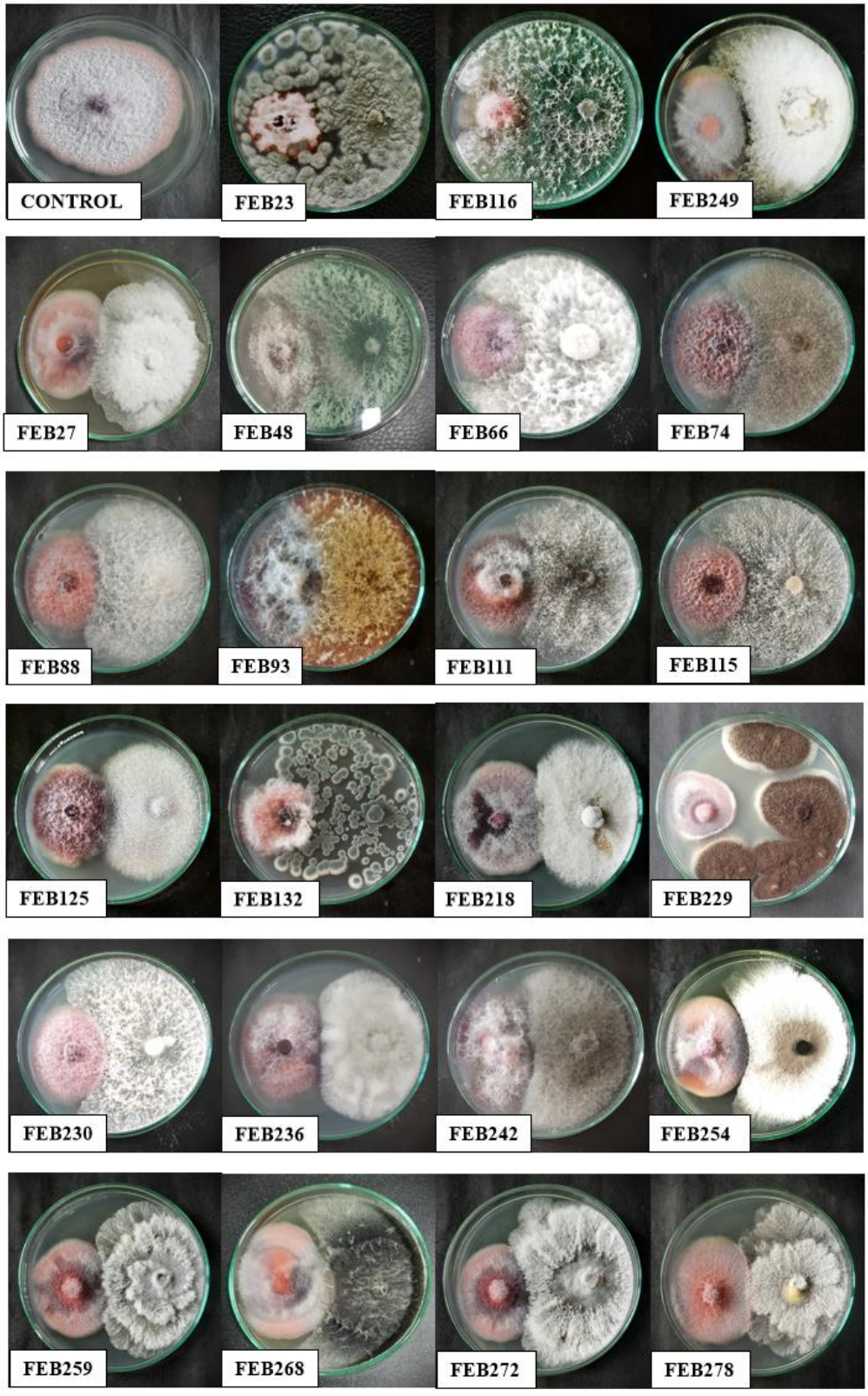


Plate 16. Antagonistic effect of the promising fungal endophyte isolates on the radial growth of the Fusarium wilt pathogen of banana

The potential mechanism of *Aspergillus* sp. maybe due to the secretion of bioactive compounds that inhibit the fungal mycelia growth through through lysis of cell wall of fungi (Gomathi and Ambikapathy, 2011). Many have accounted that the *Aspergillus*genus can make lytic enzymes like glucanase (Gao *et al*., 2008) and proteases (Sethi *et al*., 2016). Additionally, they are known to produce bioactive compounds (Tiwari *et al*., 2011 and Goutam *et al*., 2017).

Thangavelu and Gopi (2015b) reported the inhibitory effect of *Trichoderma* isolates against*Fusarium oxysporum* f. sp. *cubense* (foc). They found that six *Trichoderma* isolates from rhizosphere and 10 endophytic *Trichoderma* isolates could inhibit the pathogen growth*in vitro*. Under greenhouse condition, *Trichoderma* sp. NRCB3 + endophytic *Trichoderma asperellum* Prr2 could completely control the pathogengrowth.Related work was performed by Lalngaihawmi and Bhattacharyya (2019) who reported on the screening of 54 native rhizospheric microbes against *Fusarium oxysporum* f. sp. *cubense* (foc)*in vitro* and found that *Trichoderma reesei* and *T. harzianum* were the best performing isolates that could inhibit the growth of the pathogen. Various records have exhibited that *Trichoderma* species can successfully control the Fusarium wilt pathogen of banana (Sivan and Chet, 1986; Thangavelu *et al*., 2004).

Reports also shows *Trichoderma* species possessing the ability to manage various plant pathogenic diseases (Abdel-Fattah *et al*., 2007, Ru and Di, 2012). The potential of *Trichoderma* sp. to reduce the severity of Fusarium wilt maybe by means of mycoparasitism, competition for nutrients and space, antibiosis by enzymes and secondary metabolites, and stimulation of the plant defense system (Papavisas, 1985). *Trichoderma* species are known to produce antifungal compounds and other secondary metabolites (Thangavelu and Gopi, 2015b; Nagamani *et al*., 2017) which may show growth inhibiting properties and act as a defense mechanism against fungal pathogens.

Fungal endophytes belonging to *Diaporthe* genus from *Pachystachys lutea* were found to be antagonistic against *F. oxysporum* (Ribeiro *et al*., 2018). Abramczyk *et al*. (2022) reported that *Diaportheeres* from *Prunus dulcis* showed antagonistic activity against *Fusarium avenaceum*. Several others have reported on the antifungal and antibacterial properties of *Diaporthe* species against plant pathogens (Polonio *et al*., 2015; Carvalho *et al*., 2018, Hilario and Goncalves, 2022 and Verma *et al*., 2022).

Diaporthe genus as endophyte is known to produce secondary metabolites, and have been significantly inspected for their important compound production with various bioactivities (Abramczyk *et al*., 2022).

Xu *et al*. (2021) reported 335 secondary metabolites as bioactive compounds isolated from *Diaporthe* and *Phomopsis* species. Classification of metabolites were as polyketides, steroids, macrolides, terpenoids, alkaloids, ten-membered lactones, fatty acids and flavonoids. Taking into consideration of the numerous compounds produced by the genus *Diaporthe*, it can be said that this genus plays a vital role in the biocontrol activities against many plant pathogens.

4.8.2 Volatile metabolite production

The effects of volatile metabolite production by all the 281 isolated fungal endophytes were tested against the Fusarium wilt pathogen of banana by inverted plate method as given by Dennis and Webster (1971). The data thus obtained are presented in Table 4.9 and Plate 17. All the isolates varied in their antagonistic activity against the pathogen. Out of all the isolates tested against the pathogen, FEB81 (*Colletotrichum kahawae*) with 54.81% followed by, FEB80 (*Apiosporahydei*) with 54.07%, FEB115 (*Diaporthefructicola*) and FEB1 (*Penicillium* sp.) with 52.59%, significantly inhibited the mycelial growth of the pathogen through the production of volatile compounds. The inhibition per cent of the three fungal endophyte isolates were found to be

statistically at par. The per cent inhibition by the remaining isolates varied from 0% for FEB12

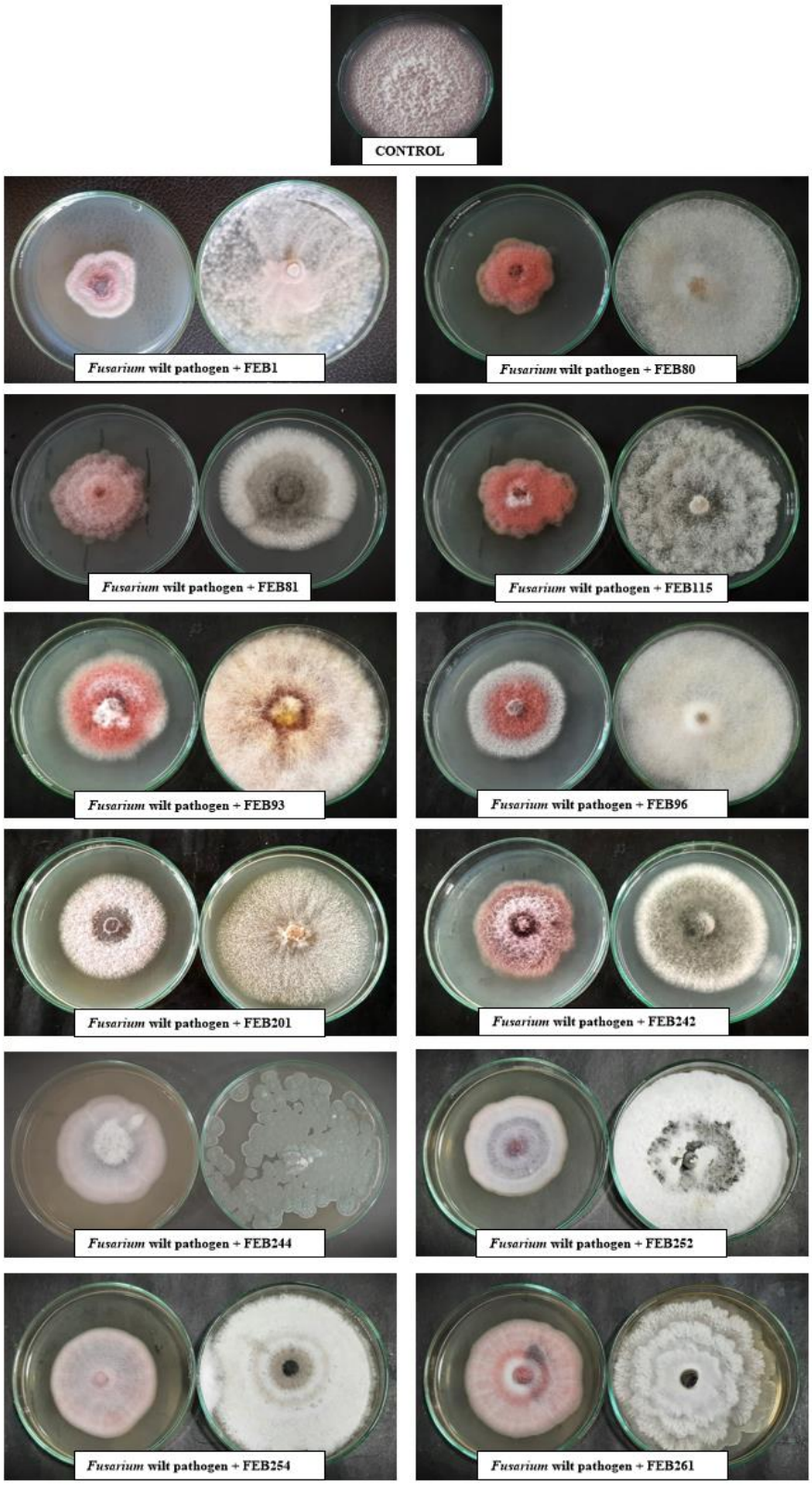


Plate 17. Antagonistic effect of volatile metabolites from the promising fungal endophyte isolates against Fusarium wilt pathogen of banana

(*Penicillium* sp.) and FEB13 (Unidentified) where no mycelial growth pathogen inhibition was observed to 45.19% for FEB201 (Unidentified).

Of the best four performing isolates, three were isolated from the leaves of wild banana plant (FEB80 and FEB81 from Kohima and FEB1 from Chumoukedima district) and one (FEB115) from the roots of wild banana plant isolated from Kohima district.

Several workers have reported on the volatile compoundsproduction by endophytes. Monggoot*et al*. (2017) reported that fungal endophytes that belonged to the genus *Colletotrichum* sp. MFLUCC16-0047, *Colletotrichum* sp. MFLUCC16-0048,*Arthrinium* sp. MFLUCC16-0042 and *Diaporthe* MFLUCC16-0051 produced a wide range of volatile compounds that had bioactivities against plant pathogens.

Fungal endophyte, *Colletotrichum truncatum* isolated from *Jatropacurcas*, an oil seed crop could effectively control *Fusarium sclerotiorum* through the production of volatile compounds (Kumar and Kaushik, 2013). Rabha *et al*. (2014) also described that the endophyte *Colletotrichum gloeosporioides* isolated from *Camellia sinensis*, (Assam, India) showed inhibitory effect as a result of volatile compound production against the pathogen, *Pestalotiopsis theae* with a per cent inhibition of 64%.

Song *et al*. (2019) isolated fungal endophyte*Diaportheapiculatum* strain FPYF 3052 which was found to inhibit 8 pathogens of plantsthrough the volatile metabolites production with a per cent inhibition range of 23.80% to 66.70%. Santra and Banerjee (2023) reported a fungal endophyte, *Diaporthe* sp. CEL3 which was isolated from leaves of an ethnomedicinal plant *Chloranthus elatior* Sw., from Arunachal Pradesh, and the endophyte isolate was found to produce volatile compounds inhibiting several important plant pathogens.

Penicillium commune (CIMO 14FM009), an endophyte has been found to obstruct the growth of several pathogens like *Botrytis cinerea* (Miles *et al*.,

2012), *Pyricularia oryzae* (Hosseyni*et al*., 2013) and *Sclerotinia* sp. (Katoch and Pull, 2017) under *in vitro* conditions.

The inhibitory effects of volatile compounds maybe due to the various volatile organic compounds produced by endophytes such as aldehydes, alcohols, cyclohexanes, benzene derivatives, hydrocarbons, heterocycles, ketones, phenols, thioalcohols and thioesters (Morath *et al*., 2012; Zhang *et al*., 2015 and Wang *et al*., 2018).

4.8.3 Non-volatile metabolites production

The non-volatile compounds produced by all the 281 isolated fungal endophytes was performed as per the procedure given by Dennis and Webster (1971). The data are presented in Table 4.9 and Plate 18. All the isolates that were assessed against the pathogen showed variation in terms of per cent inhibition over the control treatment. The inhibition per cent was observed to be highest in FEB3 (*Fusarium haematococcum*) with 69.21% followed by FEB9 (*Fusarium solani*) with 68.32% and FEB5 (*Trichoderma hamatum*) with 66.33% inhibition (Plate 15). The inhibition per cent of the three isolates were observed to be statistically at par with each other. The per cent inhibition of the rest of the isolates varied from 1.97% for FEB6 (*Trichoderma* sp.) to 54.46% for FEB105 (*Alternaria* sp.).

All the best three isolates for this test were isolated from the leaves of wild banana of Chumoukedima district.

Prior researches have exhibited that production of non-volatile compounds by endophytes imparts inhibitory effects against plant pathogens and has become a focus for new approaches for controlling various diseases in an eco-friendly manner.

Li *et al*. (2014) studied the fungal endophytes isolated from the leaf, roots and stems of cotton plants and investigated the activity of the isolated endophytes for their non-volatile metabolite production. The

endophyte*Fusarium solani*wasfound to completely inhibit the growth of *Verticillium*

Plate 18. Antagonistic effect of non-volatile metabolites from the promising fungal endophyte isolates against Fusarium wilt pathogen of banana

Table 4.9. Antagonistic Effect of the Isolated Fungal Endophytes against the Fusarium Wilt Pathogen of Banana

Isolates/Treatment	Dual Culture		Volatile		Non-Volatile	
	Radial Mycelial growth (cm)	Inhibition (%)	Radial Mycelial growth (cm)	Inhibition (%)	Radial Mycelial growth (cm)	Inhibition (%)
Control (*Fusarium oxysporum*)	3.50	0.00 (0.57)	4.50	0 $(1.28)^{n}$	3.37	0 $(0.57)^{o}$
FEB1(*Penicillium* sp.)	2.43	30.48 $(33.26)^{kl}$	**2.13**	**52.59 (46.49)^{a}**	2.53	24.63 $(29.66)^{jk}$
FEB2	2.57	26.67 $(31.08)^{lm}$	2.93	34.81 $(36.06)^{de}$	2.80	16.78 $(24.13)^{kl}$
FEB3(*Fusarium haematococcum*)	2.43	30.48 $(33.49)^{kl}$	3.80	15.56 $(23.09)^{jk}$	**1.03**	**69.21 (56.32)^{a}**
FEB4	2.67	23.81 $(29.15)^{mn}$	3.07	31.85 $(34.35)^{ef}$	3.17	5.92 $(13.79)^{mn}$
FEB5(*T. hamatum*)	1.67	52.38 $(46.37)^{cd}$	3.00	33.33 $(35.26)^{de}$	**1.13**	**66.33 (54.53)^{a}**
FEB6	2.23	36.19 $(36.98)^{ij}$	3.07	31.85 $(34.35)^{ef}$	3.30	1.97 $(5.06)^{n}$
FEB7	2.00	42.86 $(40.89)^{fg}$	4.00	11.11 $(19.47)^{kl}$	2.27	16.57 $(34.79)^{kl}$
FEB8	2.33	33.33 $(35.23)^{jk}$	4.00	11.11 $(19.47)^{kl}$	2.80	16.57 $(23.34)^{kl}$
FEB9(*Fusarium solani*)	2.27	35.24 $(36.41)^{j}$	3.83	14.81 $(22.36)^{jk}$	**1.07**	**68.32 (55.75)^{a}**
FEB10	2.70	22.86 $(28.46)^{mn}$	4.07	9.63 $(17.97)^{kl}$	3.20	4.82 $(10.54)^{n}$
FEB11	2.63	24.76 $(29.83)^{m}$	4.33	3.74 $(7.34)^{mn}$	3.13	6.73 $(12.27)^{mn}$
FEB12	2.07	40.95 $(39.75)^{gh}$	4.50	0 $(1.28)^{n}$	3.13	6.78 $(12.55)^{mn}$
FEB13	2.10	40.00 $(39.21)^{ghi}$	4.50	0 $(1.28)^{n}$	3.13	6.90 $(15.18)^{mn}$
FEB14	3.13	10.48 $(18.75)^{rs}$	4.33	3.74 $(7.34)^{mn}$	3.13	6.78 $(12.55)^{mn}$
FEB15	2.27	35.24 $(36.41)^{j}$	3.60	20 $(26.18)^{hi}$	2.57	23.62 $(29.00)^{jk}$
FEB16	2.77	20.95 $(27.08)^{no}$	4.00	11.11 $(19.47)^{kl}$	3.23	3.90 $(9.19)^{n}$
FEB17	2.53	27.62 $(31.68)^{lm}$	4.07	9.63 $(17.97)^{kl}$	2.83	15.82 $(23.43)^{kl}$

FEB18	2.67	23.81 (29.20)^{mn}	3.57	20.74 (26.70)^{hi}	2.57	23.62 (29.00)^{jk}
FEB19	2.10	40.00 (39.23)^{ghi}	3.30	26.67 (31.05)^{fg}	2.57	23.71 (29.12)^{jk}
FEB20	1.97	43.81 (41.42)^{fg}	3.27	27.41 (31.55)^{fg}	2.67	20.64 (26.91)^{jk}
FEB21	2.13	39.05 (38.61)^{hij}	3.37	25.19 (30.09)^{gh}	2.47	26.38 (30.51)^{hij}
FEB22	2.63	24.76 (29.69)^{mn}	2.80	37.78 (37.91)^{d}	2.53	24.69 (29.77)^{jk}
FEB23(*Aspergillus versicolor*)	**1.57**	**55.24 (48.01)^{bc}**	3.87	14.07 (21.84)^{jk}	2.60	22.87 (28.47)^{jk}
FEB24	2.07	40.95 (39.78)^{gh}	3.63	19.26 (26.00)^{hi}	2.53	24.75 (29.83)^{ijk}
FEB25	2.50	28.57 (32.31)^{kl}	4.07	9.63 (17.97)^{kl}	2.67	20.67 (26.95)^{jk}
FEB26	2.17	38.10 (38.07)^{hij}	4.17	7.42 (13.41)^{lm}	2.53	24.66 (29.74)^{jk}
FEB27	1.67	52.38 (46.37)^{cd}	4.17	7.42 (13.41)^{lm}	2.67	20.64 (26.91)^{jk}
FEB28	2.30	34.29 (35.83)^{jk}	3.17	29.63 (32.88)^{fg}	2.73	18.71 (25.56)^{jk}
FEB29	2.93	16.19 (23.38)^{pq}	4.17	7.42 (13.41)^{lm}	2.73	18.59 (25.14)^{jk}
FEB30	1.87	46.67 (43.09)^{ef}	3.93	12.59 (20.72)^{kl}	2.57	23.73 (29.14)^{jk}
FEB31	2.83	19.05 (25.86)^{op}	4.07	9.63 (17.97)^{kl}	2.67	20.49 (26.39)^{jk}
FEB32	3.07	12.38 (20.48)^{qr}	3.97	11.85 (19.62)^{kl}	2.73	18.74 (25.61)^{jk}
FEB33	2.57	26.67 (31.06)^{lm}	3.70	17.78 (24.91)^{ij}	2.77	17.67 (24.70)^{kl}
FEB34	2.53	27.62 (31.59)^{lm}	3.53	21.48 (27.35)^{hi}	2.77	17.67 (24.70)^{kl}
FEB35	1.87	46.67 (43.09)^{ef}	3.63	19.26 (26.00)^{hi}	2.73	18.71 (25.56)^{jk}
FEB36	2.07	40.95 (39.78)^{gh}	3.17	29.63 (32.98)^{fg}	2.73	18.71 (25.56)^{jk}
FEB37	2.00	42.86 (40.87)^{fg}	3.73	17.04 (24.18)^{ij}	2.77	17.67 (24.70)^{kl}
FEB38	1.87	46.67 (43.09)^{ef}	2.93	34.81 (36.13)^{de}	2.73	18.53 (24.99)^{jk}
FEB39	2.00	42.86	3.13	30.37	2.70	19.77

		(40.89)^{fg}		(33.43)^{ef}		(26.11)^{jk}
FEB40	2.63	24.76 (29.69)^{mn}	3.40	24.44 (29.59)^{gh}	2.73	18.83 (25.50)^{jk}
FEB41	1.80	48.57 (44.18)^{de}	3.13	30.37 (33.43)^{ef}	2.57	23.82 (29.18)^{jk}
FEB42	2.00	42.86 (40.89)^{fg}	3.47	22.96 (28.38)^{gh}	2.63	21.68 (27.56)^{jk}
FEB43	2.07	40.95 (39.78)^{gh}	3.30	26.67 (31.05)^{fg}	2.70	19.57 (25.78)^{jk}
FEB44	3.13	10.48 (18.75)^{rs}	4.07	9.63 (17.97)^{kl}	2.87	14.75 (22.34)^{kl}
FEB45	2.53	27.62 (31.70)^{lm}	4.17	7.42 (13.41)^{lm}	3.27	2.86 (6.06)^{n}
FEB46	2.00	42.86 (40.89)^{fg}	2.53	43.70 (41.37)^{bc}	3.27	2.86 (6.06)^{n}
FEB47	2.17	38.10 (38.11)^{hij}	2.87	36.30 (37.04)^{d}	3.23	3.87 (9.48)^{n}
FEB48	2.03	41.90 (40.34)^{fg}	4.17	7.42 (13.41)^{lm}	2.57	23.64 (29.03)^{jk}
FEB49	2.87	18.10 (25.11)^{op}	4.07	9.63 (17.97)^{kl}	3.20	4.77 (7.78)^{n}
FEB50	1.93	44.76 (41.99)^{fg}	3.93	12.59 (20.72)^{jk}	3.20	4.82 (10.54)^{n}
FEB51	2.30	34.29 (35.80)^{jk}	3.23	28.15 (31.97)^{fg}	2.53	24.69 (29.77)^{j}
FEB52	2.90	17.14 (24.33)^{op}	3.93	12.59 (20.72)^{jk}	2.47	26.68 (31.09)^{hij}
FEB53	2.93	16.19 (23.66)^{pq}	3.30	26.67 (31.05)^{fg}	2.63	21.51 (27.26)^{jk}
FEB54	2.87	18.10 (25.08)	4.07	9.63 (17.97)^{kl}	2.77	17.73 (24.80)^{kl}
FEB55	3.20	8.57 (16.32)st	3.87	14.07 (21.98)^{jk}	2.87	14.81 (22.58)^{kl}
FEB56	2.93	16.19 (23.66)^{pq}	4.07	9.63 (17.97)^{kl}	2.73	18.74 (25.61)^{jk}
FEB57	3.13	10.48 (18.75)^{rs}	3.93	12.59 (20.72)^{jk}	2.83	15.79 (23.30)^{kl}
FEB58	2.77	20.95 (27.23)^{no}	4.13	8.15 (16.47)^{kl}	2.63	21.68 (27.70)^{jk}
FEB59	2.07	40.95 (39.78)^{gh}	3.57	20.74 (27.06)^{hi}	2.53	24.66 (29.74)^{jk}
FEB60	2.13	39.05 (38.66)^{hij}	3.73	17.04 (24.18)^{ij}	2.57	23.59 (28.91)^{jk}

FEB61	2.47	29.52 (32.91)^{kl}	4.40	2.26 (5.84)^{mn}	2.43	27.57 (31.61)^{hij}
FEB62	2.00	42.86 (40.87)^{fg}	3.57	20.74 (27.06)^{hi}	2.40	28.58 (32.21)^{hij}
FEB63	2.07	40.95 (39.78)^{gh}	3.40	24.44 (29.59)^{gh}	2.57	23.68 (29.06)^{jk}
FEB64	1.93	44.76 (41.99)^{f}	3.80	15.56 (23.12)^{ij}	2.33	30.57 (33.52)^{hi}
FEB65	2.30	34.29 (35.81)^{jk}	3.90	13.33 (21.29)^{jk}	2.40	28.61 (32.31)^{hij}
FEB66	1.93	44.76 (41.99)^{f}	4.00	11.13 (16.29)^{jk}	2.57	23.73 (29.14)^{jk}
FEB67	1.77	49.52 (44.73)^{de}	3.83	14.81 (22.36)^{jk}	2.43	27.57 (31.61)^{hij}
FEB68	2.07	40.95 (39.78)^{gh}	3.30	26.67 (30.90)^{f}	2.50	25.64 (30.39)^{ij}
FEB69	1.87	46.67 (43.09)^{ef}	3.73	17.04 (24.18)^{ij}	2.57	23.56 (28.85)^{jk}
FEB70	2.07	40.95 (39.78)^{gh}	4.07	9.63 (17.97)^{kl}	2.40	28.64 (32.33)^{hij}
FEB71	2.13	39.05 (38.66)^{hij}	3.73	17.04 (24.18)^{ij}	2.47	26.62 (31.01)^{hij}
FEB72	1.93	44.76 (41.99)^{f}	3.53	21.48 (27.46)^{hi}	2.57	23.56 (28.85)^{jk}
FEB73	2.07	40.95 (39.78)^{gh}	3.73	17.04 (24.18)^{ij}	2.80	16.75 (23.97)^{kl}
FEB74	1.73	50.48 (45.27)^{de}	2.93	34.81 (36.15)^{de}	2.60	22.69 (28.39)^{jk}
FEB75	2.30	34.29 (35.80)^{jk}	2.80	37.78 (37.89)^{d}	2.60	22.58 (28.20)^{jk}
FEB76	2.07	40.95 (39.78)^{gh}	2.90	35.56 (36.59)^{de}	2.63	21.86 (27.85)^{jk}
FEB77	2.13	39.05 (38.66)^{hij}	3.67	18.52 (25.43)^{ij}	2.60	22.61 (28.20)^{jk}
FEB78	2.27	35.24 (36.40)^{j}	3.77	16.30 (23.80)^{ij}	2.53	24.78 (29.84)^{ij}
FEB79	2.10	40.00 (39.22)^{ghi}	3.90	13.33 (21.29)^{jk}	2.70	19.63 (26.05)^{jk}
FEB80(*Apiosporahydei*)	2.07	40.95 (39.78)^{gh}	**2.07**	**54.07 (47.34)^{a}**	2.63	21.68 (27.56)^{jk}
FEB81(*Colletotrichum kahawae*)	2.00	42.86 (40.89)^{fg}	**2.03**	**54.81 (47.76)^{a}**	2.63	21.65 (27.60)^{jk}
FEB82	1.90	45.71	2.90	35.56	2.73	18.71

		(42.53)^{ef}		(36.53)^{de}		(25.56)^{jk}
FEB83	2.07	40.95 (39.78)^{gh}	3.13	30.37 (33.43)^{ef}	2.57	23.71 (29.12)^{jk}
FEB84	2.20	37.14 (37.51)^{hij}	3.30	26.67 (31.05)^{fg}	2.73	18.74 (25.61)^{jk}
FEB85	1.87	46.67 (43.09)^{ef}	3.13	30.37 (33.43)^{ef}	2.60	22.69 (28.39)^{jk}
FEB86	2.33	33.33 (35.19)^{jk}	3.40	24.44 (29.59)^{gh}	2.67	20.61 (26.81)^{jk}
FEB87	2.53	27.62 (31.70)^{lm}	3.07	31.85 (34.35)^{ef}	2.63	21.51 (27.26)^{jk}
FEB88	1.80	48.57 (44.18)^{de}	2.67	40.74 (39.63)^{bcd}	2.47	26.77 (31.15)^{hij}
FEB89	2.03	41.90 (40.34)^{fg}	2.90	35.56 (36.59)^{d}	2.57	23.67 (29.02)^{jk}
FEB90	2.07	40.95 (39.72)^{gh}	3.57	20.74 (27.06)^{hi}	2.67	20.64 (26.91)^{jk}
FEB91	2.43	30.48 (33.44)^{k}	2.83	37.04 (37.47)^{d}	2.63	21.59 (27.53)^{jk}
FEB92	2.37	32.38 (34.65)^{jk}	3.33	25.93 (30.51)^{fg}	2.63	21.86 (27.85)^{jk}
FEB93	1.93	44.76 (41.99)^{ef}	2.73	39.26 (38.77)^{cd}	2.03	39.56 (38.97)^{efg}
FEB94	2.40	31.43 (34.06)^{jk}	3.07	31.85 (34.35)^{ef}	2.17	35.64 (36.61)^{efg}
FEB95	2.57	26.67 (31.08)^{lm}	2.77	38.52 (38.34)^{cd}	1.93	42.53 (40.70)^{cde}
FEB96	2.67	23.81 (29.17)^{mn}	2.63	41.48 (40.09)^{bcd}	2.00	40.33 (39.36)^{ef}
FEB97	1.93	44.76 (41.97)^{f}	2.53	43.70 (41.38)^{bc}	2.23	33.58 (35.40)^{gh}
FEB98	2.43	30.48 (33.44)^{k}	2.67	40.74 (39.63)^{bcd}	1.90	43.43 (41.20)^{cde}
FEB99	2.27	35.24 (36.40)^{j}	2.90	35.56 (36.60)^{de}	2.13	36.47 (37.11)^{efg}
FEB100	2.00	42.86 (40.89)^{fg}	2.53	43.70 (41.38)^{bc}	2.07	38.55 (38.37)^{efg}
FEB101	2.23	36.19 (36.96)^{ij}	2.80	37.78 (37.91)^{de}	2.17	35.51 (36.56)^{efg}
FEB102	2.40	31.43 (34.06)^{k}	3.30	26.67 (31.05)^{fg}	2.53	24.78 (29.84)^{ij}
FEB103	2.43	30.48 (33.47)^{k}	3.50	22.22 (27.96)^{gh}	2.60	22.58 (28.20)^{jk}

FEB104	2.2	37.14 (37.55)^{hij}	2.97	34.07 (35.71)^{ef}	2.10	37.54 (37.77)^{efg}
FEB105	2.27	35.24 (36.36)^{j}	3.57	20.74 (27.06)^{hi}	1.53	54.46 (47.56)^{b}
FEB106	2.33	33.33 (35.25)^{jk}	3.57	20.74 (27.09)^{hi}	2.20	34.51 (35.91)^{fg}
FEB107	2.07	40.95 (39.78)^{gh}	2.97	34.07 (35.71)^{ef}	2.47	26.68 (31.09)^{hij}
FEB108	2.3	34.29 (35.80)^{jk}	3.40	24.44 (29.59)^{gh}	2.17	35.51 (36.56)^{efg}
FEB109	2.13	39.05 (38.66)^{hij}	2.97	34.07 (35.71)^{ef}	1.73	48.40 (44.08)^{bc}
FEB110	2.23	36.19 (36.96)^{ij}	3.90	13.33 (21.29)^{jk}	2.57	23.73 (29.14)^{jk}
FEB111	1.73	50.48 (45.27)^{de}	4.13	8.15 (16.47)^{kl}	2.63	21.68 (27.56)^{jk}
FEB112	2.57	26.67 (31.06)^{lm}	3.80	15.56 (23.12)^{jk}	2.33	30.28 (32.95)^{hi}
FEB113	2.47	29.52 (32.91)^{kl}	4.07	9.63 (18.05)^{lm}	2.57	23.64 (29.03)^{jk}
FEB114	2.77	20.95 (27.23)^{no}	3.90	13.33 (21.29)^{jk}	2.63	21.86 (27.85)^{jk}
FEB115(*Diaporthefructicola*)	1.73	50.48 (45.27)^{de}	**2.13**	**52.59 (46.49)^{a}**	2.47	26.53 (30.87)^{hij}
FEB116(*T. asperellum*)	**1.33**	**61.90 (51.90)^{a}**	3.57	20.74 (27.06)^{h}	2.53	24.51 (29.45)^{jk}
FEB117	1.93	44.76 (41.99)^{f}	3.80	15.56 (23.12)^{jk}	2.67	20.67 (26.95)^{jk}
FEB118	1.93	44.76 (41.99)^{f}	3.93	12.59 (20.72)^{jk}	2.60	22.63 (28.23)^{jk}
FEB119	2.63	24.76 (29.78)^{mn}	3.83	14.81 (22.36)^{jk}	2.10	37.60 (37.81)^{efg}
FEB120	2.3	34.29 (35.80)^{k}	4.13	8.15 (16.47)^{kl}	2.57	23.71 (29.12)^{jk}
FEB121	1.87	46.67 (43.09)^{ef}	3.97	11.85 (20.12)^{kl}	2.63	21.83 (27.80)^{jk}
FEB122	2.43	30.48 (33.50)^{k}	3.67	18.52 (25.43)^{ij}	2.53	24.63 (29.66)^{jk}
FEB123	2.43	30.48 (33.50)^{k}	3.97	11.85 (20.12)^{kl}	2.63	21.59 (27.53)^{jk}
FEB124	1.93	44.76 (41.99)^{f}	3.80	15.56 (23.12)^{jk}	3.07	8.68 (14.09)^{mn}
FEB125	2	42.86	4.07	9.63	2.07	38.43

		(40.89)^{fg}		(17.97)^{lm}		(38.27)^{efg}
FEB126	2.13	39.05 (38.63)^{hij}	3.67	18.52 (25.24)^{ij}	2.63	21.77 (27.81)^{jk}
FEB127	1.93	44.76 (41.99)^{f}	3.90	13.33 (21.29)^{jk}	2.63	21.57 (27.41)^{jk}
FEB128	2.53	27.62 (31.70)^{lm}	3.90	13.33 (21.29)^{jk}	2.53	24.63 (29.66)^{jk}
FEB129	2.53	27.62 (31.70)^{lm}	4.10	8.89 (17.26)^{lm}	2.53	24.59 (29.43)^{jk}
FEB130	2.37	32.38 (34.65)^{jk}	3.67	18.52 (25.24)^{ij}	2.67	20.67 (26.95)^{jk}
FEB131	2.6	25.71 (30.45)^{mn}	3.57	20.74 (27.06)^{hi}	3.07	8.86 (17.19)^{lm}
FEB132	1.73	50.48 (45.27)^{de}	4.07	9.63 (17.97)^{l}	2.53	24.72 (29.77)^{jk}
FEB133	1.93	44.76 (41.99)^{f}	3.93	12.59 (20.59)^{jk}	2.47	26.62 (31.01)^{hi}
FEB134	1.8	48.57 (44.18)^{de}	3.97	11.85 (20.12)^{kl}	1.77	47.26 (43.41)^{cd}
FEB135	2.3	34.29 (35.80)^{jk}	3.97	11.85 (19.79)^{kl}	2.53	24.71 (29.76)^{jk}
FEB136	1.87	46.67 (43.09)^{ef}	3.60	20.00 (26.49)^{hi}	2.60	22.60 (28.24)^{jk}
FEB137	2.73	21.90 (27.90)^{n}	3.73	17.04 (24.18)^{ij}	2.63	21.66 (27.60)^{jk}
FEB138	2.67	23.81 (29.08)^{mn}	3.63	19.26 (26.00)^{hi}	2.70	19.60 (25.75)^{jk}
FEB139	2.23	36.19 (36.98)^{ij}	2.83	37.04 (37.49)^{d}	2.27	332.75 (34.88)^{ghi}
FEB140	2.2	37.14 (37.51)^{hij}	2.90	35.56 (36.56)^{de}	2.47	26.59 (30.98)^{hi}
FEB141	2.43	30.48 (33.50)^{k}	2.83	37.04 (37.47)^{d}	2.47	26.68 (31.09)^{hi}
FEB142	2.63	24.76 (29.78)^{mn}	3.67	18.52 (25.48)^{ij}	2.63	21.59 (27.53)^{jk}
FEB143	2.47	29.52 (32.91)^{kl}	3.70	17.78 (24.83)^{ij}	2.47	26.62 (31.01)^{hi}
FEB144	2.87	18.10 (25.11)^{op}	3.47	22.96 (28.53)^{hi}	2.60	22.82 (28.51)^{jk}
FEB145	3.07	12.38 (20.48)^{qr}	3.40	24.44 (29.59)^{gh}	2.77	17.67 (24.70)^{kl}
FEB146	2.63	24.76 (29.81)^{m}	3.70	17.78 (24.83)^{ij}	2.70	19.75 (26.24)^{jk}

FEB147	2.87	18.10 (25.16)^{op}	3.47	22.96 (28.63)^{hi}	2.63	21.86 (27.85)^{jk}
FEB148	2.63	24.76 (29.74)^{m}	3.50	22.22 (28.11)^{hi}	2.83	15.67 (23.08)^{kl}
FEB149	3.1	11.43 (19.42)^{rs}	3.80	15.56 (23.00)^{j}	2.83	15.80 (23.30)^{kl}
FEB150	2.07	40.95 (39.78)^{gh}	3.13	30.37 (33.43)^{ef}	2.33	30.48 (33.39)^{hi}
FEB151	3.07	12.38 (20.48)^{qr}	2.73	39.26 (38.80)^{cd}	2.30	31.44 (33.96)^{hi}
FEB152	2.83	19.05 (25.78)^{no}	3.47	22.96 (28.53)^{hi}	2.57	23.53 (28.80)^{jk}
FEB153	2.43	30.48 (33.50)^{kl}	3.40	24.44 (29.59)^{gh}	2.63	21.71 (27.75)^{jk}
FEB154	2.93	16.19 (23.66)^{pq}	3.33	25.93 (30.51)^{gh}	2.63	21.71 (27.75)^{jk}
FEB155	3.33	4.76 (12.16)^{t}	3.57	20.74 (27.06)^{hi}	2.53	24.54 (29.48)^{jk}
FEB156	2.93	16.19 (23.66)^{pq}	3.53	21.48 (27.61)^{hi}	2.53	24.71 (29.76)^{jk}
FEB157	2.8	20.00 (26.41)^{no}	3.40	24.44 (29.59)^{gh}	2.27	32.63 (34.83)^{h}
FEB158	2.27	35.24 (36.36)^{j}	3.60	20.00 (26.54)^{hi}	2.77	17.76 (24.90)^{kl}
FEB159	2.57	26.67 (31.08)^{lm}	3.67	18.52 (25.43)^{ij}	2.67	20.73 (27.07)^{jk}
FEB160	2.87	18.10 (24.96)^{op}	3.53	21.48 (27.61)^{hi}	2.70	19.75 (26.35)^{jk}
FEB161	2.93	16.19 (23.66)^{pq}	3.50	22.22 (27.96)^{hi}	2.77	17.55 (24.02)^{kl}
FEB162	3.13	10.48 (18.75)^{rs}	3.67	18.52 (25.43)^{ij}	2.63	21.68 (27.56)^{jk}
FEB163	2.7	22.86 (28.37)^{mn}	3.37	25.19 (30.06)^{gh}	2.67	20.46 (26.30)^{jk}
FEB164	2.3	34.29 (35.77)^{jk}	3.07	31.85 (34.35)^{ef}	2.20	34.50 (35.92)^{fgh}
FEB165	2.5	28.57 (32.29)^{kl}	3.53	21.48 (27.44)^{hi}	2.63	21.83 (27.80)^{jk}
FEB166	2.2	37.14 (37.55)^{hij}	3.33	25.93 (30.58)^{gh}	2.57	23.73 (29.14)^{jk}
FEB167	2.17	38.10 (38.11)^{hij}	3.87	14.07 (21.98)^{jk}	2.70	19.68 (26.00)^{jk}
FEB168	2.77	20.95	3.00	33.33	2.43	27.63

		(27.23)^{no}		(35.26)^{ef}		(31.69)^{hi}
FEB169	2.43	30.48 (33.50)^{kl}	3.03	32.59 (34.81)^{ef}	2.73	18.74 (25.61)^{jk}
FEB170	3.27	6.67 (14.59)st	3.50	22.22 (27.96)^{hi}	2.73	18.74 (25.61)^{jk}
FEB171	3	14.29 (22.21)^{qr}	3.53	21.48 (27.48)^{hi}	2.67	20.59 (26.64)^{jk}
FEB172	3.27	6.67 (14.59)st	3.83	14.81 (22.55)^{jk}	2.37	29.59 (32.92)^{hi}
FEB173	3.07	12.38 (20.48)^{qr}	3.07	31.85 (34.35)^{ef}	2.77	17.70 (24.75)^{kl}
FEB174	2.93	16.19 (23.38)^{pq}	3.90	13.33 (21.29)^{jk}	2.63	21.75 (27.73)^{jk}
FEB175	3.2	8.57 (17.02)st	3.57	20.74 (27.06)^{hi}	2.53	24.71 (29.76)^{j}
FEB176	2.37	32.38 (34.68)^{k}	3.80	15.56 (23.12)^{ij}	2.57	23.58 (28.87)^{jk}
FEB177	2.13	39.05 (38.63)^{hij}	3.30	26.67 (31.05)^{fg}	2.47	26.68 (31.09)^{hij}
FEB178	2.63	24.76 (29.83)^{m}	3.57	20.74 (27.06)^{hi}	2.77	17.58 (24.38)^{kl}
FEB179	3.07	12.38 (20.48)^{qr}	4.13	8.15 (16.47)^{lm}	2.83	15.85 (23.44)^{kl}
FEB180	2.73	21.90 (27.69)^{mn}	3.87	14.07 (21.61)^{jk}	2.90	13.77 (21.63)^{lm}
FEB181	2.57	26.67 (31.08)^{lm}	4.17	7.41 (15.76)^{lm}	2.37	29.59 (32.92)^{hi}
FEB182	2.63	24.76 (29.81)^{m}	4.17	7.41 (15.53)^{lm}	2.80	16.69 (23.94)^{kl}
FEB183	2.73	21.90 (27.69)^{mn}	4.03	10.37 (18.51)^{kl}	2.70	19.77 (26.11)^{jk}
FEB184	2.87	18.10 (25.08)^{op}	4.17	7.41 (15.53)^{lm}	2.57	23.67 (29.02)^{jk}
FEB185	2.47	29.52 (32.91)^{kl}	3.93	12.59 (20.59)^{jk}	2.70	19.81 (26.33)^{jk}
FEB186	3.1	11.43 (19.66)^{rs}	3.60	20.00 (26.54)^{hi}	2.63	21.51 (27.26)^{jk}
FEB187	3.07	12.38 (20.48)^{qr}	3.40	24.44 (29.59)^{gh}	2.43	27.63 (31.69)^{hij}
FEB188	2.9	17.14 (24.41)^{op}	3.60	20.00 (26.54)^{hi}	2.43	27.48 (31.45)^{hij}
FEB189	3.27	6.67 (14.59)st	3.83	14.81 (22.55)^{jk}	2.37	29.59 (32.92)^{hij}

FEB190	2.8	20.00 (26.53)^{no}	3.53	21.48 (27.61)^{hi}	2.70	19.75 (26.35)^{jk}
FEB191	2.5	28.57 (32.24)^{l}	4.00	11.11 (19.41)^{kl}	2.53	24.78 (29.84)^{ij}
FEB192	2.6	25.71 (30.38)^{lm}	3.07	31.85 (34.35)^{ef}	2.83	15.79 (23.30)^{kl}
FEB193	2.5	28.57 (32.24)^{kl}	3.70	17.78 (24.91)^{ij}	2.77	17.70 (24.75)^{kl}
FEB194	2.53	27.62 (31.59)^{lm}	3.73	17.04 (24.26)^{ij}	2.77	17.69 (24.29)^{kl}
FEB195	2.63	24.76 (29.81)^{m}	3.00	33.33 (35.26)^{ef}	2.63	21.57 (27.41)^{jk}
FEB196	2.77	20.95 (27.16)^{no}	3.03	32.59 (34.81)^{ef}	2.77	17.63 (24.47)^{kl}
FEB197	2.47	29.52 (32.91)^{kl}	3.97	11.85 (20.02)^{kl}	2.73	18.71 (25.56)^{jk}
FEB198	3.03	13.33 (21.23)^{qr}	4.17	7.41 (15.53)^{lm}	2.83	15.79 (23.30)^{kl}
FEB199	2.73	21.90 (27.90)^{mn}	3.67	18.52 (25.37)^{ij}	2.63	21.83 (27.80)^{jk}
FEB200	2.43	30.48 (33.50)^{k}	3.07	31.85 (34.35)^{ef}	2.37	29.66 (32.99)^{hi}
FEB201	2.83	19.05 (25.86)^{no}	2.47	45.19 (42.24)^{b}	2.87	14.63 (21.96)^{kl}
FEB202	2.57	26.67 (31.08)^{lm}	3.37	25.19 (30.09)^{fg}	2.80	16.57 (23.40)^{kl}
FEB203	2.13	39.05 (38.66)^{hij}	3.00	33.33 (35.24)^{ef}	2.83	15.79 (23.30)^{kl}
FEB204	2.5	28.57 (32.24)^{kl}	3.23	28.15 (32.02)^{fg}	2.87	14.78 (22.57)^{kl}
FEB205	2.3	34.29 (35.77)^{jk}	3.97	11.85 (20.02)^{kl}	2.87	14.63 (21.96)^{kl}
FEB206	2.73	21.90 (27.90)^{mn}	3.97	11.85 (19.87)^{kl}	2.73	18.71 (25.56)^{jk}
FEB207	2.63	24.76 (29.78)^{m}	3.83	14.81 (22.55)^{jk}	1.97	41.55 (40.14)^{de}
FEB208	2.53	27.62 (31.59)^{lm}	3.97	11.85 (19.79)^{kl}	2.83	15.79 (23.30)^{kl}
FEB209	2.33	33.33 (35.25)^{jk}	3.17	29.63 (32.88)^{f}	2.57	23.56 (28.85)^{jk}
FEB210	2.37	32.38 (34.65)^{k}	3.47	22.96 (28.38)^{gh}	2.63	21.57 (27.41)^{jk}
FEB211	2.6	25.71	3.33	25.93	2.57	23.73

		(30.40)^{m}		(30.59)^{fg}		(29.14)^{jk}
FEB212	2.4	31.43 (34.08)^{k}	3.63	19.26 (25.89)^{hi}	2.03	39.56 (38.97)^{efg}
FEB213	2.1	40.00 (39.21)^{ghi}	3.50	22.22 (28.01)^{gh}	2.23	33.46 (35.25)^{gh}
FEB214	2.63	24.76 (29.78)^{mn}	3.57	20.74 (26.70)^{hi}	2.47	26.65 (31.04)^{hij}
FEB215	2.3	34.29 (35.84)^{jk}	2.93	34.81 (36.16)^{de}	2.03	39.56 (38.97)^{efg}
FEB216	2.53	27.62 (31.68)^{lm}	3.43	23.70 (29.07)^{gh}	2.63	21.77 (27.81)^{jk}
FEB217	2.37	32.38 (34.68)^{jk}	3.70	17.78 (24.76)^{ij}	2.83	15.71 (23.11)^{kl}
FEB218	2.07	40.95 (39.79)^{gh}	3.07	31.85 (34.35)^{ef}	2.73	18.71 (25.56)^{jk}
FEB219	2.13	39.05 (38.67)^{hij}	2.87	36.30 (37.03)^{de}	2.27	332.63 (34.71)^{gh}
FEB220	2.5	28.57 (32.29)^{kl}	3.40	24.44 (29.49)^{gh}	2.73	18.65 (25.42)^{jk}
FEB221	2.47	29.52 (32.91)^{kl}	3.50	22.22 (28.01)^{hi}	2.57	23.67 (29.02)^{jk}
FEB222	2.57	26.67 (31.04)^{lm}	3.83	14.81 (22.48)^{jk}	2.53	24.72 (29.77)^{jk}
FEB223	2.63	24.76 (29.81)^{m}	3.43	23.70 (29.03)^{gh}	2.80	16.71 (23.67)^{kl}
FEB224	2.3	34.29 (35.80)^{jk}	3.00	33.33 (35.26)^{ef}	2.67	20.67 (26.95)^{jk}
FEB225	2.77	20.95 (27.23)^{no}	3.47	22.96 (28.42)^{hi}	2.87	14.81 (22.58)^{kl}
FEB226	2.53	27.62 (31.66)^{l}	3.40	24.44 (29.49)^{gh}	2.53	24.78 (29.84)^{ij}
FEB227	2.23	36.19 (36.97)^{ij}	3.37	25.19 (30.09)^{gh}	2.67	20.85 (27.12)^{jk}
FEB228	2.63	24.76 (29.83)^{m}	3.63	19.26 (26.00)^{hi}	2.60	22.69 (28.39)^{jk}
FEB229	1.8	48.57 (44.18)^{de}	3.63	19.26 (25.93)^{hi}	2.83	15.71 (23.11)^{kl}
FEB230	1.87	46.67 (43.09)^{ef}	3.57	20.74 (27.06)^{hi}	2.40	28.61 (32.31)^{hij}
FEB231	2.17	38.10 (38.07)^{hij}	3.67	18.52 (25.43)^{ij}	2.50	25.58 (30.27)^{ij}
FEB232	2.03	41.90 (40.34)^{fg}	3.50	22.22 (28.05)^{hi}	2.67	20.73 (27.07)^{jk}

FEB233	2.07	40.95 (39.78)^{gh}	3.47	22.96 (28.53)^{hi}	2.57	23.59 (28.91)^{jk}
FEB234	2.67	23.81 (29.15)^{mn}	4.30	4.46 (10.40)^{mn}	2.63	21.68 (27.56)^{jk}
FEB235	2.87	18.10 (25.11)^{op}	4.07	9.63 (17.97)^{lm}	2.67	20.77 (26.97)^{jk}
FEB236	2.07	40.95 (39.78)^{gh}	4.00	11.11 (19.41)^{kl}	2.73	18.73 (25.25)^{jk}
FEB237	2	42.86 (40.89)^{fg}	3.97	11.85 (19.79)^{kl}	2.87	14.75 (22.34)^{kl}
FEB238	2.2	37.14 (37.51)^{hij}	4.23	5.93 (13.63)^{mn}	2.60	22.75 (28.49)^{jk}
FEB239	2.07	40.95 (39.78)^{gh}	2.93	34.81 (36.16)^{de}	2.07	38.61 (38.41)^{efg}
FEB240	1.87	46.67 (43.09)^{ef}	3.33	25.93 (30.55)^{fg}	2.27	32.63 (34.83)^{gh}
FEB241	1.93	44.76 (41.99)^{f}	2.93	34.81 (36.16)^{de}	2.17	35.51 (36.56)^{efg}
FEB242	1.93	44.76 (41.99)^{f}	2.53	43.70 (41.38)^{bc}	2.27	32.48 (34.67)^{gh}
FEB243	2.33	33.33 (35.25)^{jk}	2.97	34.07 (35.71)^{ef}	2.03	39.56 (38.97)^{efg}
FEB244	1.83	47.62 (43.63)^{ef}	2.83	37.04 (37.49)^{cd}	2.13	36.47 (37.11)^{efg}
FEB245	2.13	39.05 (38.66)^{hij}	3.30	26.67 (30.90)^{fg}	2.60	22.63 (28.23)^{jk}
FEB246	2.63	24.76 (29.74)^{mn}	3.37	25.19 (30.09)^{fg}	2.77	17.72 (24.73)^{kl}
FEB247	2.43	30.48 (33.50)^{k}	3.47	22.96 (28.55)^{gh}	2.97	11.81 (20.03)^{lm}
FEB248	2.5	28.57 (32.29)^{kl}	3.20	28.89 (32.43)^{f}	2.70	19.87 (26.44)^{jk}
FEB249(*Diaporthechromolaenae*)	**1.5**	**57.14 (49.11)^{b}**	2.70	40.00 (39.23)^{bcd}	2.37	29.57 (32.89)^{hi}
FEB250	2.83	19.05 (25.86)^{no}	3.20	28.89 (32.47)^{f}	2.27	32.69 (34.83)^{gh}
FEB251	2.8	20.00 (26.33)^{n}	3.43	23.70 (29.07)^{gh}	2.63	21.51 (27.26)^{jk}
FEB252	1.93	44.76 (41.97)^{f}	2.67	40.74 (39.66)^{bcd}	2.73	18.65 (25.42)^{jk}
FEB253	2.57	26.67 (31.06)^{lm}	3.23	28.15 (31.97)^{f}	2.80	16.71 (23.67)^{kl}
FEB254	2	42.86	2.83	37.04	2.10	37.63

		(40.89)^{fg}		(37.48)^{d}		(37.78)^{ef}
FEB255	2.63	24.76 (29.74)^{mn}	3.17	29.63 (32.96)^{f}	2.17	35.51 (36.56)^{ef}
FEB256	2.4	31.43 (34.06)^{k}	3.23	28.15 (32.04)^{f}	2.10	37.63 (37.84)^{ef}
FEB257	2.2	37.14 (37.55)^{hi}	2.93	34.81 (36.16)^{de}	2.17	35.51 (36.56)^{ef}
FEB258	2.07	40.95 (39.78)^{gh}	2.93	34.81 (36.16)^{de}	2.27	32.54 (34.67)^{h}
FEB259	1.73	50.48 (45.27)^{d}	3.50	22.22 (27.93)^{gh}	2.07	38.52 (38.35)^{ef}
FEB260	2.47	29.52 (32.91)^{kl}	2.90	35.56 (36.60)^{de}	2.27	32.42 (34.54)^{gh}
FEB261	2.27	35.24 (36.40)^{j}	2.93	34.81 (36.13)^{de}	1.97	41.55 (40.14)^{de}
FEB262	2.53	27.62 (31.68)^{lm}	2.90	35.56 (36.59)^{de}	2.00	40.51 (39.52)^{def}
FEB263	2.77	20.95 (27.23)^{n}	3.17	29.63 (32.93)^{f}	2.27	32.66 (34.84)^{gh}
FEB264	3.07	12.38 (20.48)^{qr}	3.63	19.26 (25.97)^{hi}	2.17	35.64 (36.61)^{ef}
FEB265	2.57	26.67 (31.08)^{l}	3.47	22.96 (28.53)^{gh}	2.53	24.71 (29.76)^{jk}
FEB266	2.43	30.48 (33.50)^{k}	3.63	19.26 (25.87)^{hi}	2.6	22.61 (28.20)^{jk}
FEB267	2.37	32.38 (34.65)^{k}	3.30	26.67 (30.77)^{gh}	2.07	38.52 (38.35)^{efg}
FEB268	1.87	46.67 (43.09)^{ef}	3.07	31.85 (34.35)^{ef}	2.03	39.44 (38.88)^{efg}
FEB269	2.53	27.62 (31.68)^{lm}	2.87	36.30 (37.03)^{de}	2.77	17.76 (24.90)^{kl}
FEB270	1.87	46.67 (43.09)^{ef}	3.47	22.96 (28.42)^{gh}	2.07	38.58 (38.40)^{ef}
FEB271	2.43	30.48 (33.47)^{k}	3.53	21.48 (27.38)^{hi}	2.57	23.68 (29.06)^{jk}
FEB272	1.87	46.67 (43.09)^{ef}	3.43	23.70 (29.07)^{gh}	2.07	38.58 (38.40)^{efg}
FEB273	2.2	37.14 (37.55)^{hij}	3.07	31.85 (34.35)^{ef}	2.03	39.53 (38.95)^{efg}
FEB274	2.6	25.71 (30.45)^{m}	3.63	19.26 (25.93)^{hi}	2.63	21.83 (27.80)^{jk}
FEB275	2.47	29.52 (32.91)^{kl}	3.57	20.74 (26.89)^{hi}	2.63	21.47 (27.03)^{jk}

FEB276	2.57	26.67 (31.08)^{lm}	3.03	32.59 (34.81)^{ef}	2.63	21.68 (27.70)^{jk}
FEB277	2.53	27.62 (31.59)^{lm}	3.33	25.93 (30.58)^{gh}	2.73	18.65 (25.42)^{jk}
FEB278	2.03	41.90 (40.33)^{g}	3.10	31.11 (33.89)^{ef}	2.77	17.91 (24.96)^{kl}
FEB279	2.2	37.14 (37.51)^{hij}	4.10	8.89 (17.26)^{kl}	2.63	21.59 (27.53)^{jk}
FEB280	2.77	20.95 (27.23)^{no}	3.77	16.30 (23.66)^{ij}	2.63	21.77 (27.81)^{jk}
FEB281	2.53	27.62 (31.70)^{lm}	3.60	20.00 (26.50)^{hi}	2.40	28.61 (32.31)^{h}
SEm	0.079	1.503	0.113	2.076	0.084	2.459
CV (%)	5.765	7.694	5.652	13.118	5.669	14.872
CD(p= 0.05)	0.220	4.175	0.316	5.768	0.232	6.832

Data in the parenthesis are arc sine transformed values

dahlia.Ahmed *et al*. (2023) reported that *Fusarium* sp., a fungal endophyte isolated from the roots of *Mentha longifolia* L. (Labiatae) inhibited the growth of several pathogens likes *C. albicans, C. glabrata, C. krusei* and *A. fumigates* through the production of cyclodepsipeptidefusaripeptide A.

Lalngaihawmi and Bhattacharya (2019) reported the non-volatile activity of three rhizospheric*Trichoderma* isolates and it was found that all the three isolates could greatly inhibit the growth of Foc due to the metabolite production and the highest per cent inhibition was found in *T. reesei* (RMF 25) with 35.96% inhibition followed by *T. reesei* (RMF 13) with 35.22% and *T. harzianum* (RMF 28) with 34.72% inhibition, under *in vitro*.

Raza *et al*. (2013) carried out similar work on wilt disease of watermelon (*Fusarium oxysporum* f. sp. *niveum*) where *Trichoderma harzianum* SQR-T037 was observed to obstruct the pathogen growth due to non-volatile metabolite production. Thoyajakshi Bai *et al*. (2018) reported on chilli wilt caused by *Fusarium oxysporum*, where *Trichoderma* sp. was found to produce non-volatile compounds against the pathogen.

The fungal endophytes are established to produce various types of non-volatile metabolites that consist of a large-scale range of different chemical

compounds such as peptides, steroids, polyketides, alkaloids, enzymes, hormones, amino acids etc (Singh and Kumar, 2023). Non-volatile compounds production may help to obstruct the plant pathogens growth and protect the plants.

4.9 Molecular Characterization and Identification of the Best Performing Fungal Endophytes of banana

Molecular characterization and identification of the best performing three isolates from all the experiments were carried out and altogether 24 isolates were identified and characterized (Fig 4.4, Table 4.10 and 4.11). Molecular characterization was done using ITS primers, ITS1 (forward) (5' -TCC GTA GGT GAA CCT GCG G- 3') and ITS4 (reverse) (5' -TCC TCC GCT TAT TGA TAT GC- 3'). The nucleotide sequences were submitted to GenBank and the accession numbers were acquired for all the identified isolates (Table 4.10 and 4.11). Phylogenetic tree of the identified isolates was prepared using MEGA11 software and neighbor joining method (Fig. 4.5).

The identified isolates are *Apiosporalongistroma* (FEB75), *Colletotrichum horii* (FEB83), *Cladosporium tenuissimum* (FEB178), *Colletotrichum gloeosporioides* (FEB269), *Mucor circinelloides* (FEB186), *Mucor circinelloides* (FEB251), *Trichoderma asperellum* (FEB46), *Diaporthephaseolorum* (FEB27), *Diaporthe sp.* (FEB129), *Beauveria felina* (FEB143),*Aspergillus clavatonanicus* (FEB51), *Penicillium citrinum* (FEB187),*Phomopsis* sp. (FEB254), *Colletotrichum fructicola* (FEB65), *Colletotrichumgloeosporioides* (FEB68), *Aspergillus versicolor* (FEB23), *Diaporthechromolaenae*(FEB249), *Trichoderma asperellum* (FEB116), *Apiosporahydei*

Table 4.10. The ITS sequence-based identification of the endophytic fungal isolates of banana

Sl. No.	Fungal Isolates	GenBank Accession No.	Homolog Sequence	Sequence Identity %	Closest Accession Number in the database	Isolation source		
						Plant part	Wild/Cultivated	Location
1	FEB75	PP726886	*Apiosporalongistroma*	95.83%	NR_154716	Leaves	Wild	Kohima
2	FEB83	PP405942	*Colletotrichum horii*	99.83%	MT568591	Leaves	Wild	Kohima
3	FEB178	PP729470	*Cladosporium tenuissimum*	100.00%	OQ629133	Leaves	Cultivated	Peren
4	FEB269	PP726650	*Colletotrichum gloeosporioides*	100.00%	MZ823561	Roots	Wild	Mokokchung
5	FEB186	PP729469	*Mucor circinelloides*	100.00%	MH854642	Roots	Wild	Peren
6	FEB251	PP729471	*Mucor circinelloides*	100.00%	MH854642	Roots	Wild	Mokokchung
7	FEB46	PP729472	*Trichoderma asperellum*	99.83%	KT358889	Roots	Wild	Chumoukedima (Dimapur)
8	FEB27	PP766971	*Diaporthephaseolorum*	99.46%	KX815357	Leaves	Wild	Chumoukedima (Dimapur)

9	FEB129	PP726704	*Diaporthe*sp.	99.46%	ON322885	Roots	Wild	Kohima
10	FEB143	PP715981	*Beauveria felina*	100.00%	MH856642	Roots	Cultivated	Kohima
11	FEB51	PP726706	*Aspergillus clavatonanicus*	100%	DQ355025	Roots	Cultivated	Chumoukedima (Dimapur)
12	FEB187	PP726707	*Penicillium citrinum*	100%	OR354745	Roots	Wild	Peren
13	FEB254	PP726708	*Phomopsis* sp.	99.83%	MN486556	Roots	Wild	Mokokchung
14	FEB65	PP726709	*Colletotrichum fructicola*	100.00%	LC776011	Leaves	Wild	Kohima
15	FEB68	-	*Colletotrichum gloeosporioides*	89.87%	MF380748	Leaves	Wild	Kohima
16	FEB23	PP726885	*Aspergillus versicolor*	100%	MK027304	Leaves	Wild	Chumoukedima (Dimapur)
17	FEB249	PP726710	*Diaporthechromolaenae*	100.00%	MT214362	Roots	Wild	Mokokchung
18	FEB116	PP729473	*Trichoderma asperellum*	99.84%	LC075715	Roots	Wild	Kohima
19	FEB80	PP726725	*Apiosporahydei*	100.00%	KY494717	Leaves	Wild	Kohima

20	FEB81	PP726729	*Colletotrichum kahawae*	100%	MN856281	Leaves	Wild	Kohima
21	FEB115	PP726730	*Diaporthefructicola*	100%	PP542170	Roots	Wild	Kohima
22	FEB3	PP726881	*Fusarium haematococcum*	90.40%	JN088237	Leaves	Wild	Chumoukedima (Dimapur)
23	FEB5	PP726711	*Trichoderma hamatum*	100%	MN264503	Leaves	Wild	Chumoukedima (Dimapur)
24	FEB9	PP729474	*Fusarium solani*	99.59%	MG827183	Leaves	Wild	Chumoukedima (Dimapur)

Table 4.11. Internal Transcribed Spacer (ITS) region sequence of Fungal Endophytes of banana

Sl. No.	Fungal Isolates	Sequence	Base pairs	Homolog Sequence	Sequence Identity %	GenBank Accession No.	Closest Accession Number in the database
1	FEB75	TTGTTTCCCCCTTCACTCCCACACCATTTGTTACTTACTCAG TACTGCCAGGAGAATAGAGTGAGTTATCAAATGTGGGAGA GGTATAACTCTGTAATGAGTCTTTTTCCCTAGGGGGGTACG CGGAGAGATCATTTCAGAGTTATACAAATCCCACACCACTT GTTAACTTACTCAGTTATGCCTTGGCGTGAACTGCGTTCGG AGGCAGGTTGGGTGTTTCCCTGTAACCTTCCCTGTAGGTTT CCCGGTAAGTTCCCTGTAGGCTTCCCTGTAACTTTCCCTGCC CCCCTCCCGGGCAACCCGCCGGTGGTACACTAAACTCTTGT TTTATTGTATCTTCTGAGCGAATTATTTTAATAATTAAAACT TTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACG CAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGT GAATCATCGAATCTTTGAACGCACATTGCGCCCATCAGTAT TCTGGTGGGCATGCCTGTTCGAGCGTCATTTCAACCCTTAA GCCTAGCTTAGTGTTGGGAATCTACTGTATTGTAGTTCCTT AAAGACAGTGGCGGAGCGATAGTTGTCCTCTGAGCGTAGT AAATTTATTTCTCGCTTCTGCAAGGCTCTATCTTCTCGCCAT AAAACCCCCAATTTTTTAGTGGTGACCTCGGATCAGGTAGA TGCCATCGATCT	710	*Apiosporalongi stroma*	95.83%	PP726886	NR_15471 6

2	FEB83	TGCCAGAACCAAGAGATCCTTGTAAAATTTTGATTATTTGC TTGTACCACTCAGAAGAAACTTCGTAAATCAGAGTTTGTTA TCCTCCGGCGGCGCCGACCCGCCCGGGGCGGGAGGCCGGA GGTCACAGACCTGCCCGCGAAGCAACAGTTATAGTATGTTC ACAAAGTTGTAGAGCGTAAACTCAGTATTCCGTAGGGGGG ACCTGCGGAGGGATCATTACTGAGTTTACGCTCTACAACCC TTTGTGAACATACCTATAACTGTTGCTTCGGCGGGCAGGGT CTCCGTGACCCTCCCGGCCTCCCGCCCCCGGGCGGGTCGGC GCCCGCCGGAGGATAACCAAACTCTGATTTAACGACGTTTC TTCTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACG GATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG AATCTTTGAACGCACATTGCGCCCGCCAGCATTCGGCGGGC ATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTG GTGTTGGGGCCCTACAGCTGATGTAGGCCCTCAAAGGTAGT GGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTTTACG TCTCGCACTGGGATCCGGAGGGACTCTTGCCGTAAAACCCC CAATTTTCCAAAGGTTGACCTCGGATCAGGTAGGAATACCC GCTGAACTTAAGCATATCAATAAGCCGGAGGAA	768	*Colletotrichum horii*	99.83%	PP405942	MT568591

3	FEB178	GTCACTTGTAATGATTTCCGTAGGGTGAACCTGCGGAGGGA TCATTACAAGTGACCCCGGTCTAACCACCGGGATGTTCATA ACCCTTTGTTGTCCGACTCTGTTGCCTCCGGGGCGACCCTG CCTTCGGGCGGGGGCTCCGGGTGGACACTTCAAACTCTTGC GTAACTTTGCAGTCTGAGTAAACTTAATTAATAAATTAAAA CTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAA CGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCA GTGAATCATCGAATCTTTGAACGCACATTGCGCCCCCTGGT ATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTCACCACTC AAGCCTCGCTTGGTATTGGGCAACGCGGTCCGCCGCGTGCC TCAAATCGACCGGCTGGGTCTTCTGTCCCCTAAGCGTTGTG GAAACTATTCGCTAAAGGGTGCTCGGGAGGCTACGCCGTA AAACAAACCCATTTCTAAGGTTGACCTCGGATCAGGTAGG GATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA	569	*Cladosporium tenuissimum*	100.00%	PP729470	OQ629133
4	FEB269	GAACCTGCGGAGGGATCATTATCGAGTTACCACTCTATAAC CCTTTGTGAACATACCTACATGTTGCTTCGGCGGTCGGCCC CCCGGGCCCCCGGCCCCGCTCACGCGGGGCGTCCGCCGGA GGATAACCAAACTCTGATTTAACGACGTTTCTTCTGAGTGG CACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGG TTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTA ATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAAC GCACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTC GAGCGTCATTTCAACCCTCAAGCACTGCTTGGTGTTGGGGC TCTACGGTTGACGTAGGCCCCCAAAACTAGTGGCGGACCCT CTCGGAGCCTCCTTTGCGTAGTAACTTTTGTCTCGCACTGG GATTCGGAGGGATTCTAGCCGTTAAACCCCCAATTTTCTAA	543	*Colletotrichum gloeosporioides*	100.00%	PP726650	MZ823561

		AGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTA AGCATATCAATAA					
5	FEB186	TGCGGAAGGATCATTAAATAATCAATAATTTTGGCTTGTCC ATTATTATCTATTTACTGTGAACTGTATTATTACTTGACGCT TGAGGGATGCTCCACTGCTATAAGGATAGGCGATGGAGAT GCTAACCGAGTCATAATCAAGCTTAGGCTTGGTATCCTATT ATTATTTACCAAAAGAATTCAGAATTAATATTGTAACATAG ACCTAAAAAATCTATAAAACAACTTTTAACAACGGATCTCT TGGTTCTCGCATCGATGAAGAACGTAGCAAAGTGCGATAA CTAGTGTGAATTGCATATTCAGTGAATCATCGAGTCTTTGA ACGCAACTTGCGCTCATTGGTATTCCAATGAGCACGCCTGT TTCAGTATCAAAACAAACCCTCTATCCAACATTTTGTTGAA TAGGAATACTGAGAGTCTCTTGATCTATTCTGATCTCGAAC CTCTTGAAATGTACAAAGGCCTGATCTTGTTTGAATGCCTG AACTTTTTTTTAATATAAAGAGAAGCTCTTGCGGTAAACTG TGCTGGGGCCTCCCAAATAATACTTTTTTTAAATTTGATCTG AAATCAGGCGGGATTACCCGCTGAACTTAAGCATAT	610	*Mucor circinelloides*	100.00%	PP729469	MH854642

6	FEB251	GCGGAAGGATCATTAAATAATCAATAATTTTGGCTTGTCCATTATTATCTATTTACTGTGAACTGTATTATTACTTGACGCTTGAGGGATGCTCCACTGCTATAAGGATAGGCGATGGAGATGCTAACCGAGTCATAATCAAGCTTAGGCTTGGTATCCTATTATTATTTACCAAAAGAATTCAGAATTAATATTGTAACATAGACCTAAAAAATCTATAAAACAACTTTTAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGTAGCAAAGTGCGATAACTAGTGTGAATTGCATATTCAGTGAATCATCGAGTCTTTGAACGCAACTTGCGCTCATTGGTATTCCAATGAGCACGCCTGTTTCAGTATCAAAACAAACCCTCTATCCAACATTTTGTTGAATAGGAATACTGAGAGTCTCTTGATCTATTCTGATCTCGAACCTCTTGAAATGTACAAAGGCCTGATCTTGTTTGAATGCCTGAACTTTTTTTTAATATAAAGAGAAGCTCTTGCGGTAAACTGTGCTGGGGCCTCCCAAATAATACTTTTTTTAAATTTGATCTGAAATCAGGCGGGATTACCCGCTGAACTTAAGCATATCAAAAGCCGGGAGGAAAAAA	629	*Mucor circinelloides*	100.00%	PP729471	MH854642

7	FEB46	TGGGAGTTGTAAACTCGGTAAGTTCCGTAGGGTGAACCTGC GGAGGGATCATTACCGAGTTTACAACTCCCAAACCCAATGT GAACGTTACCAAACTGTTGCCTCGGCGGGGTCACGCCCCG GGTGCGTCGCAGCCCCGGAACCAGGCGCCCGCCGGAGGAA CCAACCAAACTCTTTCTGTAGTCCCCTCGCGGACGTATTTC TTACAGCTCTGAGCAAAAATTCAAAATGAATCAAAACTTTC AACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCA GCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGA ATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTC TGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAAC CCCTCCGGGGGATCGGCGTTGGGGATCGGGACCCCTCACA CGGGTGCCGGCCCCGAAATACAGTGGCGGTCTCGCCGCAG CCTCTCCTGCGCAGTAGTTTGCACAACTCGCACCGGGAGCG CGGCGCGTCCACGTCCGTAAAACACCCAACTTTCTGAAATG TTGACCTCGGATCAGGAAGGAATACCCGCTGAACTTAAGC ATAT	612	*Trichoderma asperellum*	99.83%	PP729472	KT358889
8	FEB27	TGCGGAGGGATCATTGCTGGAACGCGCTTCGGCGCACCCA GAAACCCTTTGTGAACTTATACCTATTTGTTGCCTCGGCGT AGGCCGGCCTCTTCACTGAGGCCCCCTGGAGACAGGGAGC AGCCCGCCGGCGGCCAACTAAACTCTTGTTTCTATAGTGAA TCTCTGAGTAAAAACATAAATGAATCAAAACTTTCAACAA CGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAA ATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATC GAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGG GCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCTGGCT TGGTGATGGGGCACTGCCTTCTAGCGAGGGCAGGCCCTGA	499	*Diaporthephaseolorum*	99.46%	PP766971	KX815357

		AATCTAGTGGCGAGCTCGCTAGGACCCCGAGCGTAGTAGT TATATCTCGTTCTGGAAGGCCCTGGCGGTGCCCTGCCGTTA AACC CCCAACTTC					
9	FEB129	TGCGGAGGGATCATTGCTGGAACGCGCCCCAGGCGCACCC AGAAACCCTTTGTGAACTTATACCTTTACTGTTGCCTCGGC GCATGCTGGCCCCCCTGGGGTCCCTCGGAGACGAGGAGCA GGCACGCCGGCGGCCAAGTTAACTCTTGTTTTTACACTGAA ACTCTGAGAAAAAAACACAAATGAATCAAAACTTTCAACA ACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGA AATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCA TCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGA GGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCATTG CTTGGTGTTGGGGCACTGCTTTTACCCAAAAGCAGGCCCTG AAATCTAGTGGCGAGCTCGCCAGGACCCCGAGCGCAGTAG TTAAACCCTCGCTCTGGAAGGCCCTGGCGGTGCCCTGCCGT TAAACCCCCAACTTTTGAAAATTTGACCTCGGATCAGGTAG GAATACCCGCTGAACTTAAGCATA	551	*Diaporthe*sp.	99.46%	PP726704	ON322885

10	FEB143	CTGCGGAGGGATCATTACCGAGTTTCTAACTCCATACCTTT GTGAACATACCTATCGTTGCTTCGGCGGGTCCGTCCCGGAG CTGGCAGTGCACGGCCAGCCCCGGAACCAGACGCCCGCCG AGGACCCCAAACTCTTGTTTTTATAGTGGATCTTCTGAGTC TTATACAAAATAAATTAAAACTTTCAGCAACGGATCTCTTG GTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGT AATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA CGCACATTGCGCCCGCCAGTACTCTGGCGGGCATGCCTGTC CGAGCGTCATTTCAACCCTCAGGGCCCGTCCGCGGGACCTG GCGTTGGGGATCGGCTGCCCCTGGCGGCTGCCGGCCCTGA AATACAGTGGCGGTCTCTTCGCGACCTCCCCTGCGTAGTAG TGATACCTCGCAGCCGGATAGCGGAGCGGCCACGCCGTAA AACCCCCTACTTCTCAAGGTTGACCTCGGATCA	521	*Beauveria felina*	100.00%	PP715981	MH856642

11	FEB51	GCGGAAGGATCATTACCGAGTGCGGGCCCTCTGGGTCCAA CCTCCCACCCGTGTCTATTGTACCTTGTTGCTTCGGCGGGCC CGCCGTCTTCGGACGGCCGCCGGGGAGGCCTCCGCGCCCC CGGGCCCGCGCCCGCCGAAGACCACAACATGAACTCTGTT CTGAAGTTTTGCAGTCTGAGTTGATTATCATAATCAGTTAA AACTTTCAACAACGGATCTCTTGGTTCCGGCATCCATGAAG AACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATT CAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTG GTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCC TCAAGCACGGCTTGTGTGTTGGGCCCCCGTCCCCGCCTCAC CGCGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCC GGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTTGTAGG CCCGGCCGGCGCCTGTCGACACCAACCCCAATTTTTCTAAG GTGACCTC	537	*Aspergillus clavatonanicus*	100%	PP726706	DQ355025

12	FEB187	GAAGGATCATTACCGAGTGCGGGCCCCTCGGGGCCCAACCTC CCACCCGTGTTGCCCGAACCTATGTTGCCTCGGCGGGCCCCGC GCCCGCCGACGGCCCCCCTGAACGCTGTCTGAAGTTGCAGTCT GAGACCTATAACGAAATTAGTTAAAACTTTCAACAACGGATC TCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATA ACTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGA ACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTCC GAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGCCC CGTCCCCCCCGCCGGGGGGACGGGCCCGAAAGGCAGCGGCGG CACCGCGTCCGGTCCTCGAGCGTATGGGGCTTCGTCACCCGCT CTAGTAGGCCCGGCCGGCGCCAGCCGACCCCCA	459	*Penicillium citrinum*	99.63%	PP726707	MN879404
13	FEB254	TTCCGTAGGGTGAACCTGCGGAGGGATCATTGCTGGAACGCG CCCCAGGCGCACCCAGAAACCCTTTGTGAACTTATACCTTACT GTTGCCTCGGCGCATGCCGGCCCCCAGGGGGCCCCTCGGAGA CGAGGAGCAGGCACGCCGGCGGCCAAGCTAACTCTTGTTTTT ACACTGAAACTCTGAGAGAAAAAAAACAAAATGAATCAAAA CTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACG CAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGA ATCATCGAATCTTTGAACGCACATTGCGCCCTCCGGTATTCCG GAGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACTG CTTGGTGTTGGGGCACTGCTCCTCTCGCGGGGAGCAGGCCCTG AAATCCAGTGGCGAGCTCGCCAGGACCCCGAGCGCAGTAGTT AAACCCTCGCTCTGGAAGGCCCTGGCGGTGCCCTGCCGTTAA ACCCCCAACTCTTGAAAATTTGACCTCGGATCAGGTAGGAAT ACCCGCTGAACT	562	*Phomopsis* sp.	99.83%	PP726708	MN486556

14	FEB65	CCTGCGGAGGGATCATTACTGAGTTTACGCTCTATAACCCT TTGTGAACATACCTATAACTGTTGCTTCGGCGGGTAGGGTC TCCGCGACCCTCCCGGCCTCCCGCCTCCGGGCGGGTCGGCG CCCGCCGGAGGATAACCAAACTCTGATTTAACGACGTTTCT TCTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACG GATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG AATCTTTGAACGCACATTGCGCCCGCCAGCATTCTGGCGGG CATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTT GGTGTTGGGGCCCTACAGCTGATGTAGGCCCTCAAAGGTA GTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTTTA CGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTAAAACC CCCCAATTTTCCAAAGGTTGACCTC	513	*Colletotrichum fructicola*	100.00%	PP726709	MT393756

15	FEB68	GGGTTTCAGTACCTCTATTACCCTTTGTGACATACCTACAT GTTGCTTCGGCGGTCGGCCCCCCCGGGCCCCCGGCCCCGCT CACGAGGGGCGTCCGCCGGAGGATAACCAAACTCTGATTT AACGACCTCTCTTCTGAGTGGCACAAACAAATAATCAAAA CTTTTAACAACAGATCTCTTGGCTCTGGCATCCATGAAGAA CGCACCGAAATGCAATAATGAATGTGAATTGAATAATTCA GGGATGCATGGAATCTTGGAACATACATTGTTCCCGCCAGC ATTCTGGCGGGCATGCCTGTACGAGGGCCATTTGAACCCTT TGTGAACATACTTACATGTTGCTTCGGCGGTTGCCCCGGCG GGCCTGCCAAGGATTTCACGCGGGGCGTCCGCCGGAGGAT AACCAATCTCTAACTCAAGGGCGTTTCTTCGGAGTGGCACA AGCAAATAATCAAAAGTTTAACAACGTATCTCATGGTTCTG GCATCGATGAAGAACGCAGCGTAATGCGATAAGTAATGTG AATTGCAGAATTCAGTGAATCATAGAATTTATGAGAGCAC ATGGCGCCAGCCAGCATTCTGGCGGGCATGCCTGCTTGAGC GTCATTTCACCCCTCAAGCACAGCTTGGTGTTGGGGCTATA CGGTTGACGTAGGCCCCCAAACATAGTGGCGGACCCTCTC GGAGCCTCCTGTGTGTAGTCATTTTTGTCTCGCACTGGGAT TCGGAGGGATTCTAGCCGTTAAACCCCCAAATCCAAAGGT GACCTCGATCAGTAGATGAAA ATTTAATTTGAG	804	*Colletotrichum gloeosporioides*	89.87%		MF380748

16	FEB23	TGCGGAAGGATCATTACTGAGTGCGGGCTGCCTTCGGGCG CCCAACCTCCCACCCGTGACTACCTAACACTGTTGCTTCGG CGGGGAGCCCTCTCGGGGGCGCGCCGCCGGGGACTACTGA ACTTCATGCCTGAGAGTGATGCAGTCTGAGTCTGAATATAA AATCAGTCAAAACTTTCAACAATGGATCTCTTGGTTCCGGC ATCGATGAAGAACGCAGCGAACTGCGATAAGTAATGTGAA TTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATT GCGCCCCCTGGCATTCCGGGGGGCATGCCTGTCCGAGCGTC ATTGCTGCCCATCAAGCCCGGCTTGTGTGTTGGGTCGTCGT CCCCCCCGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCG TGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTCGA TTTAGGGCCGGCCGGGCGCCAGCCGACGTCCAACCATTTTT CTTCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAAC TTAAGCATATCAATA	544	*Aspergillus versicolor*	100%	PP726885	MK027304

17	FEB249	GCGGAGGGATCATTGCTGGAACGCGCTTCGGCGCACCCAG AAACCCTTTGTGAACTTATACCTATTGTTGCCTCGGCGCAG GCCGGCCTCTTCACTGAGGCCCCCTGGAAACAGGGAGCAG CCCGCCGGCGGCCAACCAAACTCTTGTTTCTATAGTGAATC TCTGAGTAAAAAAACATAAATGAATCAAAACTTTCAACAA CGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAA ATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATC GAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGG GCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCTGGCT TGGTGATGGGGCACTGCCTGTAATAGGGCAGGCCCTGAAA TCTAGTGGCGAGCTCGCCAGGACCCCGAGCGTAGTAGTTAT ATCTCGCTCTGGAAGGCCCTGGCGGTGCCCTGCCGTTAAAC CCCCAACTTCTGAAAATTTGACCTCGGATCAGGTAGGAATA CCCGCTGAACTTAAGCATAT	548	*Diaporthechromolaenae*	100.00%	PP726710	MT214362

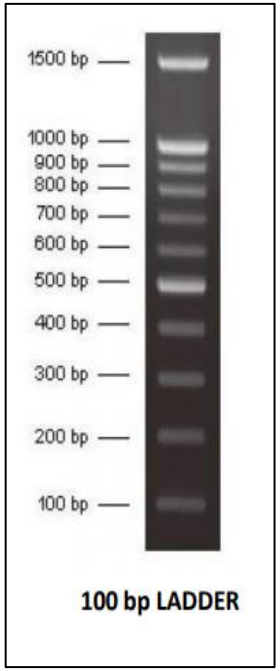
18	FEB116	GGGAGTTGTAAACTCGGTAATGTTCCGTAGGTGAACCTGCG GAGGGATCATTACCGAGTTTACAACTCCCAAACCCAATGTG AACGTTACCAAACTGTTGCCTCGGCGGGGTCACGCCCCGG GTGCGTCGCAGCCCCGGAACCAGGCGCCCGCCGGAGGAAC CAACCAAACTCTTTCTGTAGTCCCCTCGCGGACGTATTTCTT ACAGCTCTGAGCAAAAATTCAAAATGAATCAAAACTTTCA ACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAG CGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAA TCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCT GGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACC CCTCCGGGGGATCGGCGTTGGGGATCGGGACCCCTCACAC GGGTGCCGGCCCCGAAATACAGTGGCGGTCTCGCCGCAGC CTCTCCTGCGCAGTAGTTTGCACAACTCGCACCGGGAGCGC GGCGCGTCCACGTCCGTAAAACACCCAACTTTCTGAAATGT TGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCA TATCAAAAGGCCGGAGGAA	627	*Trichoderma asperellum*	99.84%	PP729473	LC075715
19	FEB80	CCTGCGGAGGGATCATTACAGAGTTATACAACTCCCATACC ATTTGCCAACTTACTCAGTTATGCCTCGGCGTAAGCTCCGT ACGGGGCTGCTGGGTGCGTTGCGGGCGACAGCTACCCTGT AGCTTACCCTGTAGCGCTACCCTGTAGCGTACCCTGCGGCG GCCCGCCGGTGGAAACGAAACTCTTGTTTTATTGTATCGTC TGAGCGTCTTATTTTAATAAGTTAAAACTTTCAACAACGGA TCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCG ATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATC TTTGAACGCACATTGCGCCCATCAGTATTCTGGTGGGCATG CCTGTTCGAGCGTCATTTCAACCCTTAAGCCTAGCTTAGTG	589	*Apiosporahydei*	100.00%	PP726725	KY494717

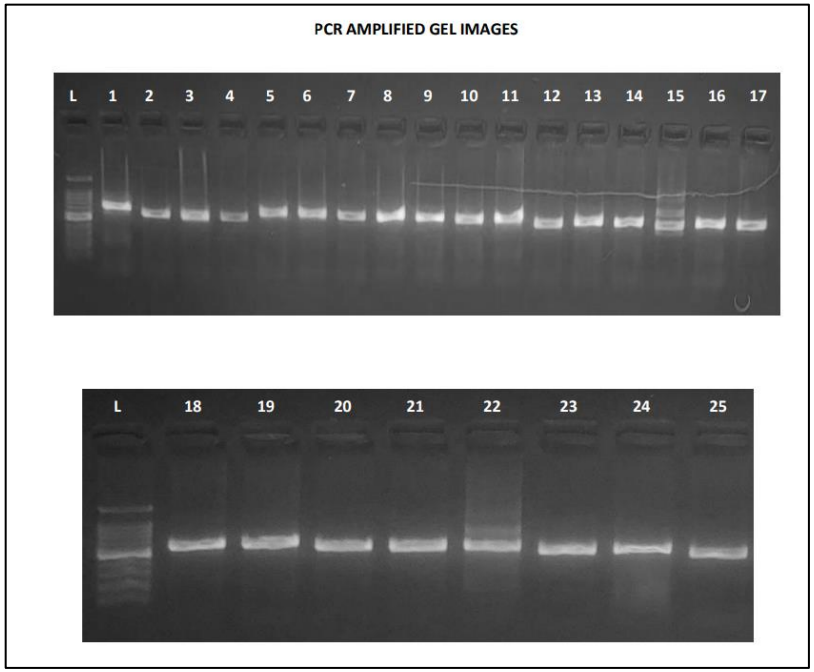
		TTGGGAATCGACTGTATTGTCGTTCCTTAAAGACAGTGGCG GAGCGGCAGTGGTCCTCTGAGCGTAGTAAATTTATTTCTCG CTTTTGTCAGGCCCTGTCCTCCCGCCATAAAACCCCCAATT TTTAGTGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAA CTTAAGCATATCAATA					
20	FEB81	CCTGCGGAGGGATCATTACTGAGTTTACGCTCTACAACCCT TTGTGAACATACCTATAACTGTTGCTTCGGCGGGCAGGGTC TCCGTGACCCTCCCGGCCTCCCGCCCCCGGGCGGGTCGGCG CCCGCCGGAGGATAACCAAACTCTGATTTAACGACGTTTCT TCTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACG GATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG AATCTTTGAACGCACATTGCGCCCGCCAGCATTCTGGCGGG CATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTT GGTGTTGGGGCCCTACAGCTGATGTAGGCCCTCAAAGGTA GTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTTTA CGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTAAAACC CCCAATTTTCCAAAGGTTGACCTCGGATCAGGTAGGAATAC CCGCTGAACTTAAGCATATCAATAA	554	*Colletotrichum kahawae*	100%	PP726729	MN856281

21	FEB115	TGCGGAGGGATCATTGCTGGAACGCGCTTCGGCGCACCCA GAAACCCTTTGTGAACTTATACCTATTGTTGCCTCGGCGCA GGCCGGCCTCTTCACTGAGGCCCCCTGGAAACAGGGAGCA GCCCGCCGGCGGCCAACCAAACTCTTGTTTCTATAGTGAAT CTCTGAGTAAAAAAACATAAATGAATCAAAACTTTCAACA ACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGA AATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCA TCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGA GGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCTGG CTTGGTGATGGGGCACTGCCTGTAATAGGGCAGGCCCTGA AATCTAGTGGCGAGCTCGCCAGGACCCCGAGCGTAGTAGT TATATCTCGCTCTGGAAGGCCCTGGCGGTGCCCTGCCGTTA AACCCCCAACTTCTGAAAATTTGACCTCGGATCAGGTAGGA ATACCCGCTGAACTTAAGCATATC	550	*Diaporthefructicola*	100%	PP726730	PP542170

22	FEB3	GGAATCAAGTTTCACTGATCACCCTGTGTGCATACCTAAAC GTTGCTTCCGCGGGAATATACGGCCCCGTGAAACGGGCCG CCCCCGCCAGAGGACCCTTAACTCTGTTTCTATAATGTTTCT TCTGAGTAAAACGAGCAAATAAATTAAAACTTTCAACAAC GGATCTCTTGGCTCTGGCATCGATGAACAACGCAGCGAGC AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTG AATCATCGAATCTTTGAACGCACATTGCGCCCGCCGGCACT CCGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCAGG ACCCCCTTTCGGGGGGGACCTGGTGCTGGGGATCAGCGGC CTCCGGGCCCCCCCAAATACAGTGGCGGTCCCGCCGCAGCT TCCATCGCGTAGTAGCTAACACCTCGCGACTGGAGAGCGG GGCGGCCACGCCGTAAAACACCCAACTCTTCTGAAGTTGA CCTCGAATCAGGAGAGCCATCTACT	511	*Fusarium haematococcum*	90.40%	PP726881	JN088237
23	FEB5	ACCTGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACC CAATGTGAACGTTACCAAACTGTTGCCTCGGCGGGGTCACG CCCCGGGTGCGTAAAAGCCCCGGAACCAGGCGCCCGCCGG AGGAACCAACCAAACTCTTTCTGTAGTCCCCTCGCGGACGT ATTTCTTACAGCTCTGAGCAAAAATTCAAAATGAATCAAAA CTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAA CGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCA GTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGT ATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTC GAACCCCTCCGGGGGATCGGCGTTGGGGATCGGGACCCCT CACCGGGTGCCGGCCCTGAAATACAGTGGCGGTCTCGCCG CAGCCTCTCCTGCGCAGTAGTTTGCACAACTCGCACCGGGA	577	*Trichoderma hamatum*	100.00%	PP726711	MH781011

		GCGCGGCGCGTCCACGTCCGTAAAACACCCAACTTCTGAA ATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAA GCATATCA					
24	FEB9	CCTGTGAACATACCTAAACGTTGCTTCGGCGGGAATAGAC GGCCCCGTGAAACGGGCCGCCCCCGCCAGAGGACCCTTAA CTCTGTTTCTATAATGTTTCTTCTGAGTAAAACAAGCAAAT AAATTAAAACTTTCAACAACGGATCTCTTGGCTCTGGCATC GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTG CAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCG CCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATT ACAACCCTCAGGCCCCCGGGCCTGGCGTTGGGGATCGGCG GAGCCCTTTGTGGGCACACGCCGTCCCCCAAATACAGTGGC GGTCCCGCCGCAGCTTCCATCGCGTACTAGCTAACACCTCG CGACTGGAGAGCGGCGCGGCCACGCCGTAAAACACCCAAC TCTTCTGAAGTTGACCTCGAATCAAGTAGGAATACCCGCTG AACTT	492	*Fusarium solani*	99.59%	PP729474	MG827183

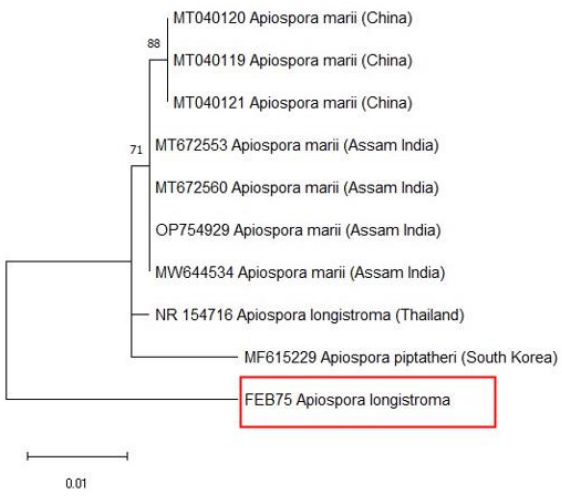




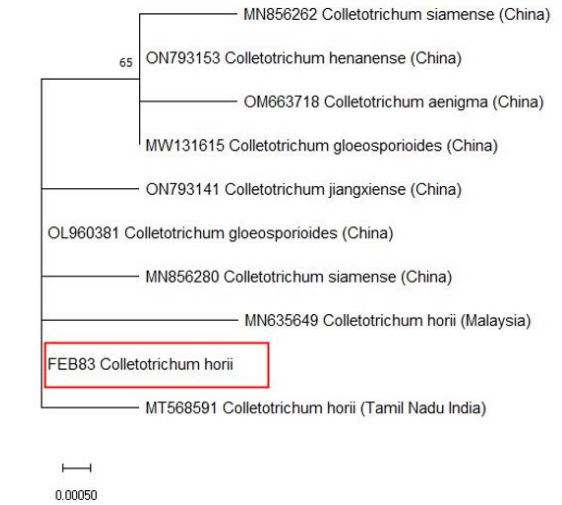
1. **FEB75**
2. **FEB83**
3. **FEB178**
4. **FEB269**
5. **FEB186**
6. **FEB251**
7. **FEB46**
8. **FEB27**
9. **FEB129**
10. **FEB143**
11. **FEB51**
12. **FEB187**

13. **FEB254**
14. **FEB65**
15. **FEB68**
16. **FEB23**
17. **FEB249**
18. **FEB116**
19. **FEB80**
20. **FEB81**
21. **FEB115**
22. **FEB3**
23. **FEB5**
24. **FEB9**
25. **Fusarium wilt pathogen**

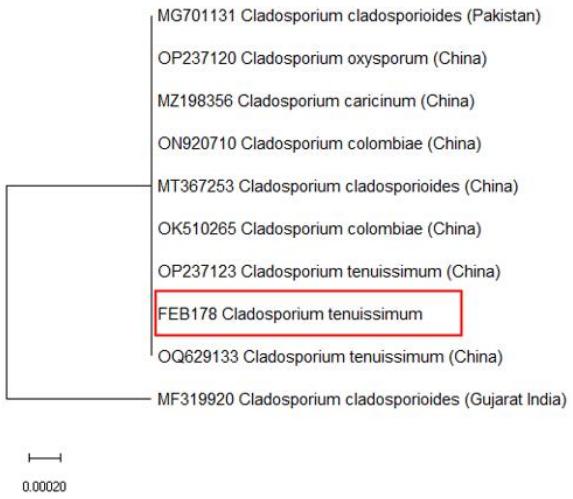
Fig 4.4. Agarose gel image of the fungal endophyte isolates and Fusarium wilt pathogen after PCR amplification using ITS1 (Forward) and ITS4 (Reverse) primers with DNA 100 bp ladder



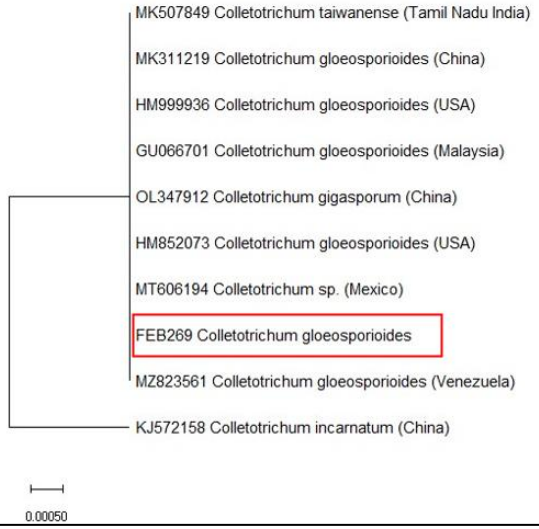
a. FEB75 *Apiospora longistroma*



b. FEB83 *Colletotrichum horii*

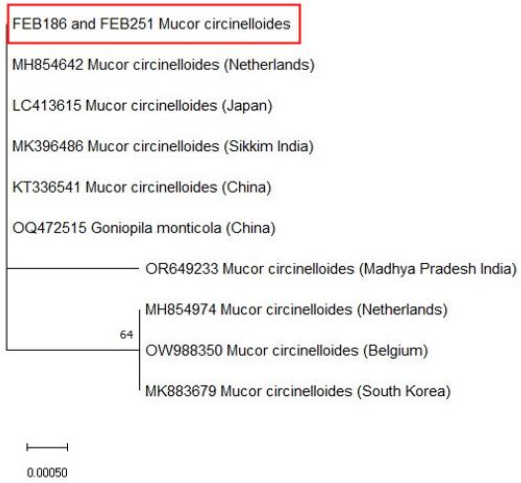


c. FEB178 *Cladosporium tenuissimum*

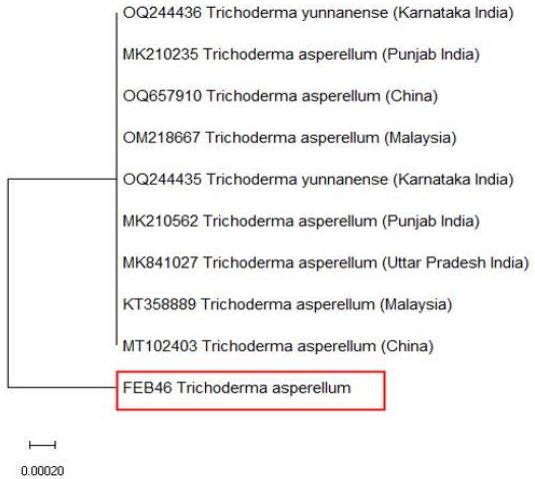


d. FEB269 *Colletotrichum gloeosporioides*

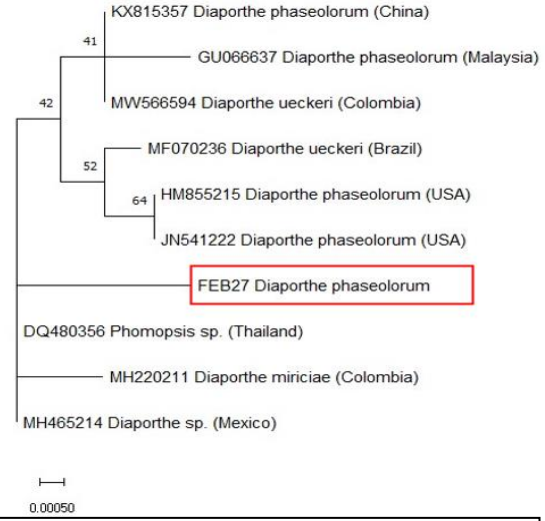
Contd.



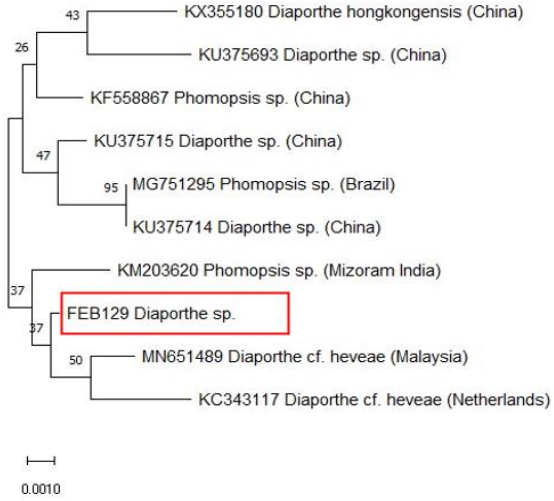
e. FEB186 and FEB251 *Mucor circinelloides*



f. FEB46 *Trichoderma asperellum*

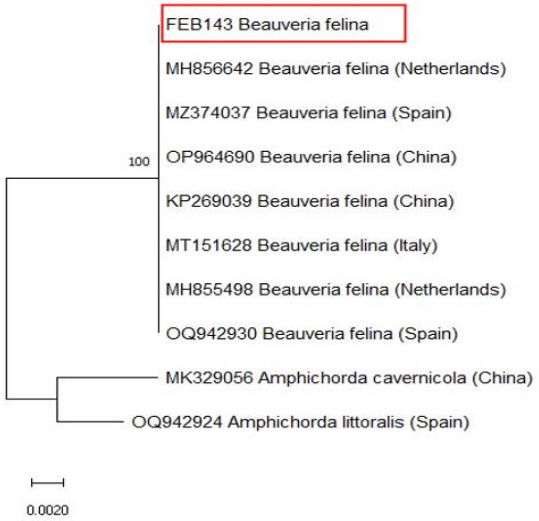


g. FEB27 *Diaporthe phaseolorum*

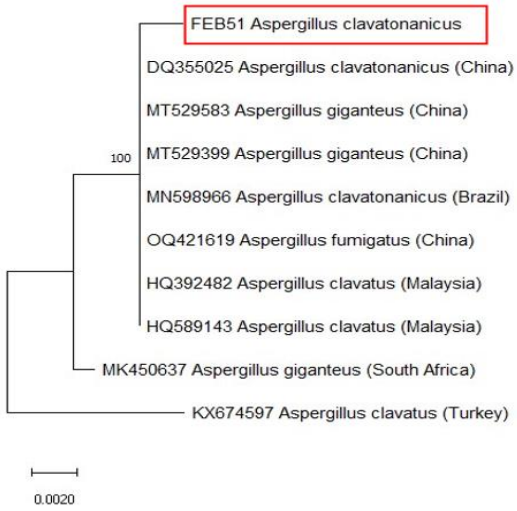


h. FEB129 *Diaporthe* sp.

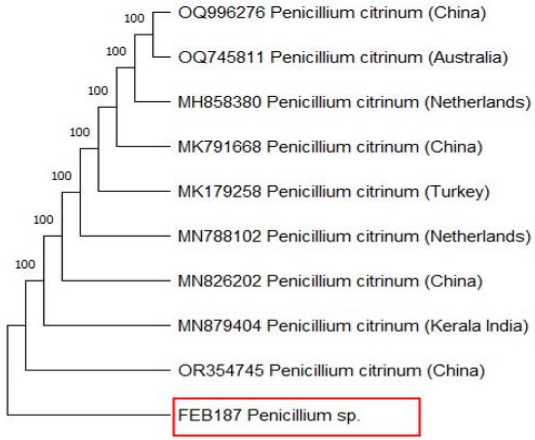
Contd.



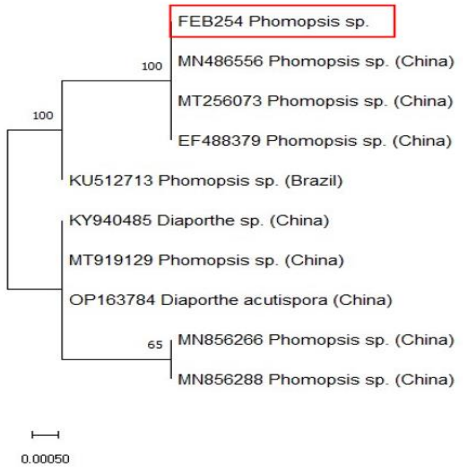
i. FEB143 *Beauveria felina*



j. FEB51 *Aspergillus clavatonanicus*

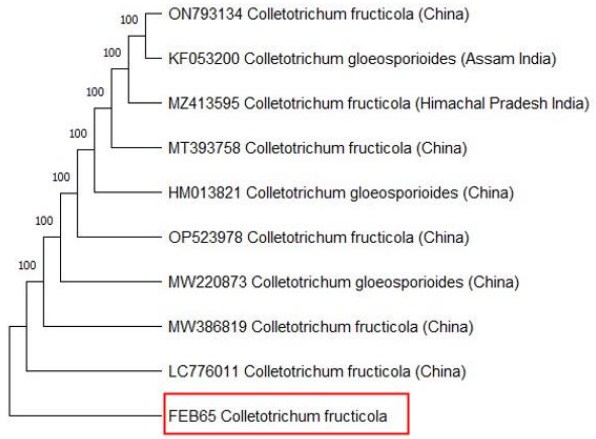


k. FEB187 *Penicillium* sp.

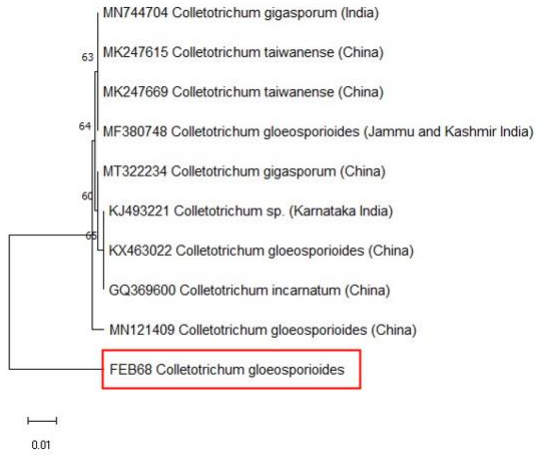


l. FEB254 *Phomopsis* sp.

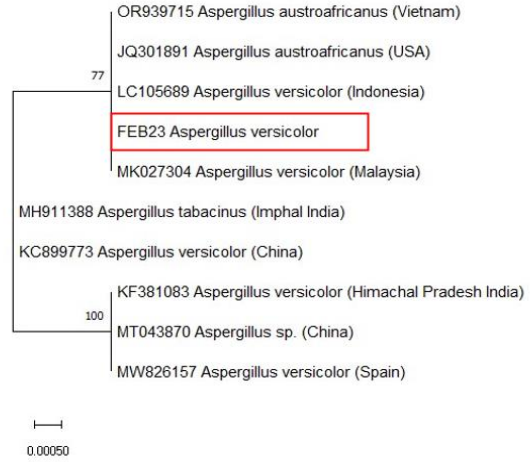
Contd.



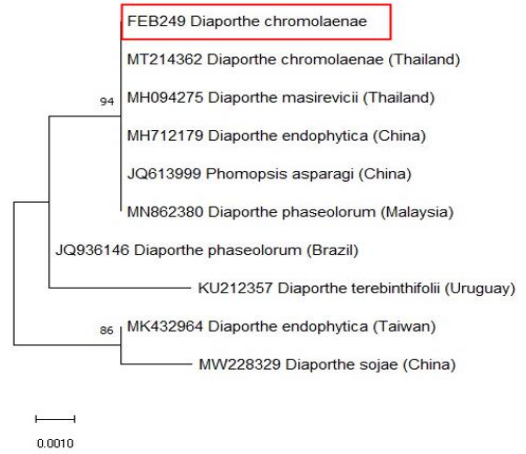
m. FEB65 *Colletotrichum fructicola*



n. FEB68 *Colletotrichum gloeosporioides*

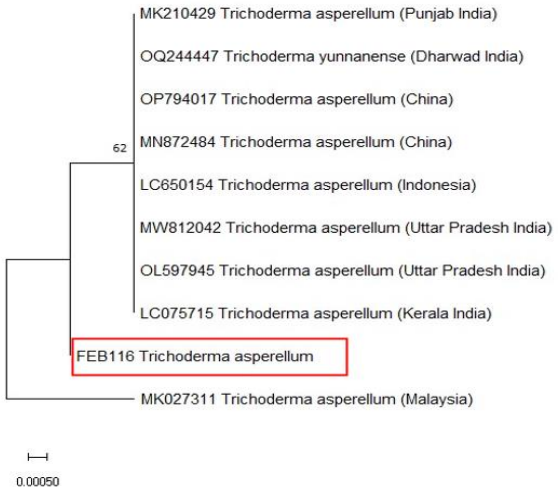


o. FEB23 *Aspergillus versicolor*

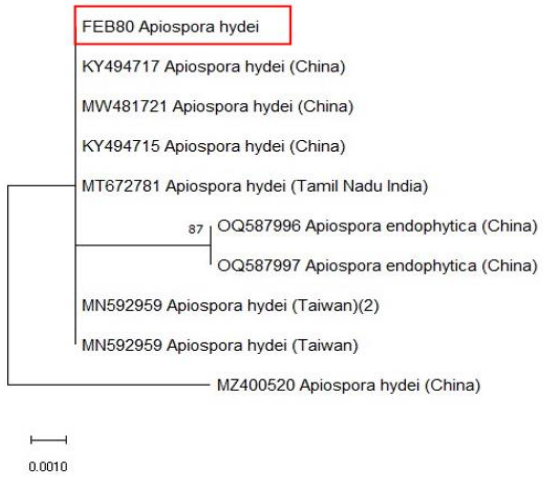


p. FEB249 *Diaporthe chromolaenae*

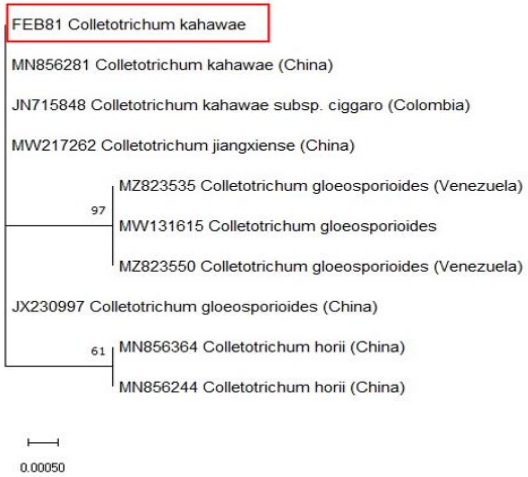
Contd.



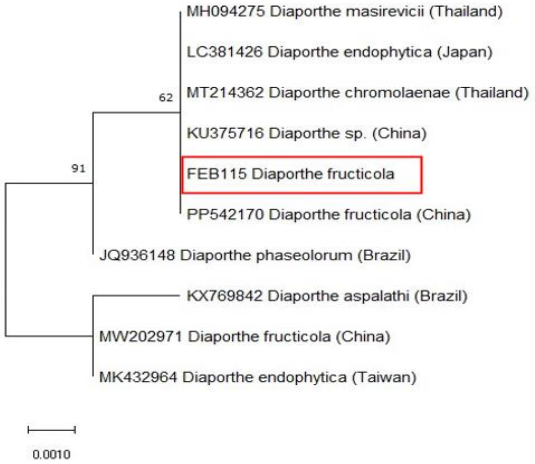
q. FEB116 *Trichoderma asperellum*



r. FEB80 *Apiospora hydei*

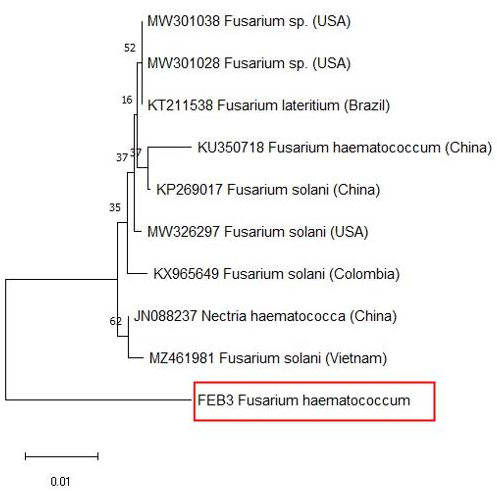


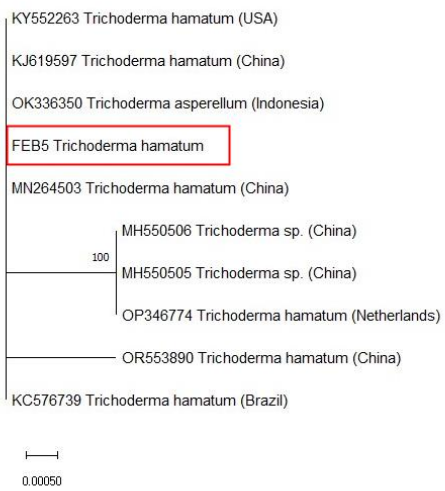
s. FEB81 *Colletotrichum kahawae*

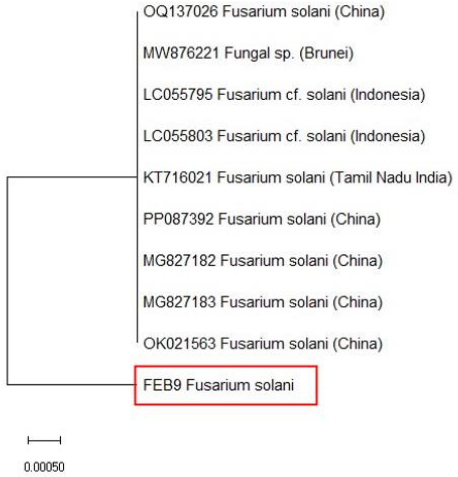


t. FEB115 *Diaporthe fructicola*

Contd.







u. FEB3 *Fusarium haematococcum*	v. FEB5 *Trichoderma hamatum*	w. FEB9 *Fusarium solani*

Fig. 4.5.Phylogenetic analysis of ITS sequences of fungal isolate with reference sequences retrieved from NCBI (National Center for Biotechnology Information). The analysis was implemented in MEGA 11 using the neighbor-joining method. The number given over branches indicate bootstrap coefficient.

a. FEB75*Apiospora longistroma*, b. FEB83 *Colletotrichum horii*, c. FEB178 *Cladosporium tenuissimum*, d. FEB269 *Colletotrichum gloeosporioides*, e. FEB186 and FEB251 *Mucor circinelloides*, f. FEB46 *Trichoderma asperellum*, g. FEB27 *Diaporthe phaseolorum*, h. FEB129 *Diaporthe* sp., i. FEB143 *Beauveria felina*, j. FEB51 *Aspergillus clavatonanicus*, k. FEB187 *Penicillium* sp., l. FEB254 *Phomopsis* sp., m. FEB65 *Colletotrichum fructicola*, n. FEB68 *Colletotrichum gloeosporioides*, o. FEB23 *Aspergillus versicolor*, p. FEB249 *Diaporthe chromolaenae*, q. FEB116 *Trichoderma asperellum*, r. FEB80 *Apiospora hydei*, s. FEB81 *Colletotrichum kahawae*, t. FEB115 *Diaporthe fructicola*, u. FEB3 *Fusarium haematococcum*, v. FEB5 *Trichoderma hamatum* and w. FEB9 *Fusarium solani*.

(FEB80), *Colletotrichum kahawae*(FEB81), *Diaporthefructicola*(FEB115), *Fusarium haematococcum* (FEB3), *Trichoderma hamatum* (FEB5) and *Fusarium solani* (FEB9).

Out of the 24 isolates that were identified, 22 belong to the Phylum Ascomycota and 2 belong to the Phylum Mucoromycota. The details have been given in Table 4.12.

The majority of the isolates were molecularly identified from the samples collected from Kohima district, where a total of nine isolates were identified from the wild banana plants (6 from leaves and 3 from roots) and one from the root of cultivated banana. It was followed by Chumoukedima (earlier under Dimapur district) district where an entirety of six isolates were identified from the wild banana plants (5 from leaves and 1 from root) and one from the root of cultivated banana. From Mokokchung district, four isolates were molecularly identified and they were all from the roots of wild banana. Lastly, from Peren district, three isolates were identified, two from the root and one from the leaves of wild banana (Table 4.10). From this present investigation, it is clear that the endophytic fungal isolates from the wild banana were found to give promising result in all the experiments conducted. Altogether, 21 isolates (11 from leaves and 10 from roots) from the wild bananaand only 3 from the leaf and roots of cultivated banana were molecularly identified and characterized.

Several workers have also identified fungal endophytes from banana plants and various other plants that have showed plant growth promotion activities and antagonistic activities against plant pathogens. Xia *et al*. (2011) collected banana roots from five sites of China and isolated and identified the *Trichoderma* species from inside and outer surface of the roots throughmolecular and AFLP identification. The largest group consisted of *T. asperellum, T. virens* and *Hypocrealixii*, which were both endophytic and epiphytic, followedby, *T. atroviride* and *T. koningiopsis*that were established

Table 4.12. Systematic position of the molecular identified fungal endophytes

Sl. No.	Fungal Isolates	Homolog Sequence	Systematic position of the isolates			
			Phylum	Class	Order	Family
1	FEB75	*Apiosporalongistroma*	Ascomycota	Sordarimycetes	Xylariales	Apiosporaceae
2	FEB83	*Colletotrichum horii*	Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae
3	FEB178	*Cladosporium tenuissimum*	Ascomycota	Dothideomycetes	Cladosporiales	Cladosporaceae
4	FEB269	*Colletotrichum gloeosporioides*	Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae
5	FEB186	*Mucor circinelloides*	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae
6	FEB251	*Mucor circinelloides*	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae
7	FEB46	*Trichoderma asperellum*	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae
8	FEB27	*Diaporthephaseolorum*	Ascomycota	Sordariomycetes	Diaporthales	Diaportheceae
9	FEB129	*Diaporthes*p.	Ascomycota	Sordariomycetes	Diaporthales	Diaportheceae
10	FEB143	*Beauveria felina*	Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae
11	FEB51	*Aspergillus clavatonanicus*	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
12	FEB187	*Penicillium citrinum*	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae

13	FEB254	*Phomopsis* sp.	Ascomycota	Sordariomycetes	Diaporthales	Valsaceae
14	FEB65	*Colletotrichum fructicola*	Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae
15	FEB68	*Colletotrichum gloeosporioides*	Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae
16	FEB23	*Aspergillus versicolor*	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
17	FEB249	*Diaporthechromolaenae*	Ascomycota	Sordariomycetes	Diaporthales	Diaportheceae
18	FEB116	*Trichoderma asperellum*	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae
19	FEB80	*Apiosporahydei*	Ascomycota	Sordarimycetes	Xylariales	Apiosporaceae
20	FEB81	*Colletotrichum kahawae*	Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae
21	FEB115	*Diaporthefructicola*	Ascomycota	Sordariomycetes	Diaporthales	Diaportheceae
22	FEB3	*Fusarium haematococcum*	Ascomycotaa	Sordariomycetes	Hypocreales	Nectriaceae
23	FEB5	*Trichoderma hamatum*	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae
24	FEB9	*Fusarium solani*	Ascomycotaa	Sordariomycetes	Hypocreales	Nectriaceae

to be epiphytic and lastly, *T. brevicompactum*, which was established to be an endophyte. Zakaria *et al*. (2016) did characterization of 31 isolates of endophytic fungi molecularly, isolated from the wild banana (*Musa acuminata*)roots. ITS regions were amplified using ITS1 and ITS4 primers for all endophytes and used the sequences of the translation elongation factor-1 α (TEF-1α) gene of *Fusarium* spp. Eighteen isolates were identified as *Fusarium* sp. with *Fusarium proliferatum* being the common one using the TEF-1α gene and 13 other isolates viz., *Curvularialunata, F. oxysporum, F. solani, Trichoderma atroviride, Calonectriagracilis, Rhizoctonia solani, Bionectriaochroleuca* and *Stromatoneurospora phoenix* (Xylariceae) were also identified using ITS primers.

Zakaria and Aziz (2018) also did characterization of endophytic fungi molecularly isolated from banana leaves using ITS1 and ITS4 primers and identified 17 species belonging to 10 genera which are *Nigrospora, Colletotrichum, Fusarium, Phoma, Pestalotiopsis, Penicillium, Bipolaris, Lasiodiplodia, Cochliobolus* and *Aspergillus*.

Tanapichatsakul*et al.* (2019) studied the fungal endophytes isolated from *Cinnamomum loureiroi* leaves and 11 isolates were isolated and identified using ITS4 and ITS5 primers. The identified isolates belonged to six genera which are *Botryosphaeria, Colletotrichum, Diaporthe, Fusarium, Neopestalotiopsis* and *Pestalotiopsis*. Sonawane *et al*. (2020) reported fungal endophyte isolated from the mangrove leaves, *Avicennia officinalis* which was molecularly identified using ITS4 and ITS5 primers as *Fusarium solani*.

Malubag*et al*. (2021) recognized endophytic fungi isolated from *Musa paradisiaca* (plantain banana) using ITS1 and ITS4 primers and identified nine isolates which are *Cladosporium cladosporioides, Fusarium chlamydosporium, Fusarium keratoplasticum, Fusarium solani* strain f2-f6, *Fusarium solani* strain ZB11263612, *Fusarium solani* strain F10-3, *Geotrichumcandidum, Nigrospora oryzae* and *Schizophyllum commune*.

Ramanujam *et al*. (2021) did molecular identification of an entomopathogen that was also isolated in this research work as one of the potential fungal endophytes, which was found to naturally infect the fall army worm in the Karnataka state of India. It was done using ITS1 and ITS4 primers and identified as *Beauveria felina*.

Ferdous *et al*. (2022) isolated and molecularly identified fungal endophytes from *Zingiber officinale*Rosc. Using ITS4 and ITS5 primers and they were *Fusarium proliferatum, Fusarium solani* and *Cladosporium cladosporoides*.

Liao *et al*. (2023) collected and studied endophytic *Apiospora* species from *Wurfbainiavillosa* and grasses in Guangdong and Yunnan provinces in China. Molecular identification was done using ITS1 and ITS4 primers, the large subunit nuclear rDNA, the partial elongation factor 1-α and d β-tubulin were also done to give a clarified phylogenetic affinity of the genus *Apiospora* and its various species. One hundred and ninety-one strains of *Apiospora* species were identified and some of which were *A. endophytica, A. guangdongensis, A. wurfbainiae, A. yunnanensis, A. guizhouensis, A. hysterina, A. longistroma, A. sorghi* etc.

The fungal endophytes diversity shows that it belongs to various groups of taxonomy. For identification through taxonomy, morphological and molecular characters are both required. Identification of endophytic fungi morphologically is regarded to be the conventional method that describes the physical attributes of fungi which gives some potential guide for identification. Regardless, there are a number of drawbacks of this method, therefore, investigation of problems in relation to classification and identification of fungal species is done using molecular technique. In scientific research, fungal identification to species level is important for ecology and taxonomy (basic) and genomics and bioprospecting (applied) applications (Ferdous *et al*., 2022).

The Internal Transcribed Spacer (ITS) and large subunit of ribosomal DNA region are particularly well regarded for identifying the fungal species. However, the ITS region is mostly more varying than the rDNA subunits and hence, may be more applicable for accurate identification if it is close match (Torres *et al*., 2015). For studies on fungal endophytes, Internal Transcribed Region (ITS) region is mostly used for molecularly identifying as this region is proposed as the general DNA barcode marker for identification of fungi (Schoch *et al*., 2012; Sun and Guo, 2012). There are many advantages for using ITS region as a marker which includes the accessibility of universal primers and datasets, abundant length of fragments and top success rate of amplification among the fungi ancestry (Vilgalys, 2003; Nilsson *et al*., 2009). Many of the fungal genes are progressively made accessible in popular databases of sequences, like GenBank which is obtainable in National Center for Biotechnology Information (NCBI) (Torres *et al*., 2015). Therefore, considering the above statement, the fungal endophytes from this experiment were molecularly identified using ITS1 and ITS4 primers.

Based on the morphological and molecular characterization and identification, 135 isolates were identified belonging to 15 genera. Out of the 15 genera, 14 belonged to the Phylum Ascomycota and 1 belonged to Mucoromycota. Of the 135 isolates, 65 was identified from leaves of wild banana plant, 54 from roots of wild banana plant, 11 from leaves of cultivated banana plant and 5 from the roots of cultivated banana plant. Altogether, 30 isolates were identified as *Diaporthe* sp. and the highest was identified from Kohima (9 isolates) and Mokokchung (8 isolates) district, isolated from the roots of wild banana. It was followed by *Penicillium* sp. with 29 isolates and highest was identified from Mokokchung (7 isolates) and Chumoukedima (6 isolates) district, isolated from the leaves of wild banana. Sixteen isolates each were identified for both *Fusarium* sp.and *Colletotrichum* sp. For *Fusarium* sp., highest identified isolates were from the roots of wild banana of

Mokokchungdistrict (5 isolates) followed by Dimapur district (4 isolates) from the leaves of wild banana. For *Colletotrichum* sp., highest isolates were identified from the leaves of wild banana from Kohima district (10 isolates). Eleven *Trichoderma* sp. were identified, with the highest isolates identified from the roots of wild banana from Peren district (5 isolates). The details of all the 24 identified isolates that belongs to distinct genera has been given in Table 4.13.

It is essential to note that an entomopathogen, *Beauveria felina* was isolated as an endophyte from the roots of cultivated banana that was collected from Kohima district. This entomopathogen may be a first report as an endophyte isolated from banana in the world.

Out of the identified isolates of fungal endophytes, it was found that *Trichoderma* sp., *Apiospora* sp., *Pestalotiopsis* sp., *Mucor* sp., *Phomopsis* sp., *Helminthosporium* sp. were only present in the wild banana isolated endophytes. *Botrytis* sp., *Beauveria felina* and *Cladosporium tenuissimum* were found only from the cultivated banana species.

Table 4.13. Fungal Endophyte Isolates Identified based on morphological and molecular characterization (District wise)

Sl. No.	Identified Isolates	District	Wild (Leaves)	Wild (Roots)	Cultivated (Leaves)	Cultivated (Roots)	Sub Total	Total
1.	***Penicillium* sp.**	Dimapur	6	-	-	1	7	29
		Kohima	1	4	-	-	5	
		Peren	1	3	-	-	4	
		Mokokchung	7	4	1	1	13	
2.	***Trichoderma* sp.**	Dimapur	2	3	-	-	5	11
		Kohima	-	1	-	-	1	
		Peren	-	5	-	-	5	
		Mokokchung	-	-	-	-	0	
3.	***Fusarium* sp.**	Dimapur	4	-	-	-	3	16
		Kohima	2	1	-	1	4	
		Peren	-	2	1	-	3	
		Mokokchung	-	5	-	-	5	
4.	***Colletotrichum* sp.**	Dimapur	1	-	2	-	3	16
		Kohima	10	-	-	-	10	
		Peren	-	-	-	-	0	
		Mokokchung	2	1	-	-	3	
5.	***Diaporthe* sp.**	Dimapur	5	-	-	-	5	30
		Kohima	-	9	-	-	9	
		Peren	1	-	-	-	1	
		Mokokchung	6	8	1	-	15	
6.	***Apiospora* sp.**	Dimapur	1	-	-	-	1	6
		Kohima	5	-	-	-	5	

		Peren	-	-	-	-	0	
		Mokokchung	-	-	-	-	0	
7.	***Aspergillus* sp.**	Dimapur	1	2	-	-	3	6
		Kohima	-	1	1	-	2	
		Peren	-	-	-	-	0	
		Mokokchung	1	-	-	-	1	
8.	***Pestalotiopsis* sp.**	Dimapur	-	-	-	-	0	6
		Kohima	2	-	-	-	2	
		Peren	3	-	-	-	3	
		Mokokchung	1	-	-	-	1	
9.	***Mucor* sp.**	Dimapur	-	-	-	-	0	3
		Kohima	-	-	-	-	0	
		Peren	-	2	-	-	2	
		Mokokchung	-	1	-	-	1	
10.	***Phomopsis* sp.**	Dimapur	-	-	-	-	0	2
		Kohima	-	1	-	-	1	
		Peren	-	-	-	-	0	
		Mokokchung	-	1	-	-	1	
11.	***Botrytis* sp.**	Dimapur	-	-	-	1	1	1
12.	***Helminthosporium* sp.**	Kohima	1	-	-	-	1	1
13.	***Alternaria* sp.**	Kohima	2	-	4	-	6	6
14.	***Beauveria felina***	Kohima	-	-	-	1	1	1
15.	***Cladosporium tenuissimum***	Peren	-	-	1	-	1	1
		Total	**65**	**54**	**11**	**5**	**135**	**135**

CHAPTER V

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

The present investigations on "**Study on the endophytic fungi from banana species of Nagaland and their effect againstthe Fusarium wilt pathogen**" was carried out in the laboratory of the Department of Plant Pathology, SAS, Medziphema Campus, Nagaland University, Nagaland. The findings obtained from various observations and recordings are already discussed in the preceding chapters.

A summary of the salient findings obtained in the present investigation is presented in this chapter.

- In the present study, fungal endophytes were isolatedfrom the healthy leaf and roots of banana species from four districts of Nagaland, which are Chumoukedima (earlier under Dimapur district), Kohima, Peren and Mokokchung districts. A total of 281 fungal endophyte isolates were isolated from the leaves and roots of banana species of Nagaland. Out of these, 246 isolates were isolated from the wild banana and 35 isolates from cultivated banana species of Nagaland. A total of 166 isolates were isolated from leaf samples and 115 isolates from root samples
- The present study identified prospective isolates with plant growth promotional activities giving excellent result. For IAA, FEB75 (*Apiosporalongistroma*), FEB83 (*Colletotrichum horii*), FEB178 (*Cladosporium tenuissimum*), FEB192 (Unidentified), FEB194 (Unidentified) and FEB222 (*Penicillium* sp.) were the best performing isolates with IAA production of 114.12 µg/ml.
- For GA3 production, FEB186 (*Mucor circinelloides*) at 113.36 µg/ml, FEB251 (*Mucor circinelloides*) at 109.64 µg/ml and FEB269 (*Colletotrichum gloeosporioides*) at 99.94 µg/ml were the best three performing isolates.

- For phosphate solubilization test, 44 isolates were found to show positive result with FEB65 (*Colletotrichum fructicola*), FEB68 (*Colletotrichum gloeosporioides*), FEB254 (*Phomopsis* sp.), FEB10 (*Penicillium* sp.), FEB23 (*Aspergillus versicolor*), FEB49 (*Aspergillus niger*), FEB71 (*Alternaria* sp.), FEB110 (Unidentified), FEB176 (Unidentified), FEB215 (Unidentified), FEB223 (*Penicillium* sp.) and FEB229 (*Aspergillus niger*) as the best performing isolates.
- For amylase test, 73 isolates were found to show positive result and the fungal isolates FEB49 (*Aspergillus niger*), FEB51 (*Aspergillus clavatonanicus*), FEB127 (*Penicillium* sp.), FEB143 (*Beauveria felina*), and FEB187 (*Penicillium citrinum*) showed the high amylase production.
- All the isolates showed negative reaction for chitinase activity test
- Siderophore production test showed 92 isolates giving positive reaction and the fungal endophytes FEB27 (*Diaporthephaseolorum*), FEB38 (Unidentified), FEB46 (*Trichoderma asperellum*), FEB49 (*Aspergillus niger*), FEB120 (Unidentified), FEB121 (*Aspergillus* sp.) and FEB129 (*Diaporthes*p.), FEB217 (*Penicillium* sp.), FEB222 (*Penicillium* sp.), FEB223 (*Penicillium* sp.) and FEB262 (Unidentified) exhibited the strongest siderophore production ability.
- The pathogen causing Fusarium wilt disease in banana was collected from an infected field showing characteristic symptoms of the disease and the collected specimen was brought to the laboratory and isolated in PDA medium. Morphological characterization revealed that the colony colour of the isolated pathogen was whitish pink and when observed under microscope, microconidia were found in abundance, non-septate or one celled, oval to kidney shaped, hyaline, 44.20 x 16 µm in size. Macroconidia were produced sparsely and were 4-6 celled, slightly sickle shaped with tapered ends, 160.12 x 20.80 µm in size.

Chlamydospores are globose, were produced singly or in pairs, found in abundance, smooth or rough walled, 40 – 44 μm in size.

- Detached leaf technique for pathogenicity test revealed that the isolated Fusarium wilt pathogen is pathogenic to banana producing symptoms similar to *Fusarium oxysporum* f. sp. *cubense*.
- The Fusarium wilt pathogen under study was identified as *Fusarium oxysporum*(with GenBank accession no. PP587552) after molecular characterization with a similarity percentage of 99.64% with the closest assession number (FJ605247).
- Antagonistic activity of the endophytic isolates against the Fusarium wilt pathogen of banana also gave promising result. In dual culture test, FEB116 (*Trichoderma asperellum*) with 61.90%, FEB249 (*Diaporthechromolaenae*) with57.14% and FEB23 (*Aspergillus versicolor*) with 55.24% inhibition gave the best result. Out of the best three isolates, two isolates were isolated from roots of wild banana plant (FEB116 from Kohima and FEB249 from Mokokchung district) and one (FEB23) from the leaves of wild banana plant from Chumoukedima district.
- For volatile production test, FEB81 (*Colletotrichum kahawae*) with 54.81%, FEB80 (*Apiosporahydei*) with 54.07%, FEB1 (*Penicillium* sp.) and FEB115 (*Diaporthefructicola*) with 52.59% inhibition gave the best result. Out of the best four performing isolates, three were isolated from the leaves of wild banana plant (FEB80 and FEB81 from Kohima and FEB1 from Chumoukedima district) and one (FEB115) from the roots of wild banana plant isolated from Kohima district.
- For non-volatile production test, FEB3 (*Fusarium haematococcum*) with 69.21%, FEB9 (*Fusarium solani*) with 68.32% and FEB5 (*Trichoderma hamatum*) with 66.33% gave the most promising result.

All the best three isolates for this test were isolated from the leaves of wild banana of Chumoukedima district.

- Molecular characterization of the best performing three isolates were done for all the experiments and a total of 24 fungal endophyte isolates have been identified which are *Apiosporalongistroma* (FEB75), *Colletotrichum horii* (FEB83), *Cladosporium tenuissimum* (FEB178), *Colletotrichum gloeosporioides* (FEB269), *Mucor circinelloides* (FEB186), *Mucor circinelloides* (FEB251), *Trichoderma asperellum* (FEB46), *Diaporthephaseolorum* (FEB27), *Diaporthe sp.* (FEB129), *Beauveria felina* (FEB143),*Aspergillus clavatonanicus* (FEB51), *Penicillium citrinum* (FEB187), *Phomopsis sp.* (FEB254), *Colletotrichum fructicola* (FEB65), *Colletotrichum gloeosporioides* (FEB68), *Aspergillus versicolor* (FEB23), *Diaporthechromolaenae*(249), *Trichoderma asperellum* (FEB116), *Apiosporahydei* (FEB80), *Colletotrichum kahawae*(FEB81), *Diaporthefructicola*(FEB115), *Fusarium haematococcum* (FEB3), *Trichoderma hamatum* (FEB5) and *Fusarium solani* (FEB9).
- Out of the 24 isolates that were identified, 22 belongs to the Phylum Ascomycota and 2 belongs to the Phylum Mucoromycota.
- Maximum isolates were molecularly identified from the samples collected from Kohima district, where a total of nine isolates were identified from the wild banana plants (6 from leaves and 3 from roots) and one from the root of cultivated banana. It was followed by Chumoukedima (earlier under Dimapur district) district where a total of six isolates were identified from the wild banana plants (5 from leaves and 1 from root) and one from the root of cultivated banana. From Mokokchung district, four isolates were molecularly identified and they were all from the roots of wild banana. Lastly, from Peren district, three

isolates were identified, two from the root and one from the leaves of wild banana.

- Morphological and molecular characterization revealed that a diversified number of fungal isolates coexist with the host plant. A total of 135 fungal species were identified belonging to 15 genera of which 119 isolates were from wild and 16 from cultivated banana.
- Out of the identified isolates of fungal endophytes, it was found that *Trichoderma* sp., *Apiospora* sp., *Pestalotiopsis* sp., *Mucor* sp., *Phomopsis* sp., *Helminthosporium* sp. were only present in the wild banana isolated endophytes. *Botrytis* sp., *Beauveria felina* and *Cladosporium tenuissimum* were found only from the cultivated banana species.

Thus, it can be concluded from the present investigation that there is a diversified number of fungal endophytes that coexist with the host plant, banana. All the isolated endophytes gave varied results in all the experiments conducted and it can be concluded that the isolated endophytes were found to have promising growth promoting abilities and antagonistic activity against the Fusarium wilt pathogen. The endophytes can be potential candidates for the management of Fusarium wilt disease in banana. These endophytes can be further explored against other disease-causing agents of banana in *in vitro* and *in vivo* conditions and we may get products to manage the diseases. An entomopathogen *viz*., *Beauveria felina* was identified from the isolated fungal endophytes and this requires further investigation against common agricultural pest. This might be a first report of *Beauveria felina* as an endophyte isolated from banana in the world. The future challenges are dependent on identifying, delineating, dissecting, and defining the mechanisms of the relationship endophytes have with their host plant. A basement- level success in this research which is reached

and further challenges might ensure the present and future successful technological applications of microbial endophytes mainly in growth promotion and in control of plant diseases.

CHAPTER V

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REFERENCES

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APPENDICES

APPENDIX

1. Potato Dextrose Agar (HiMedia composition)

Ingredients	Grams/Litre
Potatoes	200 g (peeled)
Dextrose	20 g
Agar-agar	15 g
Distilled water	1000ml
pH	7

2. ISP-2 medium

Ingredient	Grams/Litre
Yeast extract	4 g
Malt extract	10 g
Dextrose	4 g
Agar-agar	20 g
Distilled water	1000 ml
pH	7.2

3. ISP-2 broth

Ingredient	Grams/Litre
Yeast extract	4 g
Malt extract	10 g

Dextrose	4 g
L-tryptophan	2 g
Distilled water	1000 ml
pH	7.2

4. Murashige and Skoorg (MS) broth (HiMeida)

	Ingredients	**mg/L**
Macroelements	Ammonium nitrate	1650.000
	Calcium chloride	332.200
	Magnesium sulphate	180.690
	Potassium nitrate	1900.000
	Potassium phosphate monobasic	170.000
Microelements	Boric acid	6.200
	Cobalt chloride hexahydrate	0.0250
	EDTA disodium salt dihydrate	37.300
	Ferrous sulphate heptahydrate	27.800
	Manganese sulphate monohydrate	16.900
	Molybdic acid (sodium salt)	0.213
	Potassium iodide	0.830

	Zinc sulphate heptahydrate	8.600
Vitamins	Myo-Inositol	100.00
	Nicotinic acid (free acid)	0.500
	Pyridoxine HCL	0.500
	Thiamine hydrochloride	0.100
Amino acid	Glycine	2.000
Total (grams/L)		**4.4**

5. Pikovskya's agar

Ingredients	**Grams/Litre**
Glucose	10 g
$Ca_3(PO_4)_2$	5 g
$(NH_4)_2\ SO_4$	0.5 g
NaCl	0.2 g
$MgSO_4.7H_2O$	0.1 g
KCl	0.2 g
Yeast extract	0.5 g
$MnSO_4.H_2O$	0.002 g
$FeSO_4.7H_2O$	0.002 g
Agar-agar	20 g
Distilled water	1000 ml

6. Glucose yeast extract peptone (GYP) agar medium

Ingredients	Grams/Litre
Glucose	1 g
Yeast extract	0.1 g
Peptone	0.5 g
Agar-agar	15 g
Distilled water	1000 ml
pH	6

7. Colloidal chitin medium

Ingredients	Grams/Litre
Colloidal chitin	15 g
Yeast extract	0.5 g
$(NH_4)_2\ SO_4$	1 g
$MgSO_4.6H_2O$	0.3 g
KH_2PO_4	1.36 g
Agar-agar	15 g
Distilled water	1000 ml

8. Chrome Azurol S (CAS) medium

Ingredients	mg/ml or grams/litre
Chrome azurol sulfonate	60.5 mg/50 ml of distilled water
CTAB	72.9 mg/ 40 ml of distilled water
PDA medium (HiMedia)	39 g
Distilled water	900 ml

CAS solution (50 ml) was mixed with CTAB solution (40 ml) and then 10 ml of 1mM $FeCl_3.6H_2O$ solution prepared in 10 mM HCl was added. The mixture of these three solutions (100 ml) was finally added to 900 ml of melted PDA and the final volume was made up to 1000 ml. The medium was then poured into 250 ml of glassware, cotton plugged and autoclaved at 121^oC, 15 psi for 20 minutes.