ANTIMICROBIAL ACTIVITY OF ENDOPHYTES STRAIN ISOLATED FROM DIFFERENT PARTS OF *NYPA FRUTICANS* (WURMB) THUNB.

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Abstract

In this study, the samples of Nypa fruticans (wurmb)Thunb. were collected from Toung-Goke Township in Rakhine State. Eleven entophytic fungi were isolated from different parts (leaves, fruit and inflorescences) of Nypa fruticans (wurmb) Thunb. on five different media. The morphological and microscopically characters of isolated strains were carried out at the Microbiology Laboratory, Department of Botany, Dagon University. The colony appearances of isolated fungal strains were circular, irregular and filamentous. The margins of isolated fungal strains were curled, filamentous, and entire. The elevation of isolated fungal strains was raised, flat and convex. The reverse colors of isolated fungal strains were milky white, pale yellow, white, pale orange, pale green, pale brown and orange. The cultural characters of isolated fungal strains were white, deep black, pale orange, pale green, whitish green, greenish white, creaming white. Eleven isolated fungal strains were identified into possible Aspergillus, Fusarium, Cladosporium, Rhizoctonia, Penicillium and Mucor species. Strains NF-1 and 6 were identified as Aspegillus specie. Strains NF-2,4 and 9 were identified as Fusarium species. Strains NF-3 may be as Mucor species. Strains NF-7 may be Cladosporium species. Strains NF-5 may be Rhizoctonia specie. Strains NF-8 was as Penicillium sp. The antimicrobial activity was tested against eight test organisms by using paper disc diffusion method and fermentation was carried out for 7 to 10 days. The fermented broths of all isolated strains NF-5 and 8 showed good antimicrobial activity on the nine day. Secondary metabolites of eleven active strains were extracted with ethyl acetate and butanol. Their antimicrobial activity was also examined on eight test organisms. The crude extracts of two strains (NF-5 and 8) were showed excellent antimicrobial activity on seven test organisms.

Keywords: Endophytic fungi, antimicrobial activity, Nypa fruticans (wurmb) Thunb.

Introduction

Endophytes are microorganisms living within the tissue of a plant as endosymbionts, without causing symptoms of disease. Some of them are mutualistic symbionts with advantageous effects on their host, such as improved growth or resistance against disease or environmental stress, and are being used as microbial inoculants.

At the most basic level, endophyte simply means the location of an organism, with “endo” means “inside” and “phyte” means “plants”.An endophyte is an endosymbiont, often a bacterium or fungus, that lives within a plant for at least part of its life cycle without causing apparent disease. Endophytic fungal may also be used as biopesticide to prevent pathogen in plants Forchetti *et al*., (2007). Endophytic fungal normally live on intercellular spaces that contain carbohydrates, amino acids, and high amounts of inorganic nutrient. One notable endophyte with medicinal advantage to humans was discovered by Strobel (2004).

Endophytic fugal have been increased worldwide attention because of the search for new or raw biologically active compounds. The ability of endophytes to produce a great range of secondary metabolites such as antibiotics, bioinsecticides, fine chemicals, and enzymes had

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indicated some convincing results in combating pathogens and even cancer cell-lines in animals and humans Atmosukarto et al., (2005).

Nipa palm (Nypa fruticans) is a mangrove palm that thrives naturally in river estuaries and brackish water environment in which salt and fresh water mingle (Nguyen, 2014). N. fruticans is the only species present in the genus Nypa. Endophytes colonizing inside plant tissues contribute to the fitness of host and in return, they gain nutrient and protection from the host. Endophytic fungal produce a wide range of phytohormones, such as auxins, cytokinins and the gibberellins. In addition, endophytic bacteria help to enhance the nutrient ability and fix nitrogen for plants. For instance, plant hormones made by endophytic microorganisms seem to be necessary for bryophyte development. Hornschuh, et al., (2002).

Tan and Zou (2001) reported that the bioactive natural products from endophytes are promising resources for medicine, agriculture and industry Different kinds of Alkaloids are gave to plant by endophytes. Some of these alkaloids raise plants' resistance to environmental stress, and some are growth-promoting compounds.

Although many endophytic strains have been found, commercial endophytic inoculants for agriculture wider adoption of endophyte inoculants has been used by cheap synthetic fertilizers, variable responses by the endophyte depending on host genotype and environment, competition from endogenous microbes, host genotype specificity, poor establishment, and persistence. Endophytes possess significant potential to improve agriculture, but this will require further discovery of novel endophytes, genetic improvement of endophytes and their hosts, standardized testing, and formulation. Novel genes and metabolites from endophytes represent an additional largely primary resource for future agricultural biotechnology. (Website-2)

Mangroves are aggregations of ecological community with a competency to grow in extreme environmental conditions. Endophytic microorganisms alive within the plants in a symbiotic relationship where both the plants and the endophytes experience benefits.

In this investigation, altogether eleven endophytic fungal were isolated into pure culture by using five different media. Eleven isolated fungal strains are subjected into the examination microscopical character, to identify the possible genera of isolated fungal strains, and to examine antimicrobial activity of isolated fungal strains.

Materials and methods

Collection of Plant Materials

In the present research, the healthy plant parts (leaves, fruits and inflorescences of Nypa fruticans (wurmb) Thunb. were collected from Taunggoke Township in Rakhine state. The collected specimens were identified with the help of available literature. (Hundely and Chit Ko Ko, 1998; Kress, et al., 2003). Plant identification was checked with the international plant name index and world Checklist of Selected Plant Families (2022). The samples were taken and the experiments were carried out from March 2022. Samples were placed in clean plastic bags, brought to the laboratory and used for further experimental purpose.

Media for isolating endophytic Fungi

The choice of the growth medium is crucial as it directly affects the number and type of endophytic fungi that can be isolated from the leave, fruit and inflorescence 1. Nutrient agar Medium (Atlas,1993) 2. Potato Dextro Agar Medium 3. Sucrose, Yeast extract and Agar medium

4. Glucose, Yeast extract and Agar Medium 5. Lactose, Yeast extract and Agar were used for the isolation of endophytic fungi. Since there is no component in media which can suppress the growth of endophytic bacteria, so the media used for the isolation of endophytic fungi were supplemented with antibacterial agent, chloramphenicol at a concentration of 0.001 mg of each to suppress bacterial growth.

Isolation of Entophytic Fungal Strains from

Isolation of endophytic fungal strains was carried out by the scheme (Lee and Kim 2002)

Morphological Characters of Isolated Fungal Strains

The morphological and colonial characters such as colony appearance, margin, surface and reverse colours, and elevation of all isolated strains were recorded as revealed in the reference of Dubey and Maheswari, (2014).

Microscopical Characters of Isolated Fungal Strains

The microscopical characters of all isolated strains (NF-1 to NF-11) were carried out under light microscope with high magnification at Department of Botany, Dagon University. The main characters of hyphae, mycelium, sporangiophores, spore, color formation on upper as well as lower surface were comparatively studied. These are compared to those of fungi with available literatures such as Barnett, (1969).

Test Organisms

All endophytic fungi isolates were screened for antimicrobial activities. The indicator microbe included Agrobacterium tumefaciens, Bacillus subtilis, Bacillus pumilus, Candida albicans, Escherichia coli, Malassezia furfur, Pseudomonas sp., Xanthomonous sp., and Staphylococcus aureus.

Fermentation of isolated Fungal and antimicrobial activity test of liquid fermented

Nutrient, Potato Dextore, Sucrose/Yeast, Glucose/Yeast, Lactose/Yeast media were also used in this test. Each medium was heated to boiling and sterilized by autoclaving at 121 °C for 15 minutes. Each fungal isolate that has been purified in previous experiments was inoculated on the liquid medium and then fermented. The fermentation process was done in a 250 ml Erlenmeyer flask. Incubation is for 72-120 hours at a temperature of 25-27 °C on a rotary shaker incubator for fungus at a speed of 90 rpm. The ferment broth was tested its antimicrobial activity against microbes using paper disc method. Paper discs dipped in the ferment broth and planted in the medium NA containing eight test organisms and incubated at a temperature of 37 °C for 18-24 hours. Barriers to growth were observed and measured in diameter by using digital caliper.

Extraction and isolation of crude ethyl acetate extracts and butanol extract from fungal fermentation broths

Each of the pure cultures was re-cultivated on the selected media at 28°C for 5 to 7 days. Three pieces (0.5×0.5 cm^2) of mycelia agar plugs were inoculated into 500 ml Erlenmeyer flasks containing 300 ml the selective broth (NB, PDB, YSB, YGB and YLB) and incubated at room temperature for ten days under stirring conditions. The active constituents were extracted from both filtrate and mycelia after separation from the fermented culture broth. One part of the filtrate was extracted two times with equal volume of n-butanol and another part with ethyl acetate. The mycelia were extracted with only acetone. All extract was concentrated by removing the solvents

under reduced pressure at 35-40°Cat oven. The extract residue was stored at 4°C as stock solution for antimicrobial bioassay. The crude ethyl acetate and butanol extracts of the NF-1,2,3,4,5,7,8,9,10 and 11 endophytic fungi were tested for their antibacterial activity against eight test organisms.

Results

Morphological Characteristics of Collected Plant Samples

Scientific Name - Nypa fruticans (wurmb) Thunb.

Myanmar Name - Ye-Ohn-Thee

Family - Arecaceae

Habit Inflorescence Leaves Fruits

Figure 1. Habit of the Plants, Leaves, Fruits and Inflorescence

Screening of Endophytic Fungal Strains

In the present works, twelve isolated fungi designate as NF-1 to NF-11 were maintained into the pure culture for further studies. Designated fungal strains NF-1 and 7 were obtained from inflorescences, NF-3, 4, 8 and 9 were from leaves and NF-2,5,6,10 and 11 were isolated from fruits.

Table 1. Isolation of Eleven Endophytic Fungal Strains

Isolated Strains	Sources
NF-1 and NF-7	Inflorescences
NF-3, 4, 8 and 9	Leaves
NF-2, 5, 6, 10 and 11	Fruits

Table 2. Colony and morphological characterization of isolated fungi

Isolated strain	Surface color	Reverse color	Colony apperance	Margin	Elevation
NF-1	Gray	White gray	filamentous	Filiform	Raise
NF-2	White	White	Circular	Curled	Raise
NF-3	white	Light yellow	irregular	curled	Raise
NF-4	White	White	Circular	Curled	Raise
NF-5	white	Light yellow	Circular	Curled	Convex
NF-6	gray	White grey	Circular	Curled	Raise

Isolated strain	Surface color	Reverse color	Colony apperance	Margin	Elevation
NF-7	Light yellow	Light yellow	Circular	Curled	Raise
NF-8	Light grey	Light yellow	Irregular	undulate	Raise
NF-9	white	Light Yellow	rhizoid	Curled	Raise
NF-10	White	white	filamentous	Filamentous	Convex
NF-11	White	White	Circular	Filamentous	Raise

Possible Genus of Isolated Fungi According to Imperfect Fungi (Barnet ,1969)

Morphological and Microscopical character of strain NF-1

The surface color of NF-1 was pale green. Its reverse color was pale green as shown in Figure 2. Endophytic fungal strain from Inflorescences Nypa fruticans **(**Inflorescences) on NA medium.

Division - Ascomycota
Class - Ascomycetes
Order - Aspergillales
Family - Aspergillaceae
Genus - ***Aspergillus***

Surface View Reverse View Microscopical character

Figure 2. Morphological and Microscopical character of isolated Fungi (NF-1)

Conidiophores arising from the mycelium singly or less often in synnemata, branched near the apex, penicillate, ending in a group of phialides; conidia (phialospores) hyaline or brightly colored in mass, 1-celled, mostly globose or ovoid, in dry basipetal chains. This strain may be identified as Aspergillus sp. as shown in Figure 2.

Morphological and Microscopical character of strain NF-2

The surface color of NF-2 was white. Its reverse color was white as shown in Figure 3. Endophytic fungal strain from Inflorescences Nypa fruticans (fruits) on NA medium.

Division - Ascomycota
Class - Sordariomycetes
Order - Hypocreales
Family - Nectriaceae
Genus - ***Fusarium***

Figure 3. Morphological and Microscopical character of isolated Fungi. (NF-2)

Mycelium extensive and cotton-like in culture, often with some tinge of pink, in the mycelium on medium; conidiophores variable, branched irregularly, single or grouped into sporodochia; conidia (phialospore) hyaline, variable, principally of two kinds; parasitic on higher plants. These strains may be identified as Fusarium species as shown in Figure 3.

Morphological and Microscopical character of strain NF-3

The surface color of NF-3 was pale orange. Its reverse color was pale orange as shown in Figure 4 Endophytic fungal strain from Inflorescences Nypa fruticans (leaves) on YS medium.

Division - Mucoromycota
Class - Zygomycetes
Order - Mucorales
Family - Mucoraceae
Genus - ***Mucor***

Figure 4. Morphological and Microscopical character of isolated Fungi (NF-3)

Typically exhibits rapid growth, producing globose sporangia on sporangiophores that are entire solitary or branched. This strains may be identified as Mucor specie as shown in Figure (4)

Morphological and Microscopical character of strain NF-4

The surface color of NF-4 was white. Its reverse color was white as shown in Figure (5). Endophytic fungal strain from Inflorescences Nypa fruticans (leaves) on YS medium.

Division - Ascomycota
Class - Sordariomycetes
Order - Hypocreales
Family - Nectriaceae
Genus - ***Fusarium***

Figure 5. Morphological and Microscopical character of isolated Fungi (NF-4)

Mycelium extensive and cotton-like in culture, often with some tinge of pink, in the mycelium on medium; conidiophores variable, branched irregularly, single or grouped into sporodochia; conidia (phialospore) hyaline, variable, principally of two kinds; parasitic on higher plants. These strains may be identified as Fusarium species as shown in Figure (5).

Morphological and Microscopical character of strain NF-5

The surface color of NF-5 was pale green. Its reverse color was orange as shown in Figure 6. Endophytic fungal strain from Inflorescences Nypa fruticans **(**fruits) on YS medium.

Division - Basidiomycota
Class - Agaricomycetes
Order - Cantharellales

Family - Ceratobasidiaceae

Genus - ***Rhizoctonia***

Figure 6. Morphological and Microscopical character of isolated Fungi (NF-5)

Asexual fruit bodies and spores lacking; sclerotia brown or black, variable in form, frequently small and loosely formed, among and connected by mycelia thread; hyphae with long cell, septa of branch set off form main hypha. This strain may be identified as Rhizoctonia s sp. as shown in Figure (7).

Morphological and Microscopical character of strain NF-6

The surface color of NF-6 was deep black. Its reverse color was pale brown as shown in Figure 7. Endophytic fungal strain from Inflorescences Nypa fruticans (fruits) on YS medium.

Division - Ascomycota

Class - Ascomycetes

Order - Aspergillales

Family - Aspergillaceae

Genus - ***Aspergillus***

Figure 7. Morphological and Microscopical character of isolated Fungi (NF-6)

Conidiophores arising from the mycelium singly or less often in synnemata, branched near the apex, penicillate, ending in a group of phialides; conidia (phialospores) hyaline or brightly colored in mass, 1-celled, mostly globose or ovoid, in dry basipetal chains. This strain may be identified as Aspergillus sp. as shown in Figure (7).

Morphological and Microscopical character of strain NF-7

The surface color of NF-7 was central pale green, margin white. Its reverse color was pale green as shown in Figure 8. Endophytic fungal strain from Inflorescences Nypa fruticans **(**inflorescence) on YG medium.

Division - Basidiomycota

Class - Dothideomycetes

Order - Capnodiales

Family - Davidiellaceae

Genus - ***Cladosporium***

Figure 8. Morphological and Microscopical character of isolated Fungi (NF-7)

Conidiophores tall, branched variously near the apex, clustered; conidia light, 1 or 2 celled, variable in shape and size, ovoid to cylindrical and irregular, some typically lemon – shaped; often in simple or branched acropetalous chains; saprophytic. This strain may be identified as Cladosporium species as shown in Figure (8).

Morphological and Microscopical character of strain NF-8

The surface color of NF-8 was central green, margin white. Its reverse color was milky white as shown in Figure 9. Endophytic fungal strain from Inflorescences Nypa fruticans (leaves) on YG medium.

Division	-	Ascomycota
Class	-	Eurotiomycetes
Order	-	Eurotiales
Family	-	Trichocomaceae
Genus	-	***Penicillium***

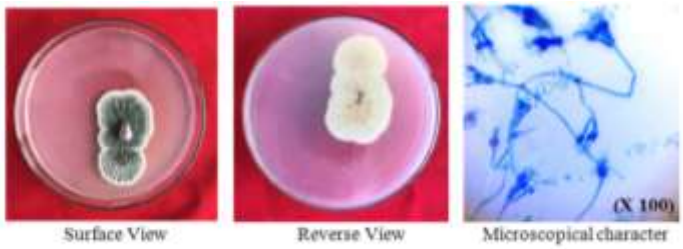

Figure 9. Morphological and Microscopical character of isolated Fungi (NF-8)

Conidiophores arising from the mycelium singly, branched near the apex to form a brush-like, conidia-bearing apparatus; conidia hyaline or brightly colored in mass, 1-celled, mostly globose produced basipetally.

Morphological and Microscopical character of strain NF-9

The surface color of NF-9 was central milky, margin white. Its reverse color was pale yellow as shown in Figure (10). Endophytic fungal strain from Inflorescences Nypa fruticans (leaves) on YG medium.

Division	-	Ascomycota
Class	-	Sordariomycetes
Order	-	Hypocreales
Family	-	Nectriaceae
Genus	-	***Fusarium***

Figure 10. Morphological and Microscopical character of isolated Fungi (NF-9)

Mycelium extensive and cotton-like in culture, often with some tinge of pink, in the mycelium on medium; conidiophores variable, branched irregularly, single or grouped into sporodochia; conidia (phialospore) hyaline, variable, principally of two kinds; parasitic on higher plants. These strains may be identified as Fusarium species as shown in Figure 10.

Morphological and Microscopical character of strain NF-10

The surface color of NF-10 was white. Its reverse color was pale orange as shown in Figure 11. It was not identified as shown in Figure (11). Endophytic fungal strain from Inflorescences Nypa fruticans (fruits) on YL medium.

Figure 11. Morphological and Microscopical character of isolated Fungi (NF-10)

Morphological and Microscopical character of strain NF-11

The surface color of NF-11 was white. Its reverse color was white as shown in Figure 12. Endophytic fungal strain from Inflorescences Nypa fruticans (fruits) on YL medium.

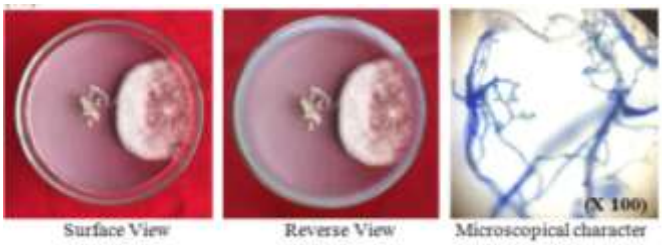

Figure 12. Morphological and Microscopical character of isolated Fungi (NF-11)

It was not identified as shown in Figure 12.

Microscopical Characters of Isolated Fungi

The microscopical characters of isolated entophytic fungi are the possible genera of Aspegillus, Cladosporium, Fusarium, Mucor, Penicillium and Rhizoctonia. These strains were identified as strains NF-1 and 6 as Aspegillus species, strain NF-7 as Cladosporium species, strains NF-2,4 and 9 as Fusarium species, strain NF-3 as Mucor, strain NF-8 as Penicillium, strain NF-5 as Rhizoctonia, strains NF-10 and 11 were being unable to identify their genus level. So, they were assumed as unidentified isolates.

Antimicrobial Activity of Fermented Broths of Isolated Entophytic Strains

At 8^{th} day fermentation strain NF-2 inhibited high activity against eight test organisms except Bacillus subtilis. Strain NF-5 stated moderate activity against on Agrobacterium tumefaciens, Bacillus subtilis, Malassezia furfur, Staphylococcus aureus, Xanthomous sp. Strain NF-8 state activity against all test organisms except Bacillus pumilus, Candida albicans, Escherichia coli. Strains NF-1, 4, 10 and NF-11 indicated activity Agrobacterium tumefaciens, Bacillus subtilis, Staphylococcus aureus, Xanthomous sp. as shown in Figure 17.

Figure 13. Antimicrobial Activity of Fermented Broths of Eight Day Old Culture

Antimicrobial activity of fermented broths of isolated entophytic strains

At 9th day fermentation, strain NF-8 indicated very high activity against on all test organism, strain NF-5 inhabited moderate activity against except Staphylococcus aureus all test organisms. Strain NF-8 expressed activity against, Bacillus pumilus, Bacillus subtilis, Candida albicans, Escherichia coli, Staphylococcus aureus, Xanthomous sp. as shown in Fig. (20).

Figure 14. Antimicrobial Activity of Fermented Broths of Nine Day Old Cult

Antimicrobial Activity of Crude Extracts of Entophytic Fungal Strains

Screening of endophytic fungi from different parts of Nypa fruticans (Wurmb) Thunb. was done by using five different media as basal isolation medium. To isolate the fungi, the chloramphenicol (0.001 mg) was put into five different media for the fungi only. Eleven isolated fungi were obtained in the present screening. The isolated fungi from different parts of Nypa fruticans (Wurmb) were designated codes NF-1 to 11. The colonies of isolated strains were shown in Table 1. The photomicrograph of morphology, mycelium and spore were displayed in Figure 3 and 13. The results of morphological cultural and biochemical test were shown in Table 3 to 7. The antimicrobial activity of all isolated against eight test organisms and indicated by size of clear zone was shown in Table 4 to 7.

The second parts of the present work mainly depend on extraction of metabolites by using ethyl acetate and butanol. According to the results of antimicrobial activity the isolated fungal strains NF-1,2,3,4,5,7,8,9,10 and NF-11 were selectively used in extraction of metabolites. The isolated fungi were grown in the 200 ml of five different broths. In the case of isolated fungi, the fermentation periods were checked 7,8,9 and 10 days and the best antimicrobial activity was detected in the 9 days. After each fermentation periods, the fermented broths were extracted with 100 ml each of ethyl-acetate and butanol in different separating funnels. The resulted crude extracts were subjected in the antimicrobial test and sizes of clear zone were tabulated in Table 4 and Figure 16. In the case of fungi, not only crude extract from fermented broth but also the mycelium biomass crude extract, by acetone was also applied in the clear zone tests. In the metabolite extraction by fungi, the isolate NF-5 and 8 which may be Rhizoctonia spp. Penicillin sp. were found to give best antibacterial activity. It was recorded that the ethyl acetate NF-5 (Rhizoctonia spp.) provide 25mm clear zone against Candida albicans, 20.0 mm against Bacillus pumilus, Bacillus subtilis, Malassezia furfur and Staphylococcus aureus. Similarly, ethyl acetate extract of NF-8 (Penicillin sp.) also showed 30 mm clear zone on Agrobacterium tumefaciens and 25.0 mm against on Candida albicans. The butanol extracts of NF-5 (Aspergillus) also provided 30 mm high antimicrobial activity on Bacillus pumilus and Bacillus subtilis such as 26 mm, 25 mm, 25 mm

and 22 mm against activity Staphylococcus aureus, Agrobacterium tumefaciens, Candida albicans and Malassezia furfur.

The mycelia acetone extract of NF-5 (Rhizoctonia sp.) also show 24 mm, 23 mm, 22 mm and 20 mm on Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis, Candida albicans, Malassezia furfur, Staphylococcus aureus, Xanthomous spp. The mycelia acetone extract of NF-8 (Penicillin.) also show against activity 27 mm, 25 mm, 26 mm and 30 mm, 20 mm and 20 mm on Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis, Candida albicans, Malassezia furfur, Staphylococcus aureus, Xanthomous spp. The results were shown in Table (8). and Figure 22 to 23.

Figure 15. Antimicrobial Activity of Nine day Crude Extract of Two Entophytic Fungi NF-5 and NF-8 Against Seven Test Organism

In the metabolite extraction by fungi, the isolate NF-9,10, 11 and 4 which may be Fusarium spp. unknown, unknown, Fusarium sp. were found to give best antibacterial activity. It was recorded that the ethyl acetate NF-9 (Fusarium spp.) provide 30 mm clear zone against Candida albicans, 27.0 mm against Malassezia furfur, 26 mm on Bacillus pumilus, 25 mm on Agrobacterium tumefaciens. Similarly, ethyl acetate extract of NF-10 showed 30, 16, 20,22 and 20 mm on Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis, Candida albicans and Xanthomous sp. The ethyl acetate extract of NF-11indicated activity against 25,15,18,16 mm on Agrobacterium tumefaciens, Bacillus pumilus, Candida albicans and Xanthomous sp. The ethyl acetate extract of NF-4 indicated activity against on Agrobacterium tumefaciens and Candida albicans. The butanol extract of NF-9 was showed 23, 20,20,20,16 and 30 mm on Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis, Candida albicans, Malassezia furfur and Xanthomous sp. The butanol extract of NF-10 was indicated activity against 25, 20, 23, 22,20,25 mm on Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis, Candida albicans, Malassezia furfur and Xanthomous spp. The butanol extract of NF-11 was indicated activity against 25, 26,22,25,15 and 26 mm on Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis, Candida albicans, Malassezia furfur and Xanthomous spp. The butanol extract of NF-4 was indicated activity against 20,28, and 20 Agrobacterium tumefaciens, Bacillus pumilus and Xanthomous spp.

The mycelia acetone extract of NF-9 also shows against activity 21, 20,22,20,20 and 21 Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis, Candida albicans, Malassezia furfur and Xanthomous spp. The mycelia acetone extract of NF-10 also shows against activity 20,19,16,16,17 and 25 Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis, Candida albicans, Malassezia furfur and Xanthomous spp. The results were shown in Table 9 and Figure 24 and 25.

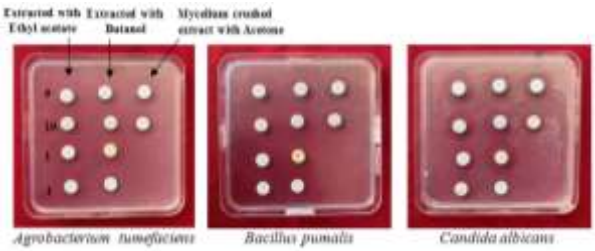

Figure 16. Antimicrobial Activity of Nine day Crude Extract of Four Entophytic Fungi NF 9,10, 11 and NF-4 Against Six Test Organisms

In the metabolite extraction by fungi, the isolate NF-2, 7,1 and 3 which may be Fusarium sp., Cladosporiumr spp., Aspegillus spp. and Mucor sp. were found to give best antibacterial activity.

It was recorded that the ethyl acetate NF-2 was indicated activity against 25,25 and 25 mm on Candida albicans, Malassezia furfur, and Xanthomous sp. The ethyl acetate NF-7 was indicated activity against 23, 23 and 25 mm on Candida albicans, Malassezia furfur and Xanthomous sp.

The ethyl acetate NF-1 was exhibited activity against 25,15 and 25mm on Candida albicans, Malassezia furfur and Xanthomous sp. The butanol extracted NF-2 was displayed activity against 20,26 and 30mm on Candida albicans, Malassezia furfur and Xanthomous sp. The butanol extracted NF-7 was indicated activity against 20, 25 and 22 on Candida albicans, Malassezia furfur, and Xanthomous sp. The butanol extracted NF-1 was indicated activity against 20,15 and 22 on Candida albicans, Malassezia furfur and Xanthomous sp.

The butanol extracted NF-3 was showed activity against 18, 25 and 22 on Candida albicans, Malassezia furfur and Xanthomous sp. The mycelium acetone extracted NF-7 was stated activity against 15, 20 and 25 on Candida albicans, Malassezia furfur and Xanthomous spp. The mycelium acetone extracted NF-1 was point to activity against 18,20 and 20 on Candida albicans, Malassezia furfur, Xanthomous spp.

The mycelium acetone extracted NF-3 was demonstrated activity against 16,25 and 15 on Candida albicans, Malassezia furfur and Xanthomous sp. as shown in Table (10) and Fig. (26 and 27).

Figure 17. Antimicrobial Activity of Nine Day Crude Extract of Four Entophytic Fungi NF-2,7,1 and NF-3 Against Two Test Organisms

Discussion and conclusion

The present investigation was 11 endophytes fungal from different parts of Nypa fruticans (wurmb) Thunb. The colony appearances of isolated fungal strains were circular, irregular and filamentous. These results were in agreement with Dubey and Maheswari, (2014). The margins of isolated fungal strains were curled, filamentous and entire. The elevation of isolated fungal strains was raised, flat and convex. These findings were in agreement with Dubey and Maheswari, (2014). The reverse colors of isolated fungal strains were white, light yellow, yellowish white, yellow,

whitish grey, brownish white and brownish gray. These results were in agreement with Davise, (1995).

In the present research among 11 isolated strains, the colony, conidiophore and conidia characteristic NF-7 was very closely to those of Cladosporium genus. Therefore, these strains were identified as possible genus Cladosporium. Zhang et al. (2014) concluded that five new compounds were isolated from the mangrove-derived endophytic fungus C.cladosporioides MA-299.

In this study Under the microscope, conidiophores arising from the mycelium singly in synnemata, branched near the apex, penicillate, ending in a group of phialides; conidia hyaline or brightly colored in mass, 1-celled, mostly globose or ovoid NF-6 were very closely to those of Aspergillus genus. Therefore, these strains were identified as possible genus Aspergillus. This strain may be identified as Aspergillus. Peng et al. (2022) and Gao et al. (2013) were stated that mangrove endophytic fungi of the genus Aspergillus have been reported to afford a wide range of secondary metabolites.

Chung et al., (2013) were reported that mangrove-derived endophytic fungi are rich sources for the production of structurally diverse and fascinating natural products with a variety of biological properties.

In this investigation, NF-3 was exhibits rapid growth, producing globose sporangia on sporangiophores that are entire solitary or branched. These characters were similar to Mucor. Shu-Shan Gao et al. (2016) were explained that Rhizovarins A–F, Indole-Diterpenes from the mangrove-derived endophytic fungus Mucor irregularis QEN-189.

Conidiophores are upright, simple, terminating in a globose, bearing phialides at the apex or radiading from the entire surface; globose often variously in mass, produce basipetally. These characters were very similar to those observed in NF8. Therefore, these strains may be identified as Penicillin. Hongju et al. (2016) were reported that polyketides with immunosuppressive activities from mangrove endophytic fungus Penicillin sp. ZJ-SY_2**.**

In the present study, Mycelium extensive and cotton-like in culture, often with some tinge of yellow in the mycelium on medium; conidiophores variable, slender, and simple, or stout, branched irregularly, single; conidia hyaline, variable, oblong, borne singly; oblong or slightly curved; parasitic on higher plants. These strains may be identified as Fusarium species. Zhongiing et al., (2011) were concluded that a new isoflavone from the mangrove endophytic fungus Fusarium sp. (ZZF60). Tauhidur et.al (2020) reported that Screening of endophytic fungi from mangrove plant with inhibitory activities against pathogenic bacteria or fungi might lead to potential novel natural products with higher antimicrobial activity. Similar to our study, Buatong et al. (2011) reported the extraction of fungal broth by ethyl acetate while the mycelia were extracted using methanol, hexane, acetone and ethyl acetate in sequence, respectively.

Ethyl acetate is widely used in extraction of endophytic fungal cultures (Bhardwaj et al. 2015) followed by methanol. As a solvent, ethyl acetate solvent possesses medium polarity so that it has the ability to dissolve both polar and non-polar active compounds and methanol solvent being a polar solvent can dissolve almost all organic compounds, even polar, semi polar and non-polar (Rahmawati et al. 2018). Li et al. (2017) stated that Identification and antifungal activity of compounds from the mangrove endophytic fungus Aspergillus clavatus R7.

In this study, we successfully isolated and identified 11 different species of endophytic fungi belonging to 5 different genera. All the isolates are moderately active against tested microorganisms. However, further studies could be initiated with Penicillum sp., Rhizoctonic sp., Cladosproiwm sp. and Fusarium spp. for potential bioactive compounds as our results showed promising activities with these endophytic fungi. The findings of this study also suggest that endophytes from mangrove ecosystem might be an attractive source for bio-prospecting of new antimicrobial compounds.

In our study, endophytic fungi isolate from eleven mangrove species were found to be diverse. In the present research among 11 isolated strains, the colony, conidiophore and conidia characteristic NF-7 was very closely to those of Cladosporium genus. Therefore, these strains were identified as possible genus Cladosporium.

In this study Under the microscope, conidiophores arising from the mycelium singly in synnemata, branched near the apex, penicillate, ending in a group of phialides; conidia hyaline or brightly colored in mass, 1-celled, mostly globose or ovoid NF 6 were very closely to those of Aspergillus genus. Therefore, these strains were identified as possible genus Aspergillus. This strain may be identified as Aspergillus.

In this investigation, NF-3 was exhibits rapid growth, producing globose sporangia on sporangiophores that are entire solitary or branched. These characters were similar to Mucor. Conidiophores are upright, simple, terminating in a globose, bearing phialides at the apex or radiading from the entire surface; globose often variously in mass, produce basipetally. These characters were very similar to those observed in NF8. Therefore, these strains may be identified as Penicillin.

In the present study, Mycelium extensive and cotton-like in culture, often with some tinge of yellow in the mycelium on medium; conidiophores variable, slender, and simple, or stout, branched irregularly, single; conidia hyaline, variable, oblong, borne singly; oblong or slightly curved; parasitic on higher plants. These strains may be identified as Fusarium species.

Ethyl acetate is widely used in extraction of endophytic fungal cultures (Bhardwaj et al., 2015) followed by methanol. As a solvent, ethyl acetate solvent possesses medium polarity so that it has the ability to dissolve both polar and non-polar active compounds and methanol solvent being a polar solvent can dissolve almost all organic compounds, even polar, semi polar and non-polar (Rahmawati et al., 2018).

Similar to our study, Buatong et al., (2011) reported the extraction of fungal broth by ethyl acetate while the mycelia were extracted using methanol, hexane, acetone and ethyl acetate in sequence, respectively.

Prihanto et al., (2011) was suggested that Isolated of Endophytic fungi from Rhizophora mucronata (Malay: bakau kurap) of the genera Penicillium, Ampelomyces, and Fusarium were showed to be active against E.coli . De Souza Sebastianes et al., (2013) were concluded that fungi from marine environments grow in habitats with unique conditions that attributed to the activation of metabolic pathways and the synthesis of distinct unknown molecules. Fox and Howlett, (2008) was concluded that production of these compounds aids in supporting the adaptation and survival of the fungi in marine ecosystems. Hence, endophytes from mangrove ecosystem are not only the bio-prospecting but also provides protection against pahogenes. An intensive search for newer and effective antimicrobial agents is needed.

This study indicates that endophytic fungi isolated from mangrove possess potential antimicrobial properties and should be further investigated to produce their active compounds as antibiotic agents.

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