Title	Isolation of Pathogenic fungi from Piper betle L.
Author	Dr. San San Maw and Naing Zaw
Issue Date	

Isolation of Pathogenic Fungi from *Piper betle* L.

San San Maw[1] & Naing Zaw[2]

Abstract

The present study deals with the isolation of pathogenic fungi from infected leaves and stem parts of *Piper betle* L. (Kun). The specimens were collected from Wa Yon Chaung Village, Nga Zun Township, Mandalay Region. Totally seven strains of pathogenic fungi were isolated from infected parts of betelvine. Stem rot and yellow leaf diseases were caused by *Aspergillus* spp. (NZ 01, NZ 07)). and leaf rot disease caused by *Fusarium* sp. (NZ 02). Leaf spot disease was infected by *Curvularia* sp. (NZ 03) and *Cladosporium* sp. (NZ 04). Anthracnose disease was formed by *Alternaria* sp. (NZ 05) and *Colletotrichum* sp. (NZ 06). The colony morphology, spore and hyphae characters were described and presented with photomicrographs. The two species of *Aspergillus* are the same genus but the different species because of the presence of septate and non-septate conidiophore and the difference color of colony.

Introduction

Betel is a shade loving perennial rooted climber which belongs to the family Piperaceae. Its scientific name is *Piper betle* L. (Srichana *et al*. 2009). Betelvine having the heart-shaped deep green leaves is an important horticultural crop of aesthetic and commercial values. It is grown throughout the country. There are about 100 varieties of betel leaf across the world. The betel leaf contains some vitamins, enzymes, thiamine, riboflavin, tannin, iodine, iron, calcium, minerals, protein and essential oil (Chopra *et al*. 1956; Khanna 1997 as cited in Jane *et al*. 2014).

Leaf juice is useful as an eyedrops in painful, ophthalmic affections and in night blindness. The leaves are traditionally used as a pan-mouth refresher and have oral hygiene due to the presence of anti - microbial components. Betel leaves are useful for the treatment of boils, abscesses, wound, itches, abrasion, cuts and injuries, ringworm, headache, hysteria, cold and cough, disease of throat, colic, dysentery and constipation, piles swelling of gum, rheumatism and join pain (Patel and Jasrai 2013).

[1.] Lecturer, Department of Botany, University of Mandalay
[2.] M Sc, Department of Botany, University of Mandalay

Leaves of betelvine are chewed along with areca nut as a masticator in many parts of the world. Betel chewing is considered to be a good and cheap source of dietary calcium (Anon 1990 as cited in Shahzad and Zareen 1999). The betelvine is grown in conservation under shady and humid conditions necessary for the growth of plant. This shady and moist atmosphere also flavors the development of many diseases, especially leaf spot disease that greatly affects the growth of plants and produce heavy losses to the farmers (Chattopadhayay and Maiti 1990 as cited in Shahzad and Zareen 1999).

Among plant microbial pathogens like bacteria, fungi, viruses etc, fungi are the most important and prevalent pathogens, infecting a wide range of host plants and are responsible to cause economical losses of crops in the field and harvest during storage and transportation. Plant-based fungal pathogens are unsafe for consumption (Patel and Jasrai 2013).

During cultivation, betelvine is very much affected by diseases and outcome of the farmer is big loss for betelvine cultivation. The most important diseases of betelvine plants are powdery mildew disease, leaf rot disease, foot rot disease and leaf spot disease. It occurs in a very powerful form and if not controlled, causes unlimited damage and even total demolition of the entire of betelvine plantations (Vijayakumar and Arumugam 2014).

The betelvine cultivation is also found in anywhere of Myanmar. The production of betelvine leaves are commonly found in this study area. The betelvine consumption with areca nuts is used as important role in traditional ceremonies and also used as medicine in local people. But, the leaves are infected by the diseases of leaf spot, leaf rot, foot rot, powdery mildew and anthracnose caused by various fungi. Therefore, many people are suffered various diseases by consuming fungus infected betelvine.

The farmers are also encountered heavy loss in leaf yield every year. The leaf is a destructive disease causing substantial yield loss in all the growing area. The diseases cause severe damage to natural leaves in the field as well as during transit and unfavorable weather conditions. Betel leaf spot, leaf rot and powdery mildew are serious disease caused by various fungal pathogens. These pathogens are necessary to control and manage because betel leaves are one of the most important cash crops in our country. Moreover, the disease infected leaves should not be used in consumption and in medicine because of harmful to health. Thus, an investigation of fungal diseases on betelvine leaves should be carried out.

Isolation of pathogenic fungi from *Piper betle* L. (betelvine) has not been conducted yet. Therefore, it is needed to fulfill the knowledge gap and have been made to study the disease of leaf rot, leaf spot, anthracnose, yellow leaf disease and stem rot disease.

The aims and objectives of this research are to investigate the symptoms of diseases on the betelvine plants, to describe what kinds of pathogenic fungus are most common in betelvine and to study their microscopical and macroscopical characters.

Materials and Methods

Collection of Plant Samples

The disease infected parts of *Piper betle* L. were collected from Wa Yon Chaung Village, Nga Zun Township, and Mandalay Region. The infected stems and leaves in the field of study were cut and then put into the plastic bags. The specimens were also recorded by photographs. The sample preparation was performed at Microbiology laboratory of Botany Department, University of Mandalay. Plant identification was done by Hooker (1885), Backer & Brink (1965) and Dassanayake (1987).

Preparation of Potato Dextrose Agar (PDA)

Potato Dextrose Agar (PDA)	3.9 g
Distilled water	100 ml
pH	6.5

After autoclaving chloramphenicol (0.1 g) was added to the medium.

Preparation of Water Glucose Agar (WGA)

Glucose	1.8 g
Agar	2.0 g
Distilled water	100 ml
pH	6.5

After autoclaving chloramphenicol (0.1 g) was added to the medium.

Direct Isolation Method

The disease infected parts were washed in tap water for 5 minutes. The leaves were cut into about 1 cm pieces. They were washed with distilled water and then dried on the sterilized filter paper for 15 minutes. Infected parts were placed on the PDA medium for one hour to obtain proper deposition of fungal spores from the infected parts. After that they were removed from the plate. The pathogenic spores were inoculated for 3 - 10 days in the room temperature. The pathogenic fungal colonies developing on PDA medium were subcultured on fresh PDA medium to get

pure colony. The subcultures were carried out four to six times. The diameter of each colony was measured at 3 - 8 days after inoculation and also studied the morphological characters. Again, the pure fungal colony was subcultured to identify the microscopical characters on WGA medium for 5-15 days.

Forcible Spore Discharge Method

The infected plant parts were fixed with double-faced sticky tape inside the lip of a petridish. The lip of a petridish was placed on Potato Dextrose Agar (PDA) plate. Then, the pathogenic spores were inoculated at room temperature for 3 to 10 days.

Identification of Pathogenic Fungi

The most significant characteristics of fungus used for identification are spore - bearing structure and some extent characteristics of fungus body. These fungus were examined under the electric microscope directly. The conidia shape, size and color, and arrangement of spores on the sporangiophore from each replicate were noted under electric microscope with 40 X magnifire. The fungal spores were measured according to the method of Kokate (2000). The isolated pathogenic fungi were identified by the literatures; Barnett (1955), Funder (1961) and Dube (1990).

Results

Outstanding Character of *Piper betle* L.

Family - Piperaceae
Myanmar name - Kun
English name - Betelvine

Perennial, rooted climbing herbs, up to 27 m tall depending on the nature of the support; the older stems faintly ridged, the younger ones smooth, strongly swollen at the node. Leaves simple, alternate, stipules adnating about half of the petiole length; the petiole 1.2 - 2.5 cm long; blades broadly ovate, 12.0 - 20.5 cm by 6.5 - 15.8 cm, rounded or shallowly cordate at the base, entire along the margin, acuminate at the apex, dark green and shining above, pale green beneath, coriaceous. Inflorescences axillary spikes; female spike 3.5 - 5.0 cm long pendulous; peduncles 1.8 - 2.5 cm long, glabrous. Flowers crowded, sessile, pale yellow, unisexual, minute, without perianth; bracts orbicular, scale-like, pale yellow, broadly stipitate, with a membranous margin. Female flowers pale yellow; ovary immersed in rachis of spike, stigmatic branches 5-lobed. Male spike is not available in the present study.

Figure 1. Habit of *Piper betle* L.

Stem rot disease symptoms

The betelvines of all stages are susceptible to stem rot disease. The disease infected stems are firstly brown and turn to black later. Whitish cottony mycelium is seen on the stem and roots. The stem portion gradually shows rotting of stem at the point of attack. Then, the plants show dropping of leaves and withering finally dry up (Figure 2 A). This disease is caused by NZ 01.

Macroscopical Characters of NZ 01

After 3 - 7 days of cultivation, the colony of NZ 01 is white black colour at 25ºC on PDA medium. Firstly, the color of the colony is white and turn to black and finally black when mature. The colony is flat, like powdery, the peripherial white and the central black. The best sporulation occured at 25ºC and the optimum pH range is 6.5 - 7.0 (Figure 2 B).

Microscopical Characters of NZ 01

The hyphae are septate and branches. Conidiophores are long upright, non septate, branched, colourless, terminating in globose or subglobose. Conidia 1-celled, globose or subglobose, dark color, 3-5 µm in diameter. According to macroscopical and microscopical characters, the fungus NZ 01 may be *Aspergillus* sp. (Figure 2 C).

Leaf rot disease symptoms

The disease infected leaves show small circular to irregular water soaked spots. This spots are rapidly large and cover a part or whole of the leaf blade, which show rotting. The leaves turn brown to dark brown or dirty black and defoliation occurs. They are found mostly near the soil region. The leaves which have about 2 - 3 feet height of the vine show the leaf rot symptom (Figure 2 D). It is caused by the fungus NZ 02.

Macroscopical Characters of NZ 02

After 4 - 7 days of cultivation, it was observed that NZ 02 is pinkish white colony at 25ºC on PDA medium. Initially, the color of the colony is pale pink and change to white when mature. It is circular fluffy and cottony like colony. Good growth was at 25ºC and the optimum pH is 6.5-7.0 (Figure 2 E).

Microscopical Characters of NZ 02

Hyphae are septate, hyaline and branched. Conidiophores are short, each bearing a single apical conidium. Conidia are slimy, effuse sporodochia called pionnotes or sometimes scattered on the mycelium. Conidia were slightly canoe-shaped, 3 - 6 septate and 10 - 30 µm in length and 3 - 5 µm in width. According to macroscopical and microscopical characters, the fungus NZ 02 may be *Fusarium* sp. (Figure 2 F).

Leaf spot disease symptoms

The disease infected leaves are severely stunted brown spot about 0.1 - 0.2 cm in diameter on the leaves. Brown spot is seen on both the surface of leaves which later abundant entire the portion of the leaves. When the disease advances, the brown spots growth turn to black blotches in severe cases. The leaves turn yellow and defoliation occurs (Figure 3 A). This disease is formed due to the fungi NZ 03 and NZ 04.

Macroscopical Characters of NZ 03

After 4 - 7 days of cultivation, it was seen that NZ 03 is grey color colony at 25ºC on PDA medium. Firstly, the color of the colony is pale grey and turn to brown when mature. The colony is woolly. The sporulation was best at 25ºC and the optimum pH range is 6.5 - 7.0 (Figure 3 B).

Microscopical Characters of NZ 03

The hyphae are septate and brown. Conidiophores are brown, simple or sometimes branched.The conidia are originated from the bent at the point of the conidiophore. The conidia are brown, 3 - 4 celled, central cell of the conidium darker and end cells lighter. The central cell of conidia are swelling and curve the lateral cells in appearance and 12 - 24 µm in length

and 5 -10 μm in width. According to these macroscopical and microscopical characters, the fungus NZ 03 may be *Curvularia* sp. (Figure 3 C).

Macroscopical Characters of NZ 04

After 6 - 10 days of cultivation, it was found that NZ 04 is olivaceous brown colony at 25ºC on PDA medium. Firstly, the color of the colony is olive green. The colony is velvet, the peripherial whitish and the central olivaceous color. The sporulation was best at 25ºC and the optimum pH range was 6.5 - 7.0 (Figure 3 D).

Microscopical Characters of NZ 04

The hyphae are septate. The conidiophore are dark, branched variously near the upper or middle position, cluster or single. Conidia are dark 1 or 2 celled, variable in shape and size, ovoid, to cylindrical and irregular 5 - 10 μm in length and 2 - 5 μm in width. According to these macroscopical and microscopical characters, the fungus NZ 04 may be *Cladosporium* sp. (Figure 3 E).

Anthracnose disease symptoms

The leaves show small black circular spots initially which later enlarge and develop to a size of 1 - 2 cm in size, become concentric and covered with a yellow color. The affected leaves turn pale yellow and dry up with large black dots in the centre of the spots. This disease infected leaves are defoliation and finally show rotting (Figure 4 A).

Macroscopical Characters of NZ 05

After 4 - 7 days of cultivation, it was observed that NZ 05 is pale greyish colony at 25ºC on PDA medium. Initially, the color of the colony is white and turns to brown. The colony of the hyphal mass is cottony. The sporulation was best at 25ºC and the optimum pH range was 6.5 –7.0 (Figure 4 B).

Microscopical Characters of NZ 05

The hyphae are septate. The conidiophore are septate, simple, not branched, brown, rather short or elongate. Conidia are singly, holoblastic, dictyospore, variously shaped, obclavate to elliptical produce germ tubes and 10 - 30 μm in length and 7 - 15 μm in width. According to these macroscopical and microscopical characters, the fungus NZ 05 may be *Alternaria* sp. (Figure 4 C).

Macroscopical Characters of NZ 06

The colony of NZ 06 was white creamy color after 3 - 7 days of cultivation on potato dextrose agar (PDA) medium, at 25ºC. It is circular

and cotton like colony. The sporulation was best at 25ºC and the optimum pH range 6.5 - 7.0 (Figure 4 D).

Microscopical Characters of NZ 06

The hyphae are septate. Conidiophores are septate, hyaline, simple, cylindrical. The conidia are hyaline, 1 - celled, ovoid or oblong, 6 - 12 µm in length and 2-4 µm in width. According to these macroscopical and microscopical characters, the fungus NZ 06 may be *Colletotrichum* sp. (Figure 4 E).

Yellow Leaf disease symptoms

The disease infected leaf show yellow half of the leaf blade. This disease rapidly enlarges and covers whole of the leaf blades. Finally, the leaf show rotting and defoliation occured (Figure 5 A).

Macroscopical Characters of NZ 07

After 4 - 10 days of cultivation, it was occurred that NZ 07 is whitish green colony at 25ºC on PDA medium. Firstly, the color of the colony is white and turns to green when mature. The colony is flat, like powdery the peripherial white and the central green. The sporulation was best at 25ºC and the optimum pH range is 6.5 - 7.0 (Figure 5 B).

Microscopical Characters of NZ 07

The hyphae are septate and branched. Conidiophores are long, septate, colorless, not constricted below the vesicle. Conidia are 1-celled globose, pale green, smooth, 1 - 4 µm in diameter. According to these macroscopical and microscopical characters, the fungus NZ 07 may be *Aspergillus* sp. (Figure 5 C).

Discussion and Conclusion

In the present study, seven species belonging to six genera of fungal pathogens namely *Aspergillus* spp., *Fusarium* sp., *Curvularia* sp., *Cladosporium* sp., *Alternaria* sp., and *Colletotrichum* sp. dealing with the five kinds of disease, were isolated from the infected parts of the betelvine. The macroscopical, microscopical and morphological characters of these pathogenic fungi were presented.

In this study, Forcible spore discharge method was observed to be good potential for obtaining pure isolation of free fungal pathogens from endophytic fungi. Because direct isolation method was more difficult in order to isolate pure pathogenic fungi. A mass tangle of heavily colonies was obtained after being isolated from first culture. Thus, subculture isolations were carried out after being isolated from first culture.

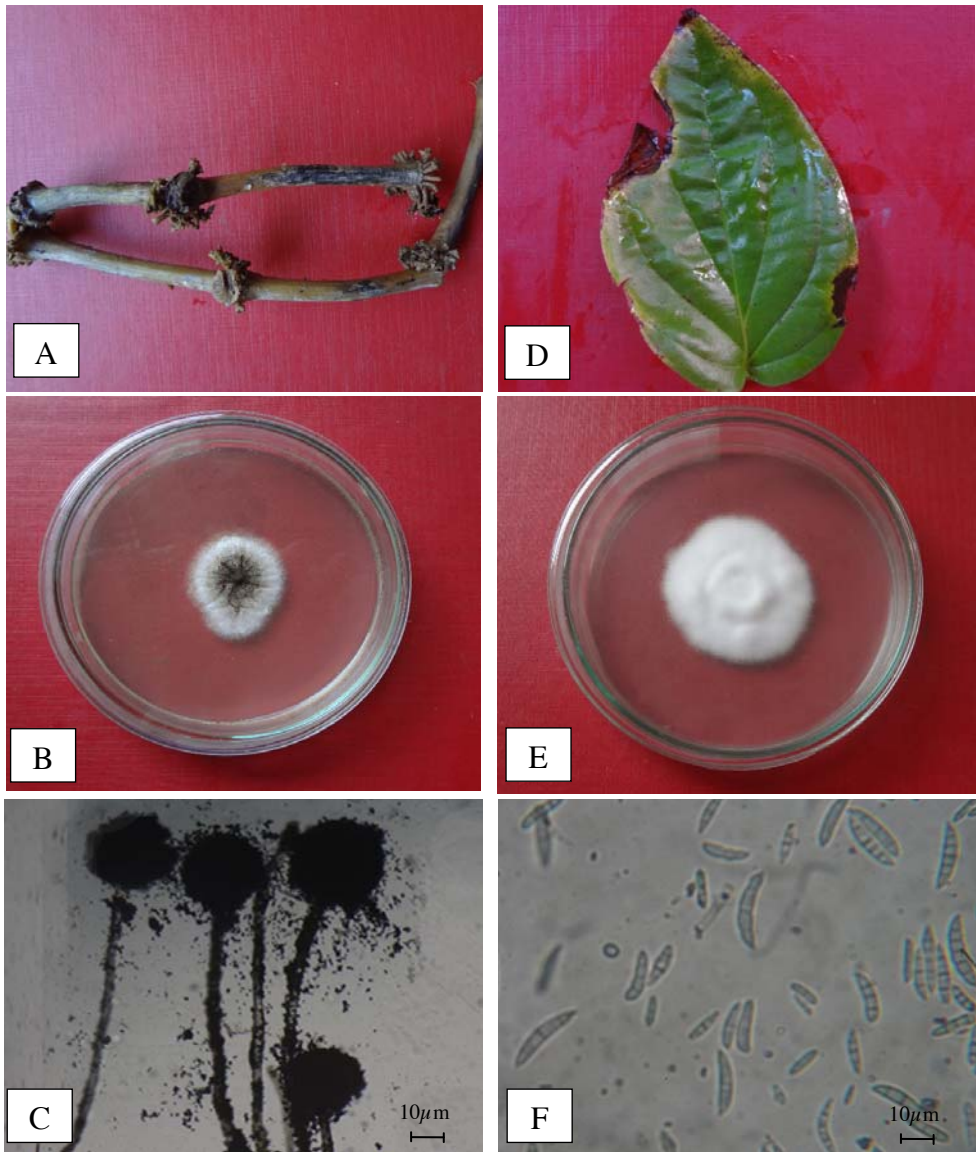

Figure 2. Disease symptom and characters of isolated fungi

A. Disease symptom of stem rot
B. Colony character of NZ 01
C. Photomicrograph of NZ 01, *Aspergillus* sp.
D. Disease symptom of leaf rot
E. Colony character of NZ 02
F. Photomicrograph of NZ 02, *Fusarium* sp.

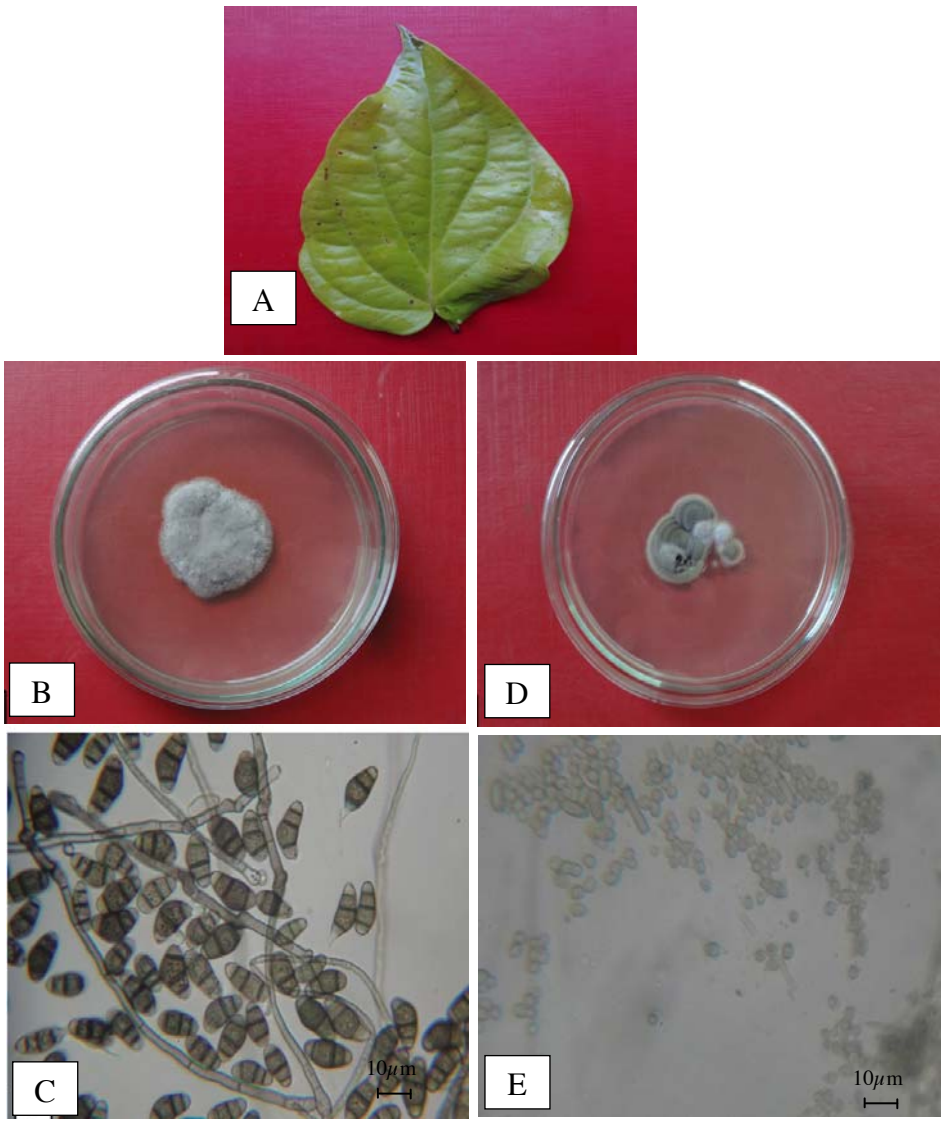

Figure 3. Disease symptom and characters of isolated fungi

- **A. Disease symptom of leaf spot**
- **B. Colony character of NZ 03**
- **C. Photomicrograph of NZ 03, *Curvularia* sp.**
- **D. Colony character of NZ 04**
- **E. Photomicrograph of NZ 04, *Cladosporium* sp.**

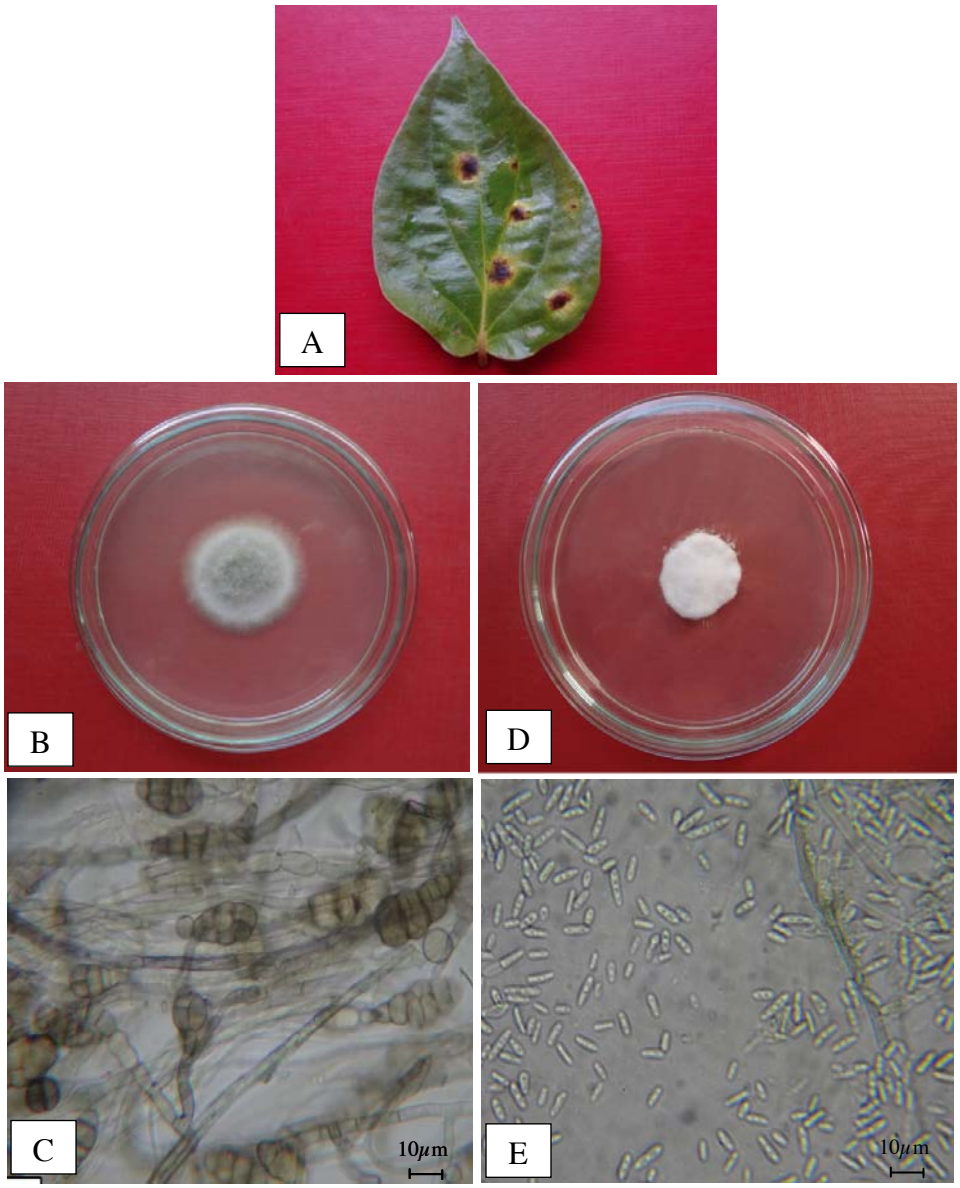

Figure 4. Disease symptom and characters of isolated fungi

- **A. Disease symptom of antharonose**
- **B. Colony character of NZ 05**
- **C. Photomicrograph of NZ 05, *Alternaria* sp.**
- **D. Colony character of NZ 06**
- **E. Photomicrograph of NZ 06, *Colletotrichum* sp.**

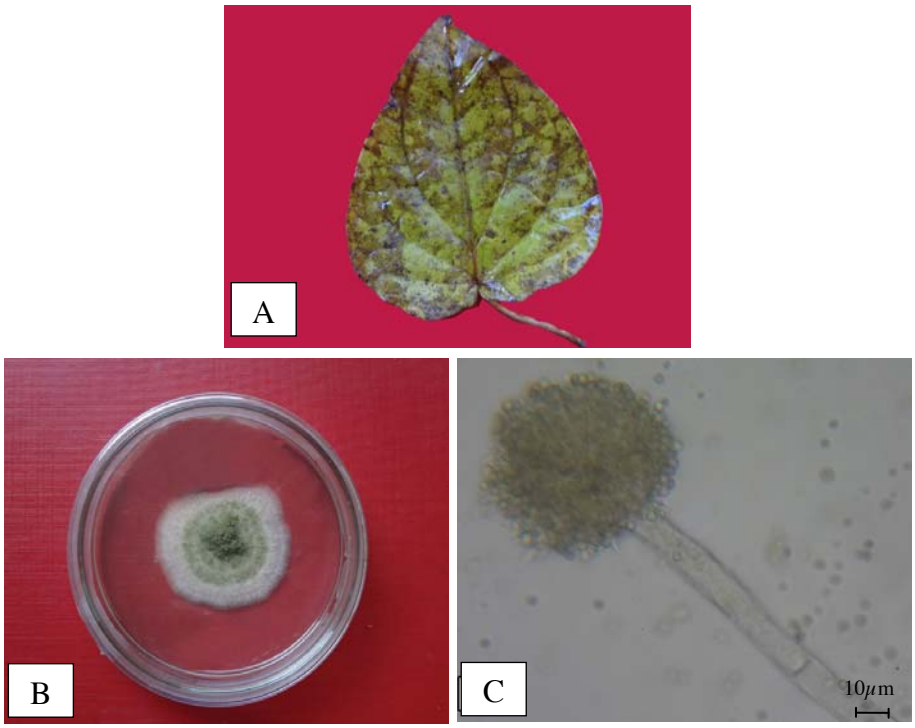

Figure 5. Disease symptom and characters of isolated fungi
A. Disease symptom of yellow leaf
B. Colony character of NZ 07
C. Photomicrograph of NZ 07, *Aspergillus* sp.

The macroscopical characters of NZ 01 fungus was white-black colony on PDA medium. It was found infected on betel stem named stem rot disease. The microscopical characters were conidiophore long, non-septate, hyaline, broadening upwards and enlarging in globose vesicles. The hyphae were septate and branched. The conidia were dark brown, globose. These data are similar to the literatures reported by Gilmen (1957). Therefore, NZ 01 strain may be *Aspergillus* sp.

The macroscopical characters of NZ 02 fungus was pinkish white colony on PDA medium. NZ 02 strain caused to betel leaf rot disease. The microscopical characters were simple or branched conidiophores and slimy, canoe-shaped, 3 to 6 septate conidia. The present data are in agreement with

those of Barnett (1955) and Dube (1990). Therefore, NZ 02 strain may be *Fusarium* sp.

The macroscopical characters of NZ 03 fungus was pale gray colony on PDA medium. NZ 03 and NZ 04 fungus were found infected on betel leaves named leaf spot disease. The microscopical characters of NZ 03 fungus were brown, simple or sometimes branched conidiophores. Conidia were brown and bent. The most conspicuous appear of *Curvularia* sp. was that the central cell was expended from the pore end of the conidium. The above data are similar to the literatures reported by Dube (1990) and Webster (1999). Therefore, NZ 03 strain may be *Curvularia* sp.

The macroscopical characters of NZ 04 fungus was olivaceous brown colony on PDA medium. The microscopical characters were singly or grouped conidiophore and 1 or 2 celled conidia that were cluster or single, variable in shape and size, ovoid to cylindrical and irregular. These results are similar to that of Ogorek *et al*. (2012). Therefore, NZ 04 strain may be *Cladosporium* sp.

The macroscopical characters of NZ 05 fungus was pale greywish colony on PDA medium. The microscopical characters were rather short or elongate conidiophores and singly conidia with various shaped. The most distinct appearance of *Alternaria* sp. was dictyospore and contains both transverse and longitudinal septa. The above data are similar to those of Barnett (1955) and Dube (1990). Thus, NZ 05 strain may be *Alternaria* sp.

The macroscopical characters of NZ 06 were white creamy, circular and cotton like colony on PDA medium. The microscopical characters were simple septate conidiophores and conidia are hyaline, 1-celled, ovoid or oblong. These results are similar to the literatures reported by Barnett (1955) and Dube (1990). Therefore, NZ 06 strain may be *Colletotrichum* sp.

The macroscopical character of NZ 07 was greenish white colony on PDA medium. The microscopical characters were upright, simple, long, septate conidiophores, the streigmata uniseriate, conidia 1-celled, globose and catenulate. The above data are in agreement with those of Barnett (1955) and Gilman (1959). Therefore, NZ 07 strain may be *Aspergillus* sp.

In this study, NZ 01 and NZ 07 fungi were different in the presence of septate and non septate conidiophores, and the color of colony. Thus, these fungus may be the same genus, *Aspergillus* but the different species.

Fungus diseases infected on betel plants can cause several damage and loss of yield in betel leaves production in Nga Zun Township. The most important disease, leaf-spots disease was reported to the most destructive serious diseases in this study area. Among the several diseases, the above

five disease caused infection on betel, especially on leaves. Fungi infected not only to leaves but also together to stems and roots.

Stem rot and yellow leaf diseases were caused by *Aspergillus* spp. and leaf rot disease by *Fusarium* sp.. Leaf spot disease was infected by *Curvularia* sp. and *Cladosporium* sp.. Anthracnose disease was caused by *Alternaria* sp. and *Colletotrichum* sp. Srichana *et al*. (2009) stated that *Aspergillus flavus* can be pathogenic for plants and animals. Due to Gogoi *et al*. (2008) anthracnose disease on leaves are caused by *Colletotrichum* sp. Again, Bashar *et al*. (2014) stated that *Alternaria* sp. *Curvularia* sp. and *Fusarium* sp. found associated with anthracnose infected stem as secondary invaders. Thus, the present data were agreement with those of Gogoi *et al*. (2008), Srichana *et al*. (2009) and Bashar *et al*. (2014). But, It was not known that *Cladosporium* sp. can cause what kind of disease on betel plant.

Srichana *et al*. (2009) revealed that the fungus *Fusarium verticillioides* produces a toxic substance, fumoinsin has been found to cause esophageal cancer in human and the fungus *Aspergillus flavus* produces aflotoxin, a toxic and carcinogenic compound. Thus, the infected betel leaves should not be used. Again, the method of detecting the disease and method of disease control should be done.

It is difficult to achieve control of disease at a time where both fungal and bacterial pathogens are associated. But, application of chemical fungicides is, no doubt, an effective method of plant disease control. However, indiscriminate use of fungicides could produce environmental and health hazards especially on a crop like betelvine because the leaves are chewed directly by human beings. Therefore, there is need to evaluate the pesticide and fungicides residues in leaves and the time period require to reduce the residue level below the permissible limits before human consumption.

The present study can be confirmed scientifically that what kinds of pathogenic fungus were responsible what kinds of disease. Therefore, it can also fullfill the knowledge concerning with fungal diseases on betelvine for betel researcher.

References

Backer, C.A. & R.C.B.V.D. Brink. 1968. Flora of Java. Vol III, Rijksherbarium, Layden, N.V.P. Noordhoof. Croningen Company, The Netherland.

Barnett, H. L. 1955. Illustrated genera of imperfect fungi. Burgess Publishing Co., West.

Bashar, Ma. A and J. Ahmed .K.S Hossain. 2014. Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh.

Dassanayake, M.D. 1987. A Revised Handbook to the flora of Ceylon. Vol. VI. University of Peradeniya, Department of Agriculture, Washington D. C., U.S.A.

Dube, H.C. 1990. An introduction to fungi. 2nd Edition, Vikas Publishing House, Pvt Ltd. New Delhi.

Funder, S. 1961. Practical Mycology. Munual for Identification of Fungi. Norway.

Gilman, J. C. 1959. A Manual of soil fungi. Oxford & IBH Publishing Co. Calutta, Bombay, New Delhi.

Gogoi, R., U. K. Deka, P. K. Dutta and P. K. Borah. 2008. Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110 012.

Hooker, J.D. 1885. The Flora of British India. Vol VI, L. Reeve & Co, 5 Henrietta Street, Coinvent Gradne, London.

Jane, N. S., A.P. Deshmukh and M. S. Joshi. 2014. Review of Study of Different Diseases on Betelvine Plant and Control Measure. Research Scholar, Department of Electronics & Telecommunication, Institute of Technology & Research, Badnera, Amravati, India.

Kokate, C.K. 2000. Practical pharmaconosy. Jain for Vallabh Pakashan, Pitanpura, Delhi India. University of Mandalay.

Ogorek R., A. Lejman, W. Pusz, A. Miluch and P. Miodynska. 2012. Characteristics and taxonomy of *Cladosporium* fungi. Division of Plant Pathology and Mycology, Department of Plant Portection, Wroclaw University of Environmental and Life Sciences, Poland.

Patel, R.M. and Y.T Jasrai. 2013. Evaluation of Fungitoxic Potency of *Piper betle* L. (Mysore Variety). Leaf Extracts Against Eleven Phytopathogenic Fungal Strains. Department of Botany, University School of Sciences, Gujarat University, Ahmedabad - 380009, Gujarat, India.

Shahzad, S. and A.Zareen. 1999. Leaf Spot of Betelvine in Pakistan. Department of Botany, University of Karachi, Karachi - 75270, Pakistan.

Srichana, D, A. Phumruang and B. Chongkid. 2009. Inhibition Effect of betel Leaf Extract on the Growth of *Aspergillus flavus* and *Fusarium verticillioides*. Department of Agricultural Technology, Faculty of Science and Techrology, Thammasat University, Pathum Thani, Thailand.

Vijayakumar, J. and S. Arumugam. 2014. *Odium piperis* Fungus Identification for *Piper betle* plants Using Digital Image Processing. Department of ECE, Nandha College of Technology, Tamil Nadu, India.

Webster, J. 1999. Introduction to fungi. 2nd Edition, Cambridge University Press.