Isolation and Identification of Pathogenic Fungi from Selected Bean Species in Yegyi Township

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Abstract

The disease infected plants of two bean species were collected from cultivated field of Yegyi Township, Ayeyarwady Region, Myanmar. Pathogenic microbes from diseased parts of plants were isolated by various isolation methods. These microbes were identified by methods such as morphology and spore formation. These microbes cause the symptoms of alternaria leaf spots, fusarium yellow, cercospora leafs spot and yellowing leaf spots in bean species. The developing fungial colony were examined after 3-7 days for fungal colony, and then isolated into slants with Potato-Dextrose Agar (PDA). Subculture of fungal isolates were carried out 5-7 times successfully with Potato-Dextrose Agar (PDA) slants medium and Czapex (Dox) Agar medium, respectively, until the pure culture was obtained. Pure colonies were cultured in Petridishes with Czapex (Dox) Agar medium for fungus to examine color, form of colony, cell structure and spore formation. Six fungi from infected leaves were recorded and examined under microscope. According to their morphological, these six fungal isolates were designated as *fusarium* species, *Alternaria* species, *cercospora* species and *curvularia* species from *Vigna mungo* (L.) Hepper (met-pe) and *fusarium* species and *curvularia* species from *Vigna radiata* (L.)R.Wilczek (pe-te-sein) respectively.

Keywords: *fusarium* species, *Alternaria* species, *cercospora* species and *curvularia* species., *Vigna mungo* (L.) Hepper and *Vigna radiata* (L.)R.Wilczek

Introduction

Yegyi Township is situated in the northern part of Ayeyarwady Region. It is located between Ayeyarwady River and Ngawun (or) Pathein River. Yegyi Township extends from latitude 17°34' North and longitude 95° 12' East with an area of about (1297km^2).

Vigna mungo (L.) Hepper and *Vigna radiata* (L.)R.Wilczek are Fabaceae (Leguminosae) family. These beans are winter season grain legume crop in Myanmar. *Vigna mungo* (L.) Hepper has high demand in international market and is exported to India, Pakiston, Malaysia, UAE, Singapore, Japan, Srilanka and Korea. It is one of the most highly prized pulses of India, more particularly in the vegetarian diet of high caste Hindus. *Vigna mungo* (L.) Hepper and *Vigna radiata* (L.) R.Wilczek are cultivated as a second crop after rice and also on the riverbanks when the water subsides in lower Myanmar.*Vigna radiata* (L.)R.Wilczek is exported to many countries including India, Indonesia, Netherlands and Saudi Arabia. In China and the United States, it is used for bean sprouts. (Pluses Training Manual).

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The diseases are found in *Vigna mungo* (L.) Hepper and *Vigna radiata* (L.) R.Wilczek including, powdery mildew disease, cercospora leaf spots disease, anthracnose disease and bean yellow mosaic disease. But in Yegyi Township, *Alternaria* leaf spots, fusarium yellow diseases, cercospora leaf spots and yellowing leaf spots were observed. So many diseases are cause to loss the crops production. The serious disease greatly affected the growth of plants and produce heavy losses to the farmers.

The objectives of the present study are infected leaves on *Vigna mungo* (L.) Hepper and *Vigna radiata* (L.)R.Wilczek of cultivated fields (Yegyi Townships) and the different pathogenic fungi are identified and classify these pathogenic agents.

Materials and Methods

Collection of bacterial and fungal pathogens

The fungal pathogens were collected from diseased *Vigna mungo* (L.) Hepper and *Vigna radiata* (L.)R.Wilczek from Yegyi Townships Area, Pathein Division, Ayeyarwady Region.

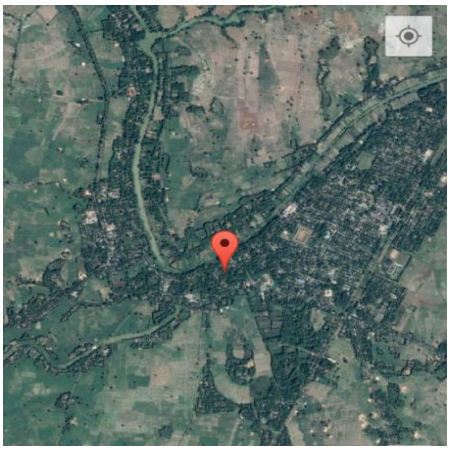


Figure 1. Location Map of the study area (Base map - Setellite Image)

Isolation of Fungal pathogens

Pathogens have to be isolated and cultured from diseased specimens before they can be identified. For surface lesions on leaves, small pieces of diseased tissue of a few cubic millimeters excised from the lesion margin are surface sterilized in 5 % Clorox for 5 min. And then placed on to the agar growth medium in sterile petridishes, when fungi are growing on the agar medium. The developing fungal colony were examined after 3-7 days for fungal colony, and then isolated into slants with Potato-Dextrose Agar (PDA). Subculture of fungal isolates were carried out 5-7 times successfully with Potato-Dextrose Agar (PDA) slants medium and Czapex Dox Agar medium, respectively, until the pure culture was obtained. Pure colonies were cultured in petridishes with Czapex Dox Agar medium for fungus to examine color, form of colony, cell structure and spore formation.

Cultivation of Fungi

It is also the subculture medium and pure colony medium (Czapek Dox Agar) for the maintenance the fungus (Ronald M, 1993).

PDA medium

Fleshy peeled Potato - 40 g

Dextrose - 2.5 g

Agar - 2 g

Distilled water - 100 ml

(Add chloramphenicol (0.001 /l) for antibacterial activity)

pH - 5.6 at 25˚C

Czapek Dox Agar medium

$NaNO_3$ - 0.2 g

K_2HPO_4 - 0.1 g

$MgSO_4$- $7H_2O$ - 0.05 g

KCl - 0.05 g

$FeSO_4$-$7H_2O$ - 0.001g

Sucrose - 3.0 g

Agar - 2.0 g

Distilled water - 100 ml

pH - 7.3 at 25˚C

Test for the characterization of fungus isolates

Each pure isolated fungi species was mounted on the clear glass slides and stained with lacto phenol cotton blue by Needle-mount method. (Leck A, 1999)

Results

Outstanding character

Scientific Name - *Vigna mungo* (L.) Hepper

Family - Fabaceae

Common Name - Black gram

Myanmar name - Mat-pe

Vigna mungo (L.) Hepper

Scientific Name - *Vigna radiata* (L.) R. Wilczek

Family - Fabaceae

Common Name -Green gram

Myanmar name - Pe-te-sein

Vigna radiata (L.) R. Wilczek

Characteristic of Fungal pathogens

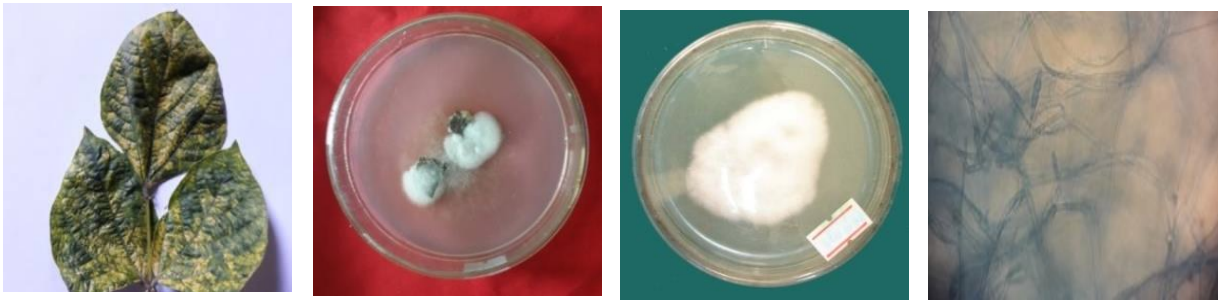
According to Barnett *et.al* (1998), disease symptom, characters of mycelium, spore formation and identification of each fungal pathogen is presented in figure (1 and 6). The study showed that different kinds of fungal pathogen were observed on two diseased bean plants from Yegyi Townships Area. According to the results, diseases systems of *Fusarium* yellow are caused by *Fusarium* sp., yellowish leaf spot caused by are *Alternaria* sp. Cercospora leaf spots are caused by *Cercospora* sp. and yellow leaf spots are caused by *Curvularia* sp. respectively.

Symptom of Fusarium Yellow

Infected leaf becomes pale-green and then conspicuously bright-yellow. The leaves eventually drop off and the plant dies. Lower leaves are typically affected first and the symptoms progress upwards in the plant. Infected young plants may appear stunted.

Character of mycelium and spore formation of *Fusarium* yellow

Mycelium extensive and cotton-like in culture, often with some tinge of pink in the mycelium on medium; conidiophores variable, slender, simple and short.Conidia hyaline, several-cell slightly curved at the pointed ends, typically canoe-shaped in observed.



Disease symptom | Fungal isolate from diseased leaf | Pure fungal colony | Mycelium and conidia

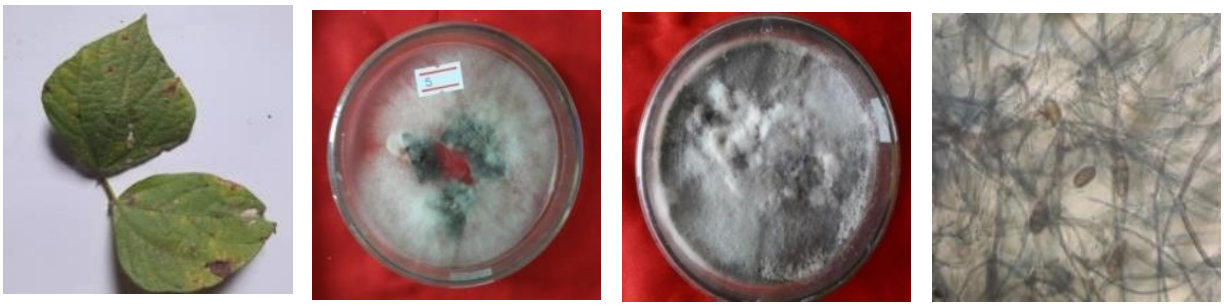
Figure 1. Fungal Pathogen Causing *Fusarium* yellow in *Vigna mungo* (L.)

Symptom of *Alternaria* Leaf Spot

Lesions tend to appear first on the older leaves as small circular spots. The spots are light brown with a light center and form concentric dark rings as they enlarge. Lesions which form on the lower leaf surface tend to be more diffuse.

Character of mycelium and spore formation of *Alternaria* Leaf Spot

Mycelium initially hyaline, becoming olive-buff to deep olive-buff, branched, septate. Conidiophores similar to mycelium in colour, septate, unbranched, erect, broader towards the distal end, on the host. Conidia acrogenous, borne singly or in chains of 2-4, smooth, irregularly ovoid, both ends rounded, gradually tapering into a beak. Spore body pale brown to dark olive-buff, becoming darker with age.



Disease symptom | Fungal isolate from diseased leaf | Pure fungal colony | Mycelium and conidia (×40)

Figure 2. Fungal Pathogen Causing *Alternaria* Leaf Spot in *Vigna mungo* (L.)

Symptom of *Cercospora* Leaf Spot

Cercospora leaf spot is caused by the fungus *Cercospora* sp. Leaf spots are initially small and reddish, but they enlarge rapidly with center of the spot turning ash gray color. Spots enlarge, coalesce and cause defoliation.

Character of mycelium and spore formation of *Cercospora* Leaf Spot

Conidiophores dark, simple, arising in clusters and bursting out of leaf tissue, bearing conidia successively on new growing tips; conidia hyaline or gray, long cylindrical to filiform, several celled; commonly causing leaf spots.

Disease symptom | Fungal isolate from diseased leaf | Pure fungal colony | Mycelium and conidia (×40)

Figure 3. Fungal Pathogen Causing Cercospora leaf spots in *Vigna mungo* (L.)

Symptom of yellowing leaf spots

Yellowing leaf spot is caused by the fungus *Curvularia* sp. Small yellowish spots form at first lower leaves, becoming oval longitudinally; coalescence of the spots lead to death of the leaves; leaf sheaths and ears can be spotted.

Character of mycelium and spore formation of yellowing leaf spots

Conidiophores brown, mostly simple, bearing conidia apically growing points; conidia dark, end cells lighter, 3-5 celled, more or less fusiform, typically bent, with one of the central cells enlarged.

Disease symptom | Fungal isolate from diseased leaf | Pure fungal colony | Mycelium and conidia (×40)

Figure 4. Fungal Pathogen Causing yellowing leaf spots in *Vigna mungo* (L.)

Disease symptom | Fungal isolate from diseased leaf | Pure fungal colony | Mycelium and conidia (×40)

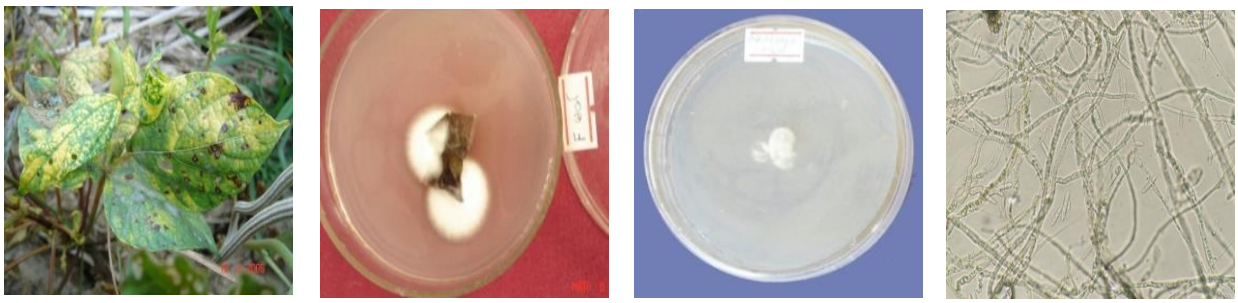
Figure 5. Fungal Pathogen Causing yellowing leaf spot in *Vigna radiata* (L.)

Symptom of Fusarium Yellow

Foliage of infected Bean becomes pale-green and then conspicuously bright-yellow. The leaves eventually drop off and the plant dies. Lower leaves are typically affected first and the symptoms progress upwards in the plant. Infected young plants may appear stunted.

Character of mycelium and spore formation of *Fusarium* yellow

Mycelium extensive and cotton-like in culture, often with some tinge of pink in the mycelium on medium; conidiophores variable, slender and simple, short, branch irregularly. Conidia hyaline, several-cell slightly curved at the pointed ends, typically canoe-shaped in observed.



Disease symptom | Fungal isolate from diseased leaf | Pure fungal colony | Mycelium and conidia (×40)

Figure 6. Fungal Pathogen Causing Fusarium yellow in *Vigna radiata* (L.)

Discussion and Conclusion

The diseased plant samples were examined according to the disease symptom. And then it is identified by morphological characters of mycelium, pure colony and spore formation for fungi. The fungi were cultivated on PDA medium as well as Czapak Dox agar medium. In this study, *Alternaria* leaf spots, *fusarium* yellow diseases, *cercospora* leaf spots and yellowing leaf spots caused by fungus were observed in Yegyi Township. Heavy infections of foliage reduce plant vigor and yield. Pod infections cause distortion, premature shattering, and shriveled, diseased seed that germinate poorly. (Helene,2008)

Among them Alternaria leaf spot diseases are most important diseases that produce heavy losses to the farmer. Fusarium yellow diseases are the second most important disease that severely reduces the yield of bean plants. If there are seriously infected mixed planting, transgenic methods, biotic and abiotic elicitors, biocontrol methods, fungicides and pesticides may be used for controlling disease (Kessmann et al, 1994).

In the present studies, six different kinds of pathogenic fungi from the collected diseased plant samples were observed in two bean species. These fungal diseases symptoms may be occurred about one month after planting of bean seedlings. The disease causes reduction in quantity and quality of yield. According to the results, *fusarium* species, *Alternaria* species, *cercospora* species and *curvularia* species from *Vigna mungo* (L.) Hepper (met-pe) and *fusarium* species and *curvularia* species from *Vigna radiata* (L.)R.Wilczek (pe-te-sein) respectively, can be isolated from diseased bean plants.

Since Irrawaddy is an agriculture-based region, this research paper is presented with the aim of information the bean farmers in Yegyi Township that the beans are affected by fungal diseases and the yield of beans has decreased.

We believe that this information of some diseased plants in *Vigna mungo* (L.) Hepper (met-pe) and *Vigna radiata* (L.) R.Wilczek (pe-te-sein) infected by the results of fungi which cause the major economic losses for farmers can be provided by this research work.

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