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Isolation and Identification of Fungal Pathogens from Five Flowering Plants in Jashore Region of Bangladesh

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ABSTRACT

Bangladesh is a great market for the flower trade. It produces a lot of different types of flowers as well as imports from neighboring countries which cost 3 million BDT currencies every year. The Jashore district of plants viz. Gladiolus, Gerbera, Rose, Tuberose and Marigold. The infected part of the plant samples was collected from five separate flower gardens. The precisely prepared infected sample was cultured on Potato Dextrose Agar media at 28ºC in an incubator for 48 hours and sub-cultured several times of each sample of distinct features to get a fresh culture of fungal pathogens. The isolates were identified based on their morphological features of the colony and observation of mycelia structure. The infection by fungal pathogens is considered a great barrier to flower cultivation. Therefore, the present study was attempted to the isolation infecting fungal pathogen from five different flowering crystal violet dyes to analyze the spore structure, the shape of the tips, conidial structure, and we identified three different types of fungus from five flowering plants *Aspergillus niger* identified from gerbera and rose, *Colletotrichum gloeosporioides* (pinkish) from tuberose, *Colletotrichum gloeosporioides* (whitish) from gladiolus and *Alternaria alternate* from marigold. This study has provided the primary alarm of fungal infection by following a less expensive technique. This study will be helpful to identify and management of phyto-pathogen for floriculture.

Keywords: Flowering plants, Mycelia, Incubator, Fungal pathogen, Jashore region, and Phyto-pathogen.

INTRODUCTION:

Flower cultivation is becoming very popular day by day. Low investment but higher rate of profit encourages the farmers for large scale cultivation of different flowers. Large-scale commercial production started from mid eighties in Jhikargacha upazila of Jashore district (Sultana, 2003). At present, 10,000 hectares of land covers flower cultivation taking the lead by the Jashore district (Nusrat, 2012). More than 5,000 resilient farmers are growing flower and foliage in the country and about 150,000 people are directly or indirectly involved in floriculture business as their sole livelihood (Chowdhury, 2010). The area coverage under commercial flower cultivation is approximately 10,000 hectares of land while commercial nurseries have covered approximately 2,000 to 2,500 hectares of land (Momin, 2006). Bangladesh has to spend roughly Tk. 2-3 million in importing flowers and ornamental

plants from abroad. The flowers originated from other countries are higher susceptible for plant pathogens. The fungal pathogens are primary enemies of flowering plants. Generally bacterial infections are very less common incidents than fungal infection. A wide range of fungal pathogens are recorded for infection. The type of fungal infections widely depends on geographical locations. Some of the changes in endemic fungal infections can be attributed to climate changes, extension of human habitats, ease of travel, and shifting populations (Jeannette *et al*., 2011).

A wide range of fungal diseases are recorded like battling mildew, mold and black spot, powdery mildew, gray mold, black spot, cankers, etc. Each case, different microorganisms are involved for the development symptoms. But the black spot may be generated by different fungal species. The air born fungus is mostly involved for development of diseases. The mal-nutrition and lower immunized flowering plants are worse victim by air born fungus, because they can easily colonize and finally further reproduction. There are several techniques have been developed for identification, such as enriched morphological features, textures. The précised way for identification is to determine the specific agent present in the histopathologic specimen, including immunohistochemistry, *in situ* hybridization, polymerase chain reaction (PCR) and DNA sequencing using bioinformatics tools. In addition, techniques such as laser microdissection will be useful to detect the now more frequently recognized dual fungal infections and the local environment in which this phenomenon occurs (Sharif *et al*., 2019; Jeannette *et al*., 2011).

Management of fungal pathogens becomes an ongoing battle, the scientist have developed a new fungicide but becomes resistant after some generation of applications. Fungus is very pathogenic for flowering plant. A very small colony can change the appearance of flower and fragrance. For proper management fungus infection, the identification process is very important. Our research carried out based on several aims and objectives. My primary objective of research is to make well conscious of our farmer. It focuses some focuses to identify the unknown fungal pathogens, to analyze the structure and function of a fungal mycelium and discuss its adaptive significance, to explore the nature and characteristics of the pathogenesis of fungal infections and find out the appropriate application of fungicide.

MATERIALS AND METHODS:

Sample Collection and Preparation - The different living and nonliving materials were used in this investigation, different plant materials such as stems, leaves and flowers where we found visual characterized symptoms like rots, black spots, blights, wilts and anthracnose are used for isolation of fungus from different nurseries in the Jashore region of Bangladesh. The investigation focused on flowering plants like gerbera, tuberose, rose, marigold and gladiolus. The infected parts were taken in the white transparent polythene bags in separately and tied each bag with cotton to prevent any type of contamination in these bags (Delhove *et al*., 2013, Rebecca *et al*., 2012, Shahen *et al*., 2019; Soni and Sharma, 2014).

The preparation of sample was done in the laminar air flow cabinet of the microbiology laboratory. The uninfected parts of the leaves were discarded and infected parts are taken for further procedure. The infected parts were cleaned very carefully with 0.5 % hypochlorite solution (Larran *et al*., 2001, Amsalu Abera *et al*., 2016) and 0.5 % $HgCl_2$. To perform this research in a clean room having provision for working space, free of dust and convection of current that carry spores of contaminating microorganisms. The preparation of room was equipped with cabinet and shelf space for safe storage of chemical compounds and dust free storage facilities, transfer areas for aseptic manipulations, culture room where cultures incubated under controlled light and temperatures.

Preparation of Media - For the preparation of 250 ml of potato dextrose agar media 9.75 grams of powder weighted with top pan balance and taken in to a conical flask. Then 250 ml of autoclaved distilled water was added. To mix properly, the gentle stirring was done. For making a fine suspension, the conical flask was placed on water bath for 15 minutes around 70ºC and finally autoclaved at 121ºC for 15 minutes and sterilized media poured on Petri-dish.

Culture and Isolation - The prepared sample was inoculated in the surface of PDA media in the biosafety cabinet. The plates containing sample were

placed in an incubator for 3 to 5 days at 27±1ºC temperatures. After successful incubation period, the visual mycelial structure was found. That visualized mycelia was sub-cultured in fresh PDA media, 48 hours later, the pure fungus was found on the surface of the plate. Again re-subculture of fungus was done to obtain final pure fungal culture (Javadi *et al*., 2012; Jasuja *et al*., 2013; Gaddeyya *et al*., 2012).

Identification - The fungus was identified based on colony characteristics and micro-morphological and macro-morphological appearances both on agar surface, microscopic slide observation and shape of spore and nature of the sporulation, shape of the tips and mycelia structure. For measuring the diameter of mycelia, we used ocular and stage micrometers. Pure cultures of the isolates were sub-cultured at-least for triple times of every sample for purification and a microscopic view of every slide also tabulated for confirmation and confirmed based on morphological features with our existing fungal sample in microbiology laboratory, Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh and comparing with the features (Josep Cano *et al*., 2004, N. MacClenny, 2005 and Ramjegathesh *et al*., 2012).

RESULTS AND DISCUSSION:

We collected different infected parts of five flowering plants *Gladiolus dalenii, Gerbera jamesonii, Rosa macdub*, *Polianthes tuberose* and *Tagete serecta*. We confirmed two isolates as *Aspergillus niger* by morphological and cultural features by observing microscopic slide and agar surface. We found typical features of hyphae of both isolates of *Aspergillus niger* from *Gerbera jamesonii and Rosa macdub*, where average width of hyphae was 2.6 to8 mm and tree like branching and some slide acute angle branching, hyaline and septate as described by N. McClenny. To differentiate between *A. fumigates* and *Aspergillus niger*, we monitored growth rate and color. We didn't observe any grey-blue-green colonies and uniseriate conidial heads within 24 to 48 hours incubation periods (N. McClenny, 2005).

For isolates of *Tagete serecta* we confirmed as *Alternaria alternata,* where the conidia were muriform shaped and colour was light brown and the length of conidia was 31 to 35 µm and the mean diameter of mycelia growth on Potato Dextrose Agar (PDA) was 8.15 cm as described by Ramjegathesh *et al*. 2012.

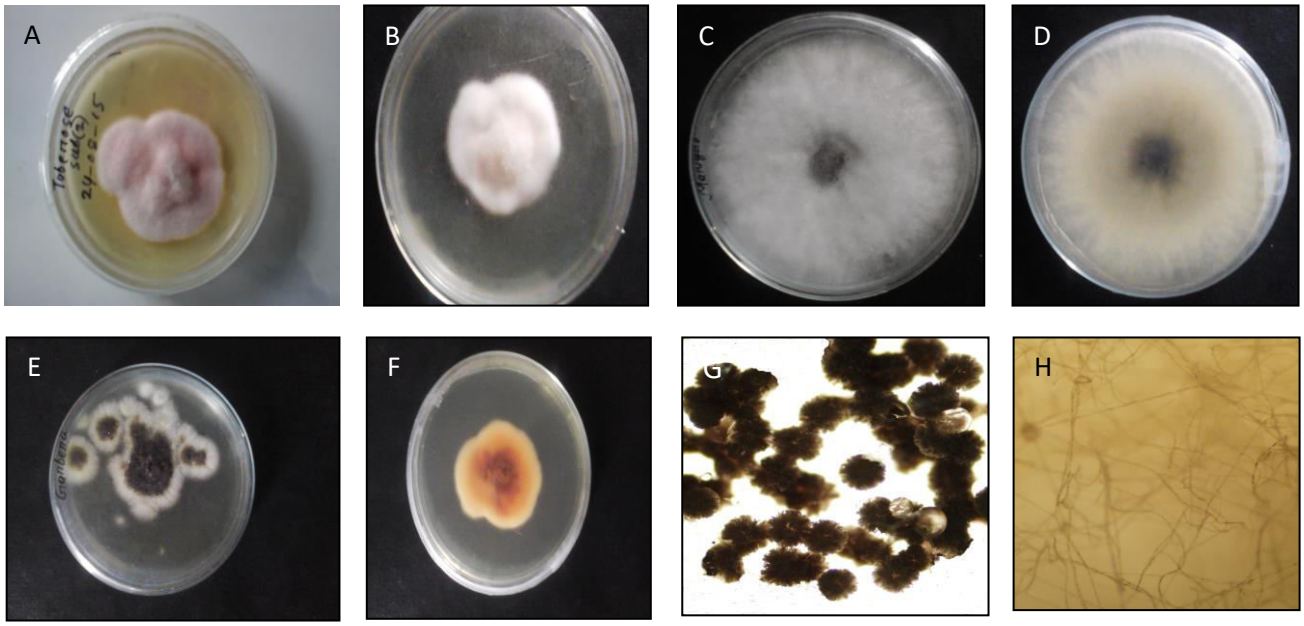

Fig. 1: A); Front view of *Colletotrichum gloeosporioides from Polianthes tuberosa,* B); Front view and F back view of *Colletotrichum gloeosporioides* from *Polianthes tuberose*. C); and D); are the front view and back view of *Alternaria alternata* and *Tagete serecta.* E); and G); are front view of plate and microscopic view of *Aspergillus niger* respectively and H is the mycelia structure of *Alternaria alternata.*

Two isolates were identified as *Colletotrichum gloeosporioides* both from *Gladiolus dalenii* and *Polianthes tuberose*. *Colletotrichum gloeosporioides* species have a complex genetically; morphological characteristics are more or less typical (Weir *et al*., 2012). All the cultures plate produced conidiogenous cells and aerial mycelium of the colony directly on the agar surface and conidiogenous cells were cylindrical shape and glassy and translucent in appearance, tapered and measured up to 20 by 3 to 4 µm. The setae were dark brown, acicular, thick walled and up to 200 µm long as described by Josep Cano et al. 2004. Both isolates of *Colletotrichum gloeosporioides* were slow growing comparatively of all isolates.

The *Aspergillus niger*, isolated from gerbera were very fast growing. It was difficult to store this isolates in the refrigerator for long duration, because it turns into black within a very short period of time. As a result, a frequent subculture had to done.

Table 1: List of isolated fungus.

Serial No.	Host Flowering Plant	Isolated Fungus
1	*Gladiolus dalenii*	*Colletotrichum gloeosporioides*
2	*Gerbera jamesonii*	*Aspergillus niger*
3	*Rosa macdub*	*Aspergillus niger*
4	*Polianthes tuberosa*	*Colletotrichum gloeosporioides*
5	*Tagete serecta*	*Alternaria alternata*

CONCLUSION:

Commercial flower cultivation has increased dramatically over the last few decades. A number of new farmers are getting involved every year. For making more economic of floriculture, the appropriate disease management should be practiced. Necessary information about fungal pathogens is integral part of disease management. Identification by phenotypic features and culture is quite easy and fast (N. McClenny, 2005). Our current research would be helpful for diagnosis of infecting fungus of phyto-pathogenecity. The morphological diagnosis of fungi is considered as the powerful weapon for early diagnosis. It is the pre-condition for proper management of infecting fungal pathogens. The infecting fungus like *Aspergillus niger, Colletotrichum gloeosporioides* and *Alternaria alternata* have their own distinguished morphological features.

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CONFLICTS OF INTEREST:

The author(s) declare that there is no potential conflict of interest.

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