LEAF BLIGHT OF *CATHARANTHUS ROSEUS* (L). G. DON CAUSED BY *MACROPHOMINA PHASEOLINA* (TASSI) GOID AND ITS *IN VITRO* CONTROL THROUGH BIO-PESTICIDES

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Abstract

Catharanthus roseus (L.).G. Don, a highly valued medicinal plant suffers from a serious disease. A survey on the symptom and severity of the leaf blight of *C. roseus* was performed in the nurseries of the Institute of Forestry and Environmental Sciences (IFESCU), Bangladesh Council of Industrial Research (BCSIR) and Bangladesh Forest Research Institute (BFRI), Bangladesh. An observation was also made in the avenues and garden of King Saud University (KSU), Kingdom of Saudi Arabia. No disease was recorded in KSU, but severe infections were found in all the locations surveyed in Bangladesh. The highest infection percentage and the highest disease index were found in BCSIR nursery followed by BFRI and the lowest was recorded in IFESCU nursery. *Macrophomina phaseolina* (Tassi) Goid was isolated and it was proved to be pathogenic. This is the first report of leaf blight of *C. roseus* caused by *M. phaseolina*. *Azadirachta indica, Ocimum sanctum Vitex negundo, Mucuna pruriens, Calotropis procera, Terminalia arjuna, Allium sativum, Zingiber officinalis* and *Allium cepa* were used as biopesticides. Out of the nine plant species screened, *T. arjuna* showed the highest (58.37%) inhibition percentage which was followed by *A. indica* (55.72%) and the lowest (27.4%) inhibition percentage were obtained with *V. negundo* whereas the rest of the plant extracts showed more or less same inhibitory effect.

Key words: Leaf blight; *Catharanthus roseus; Macrophomina phaseolina;* Biopesticidal control.

Introduction

The importance of medicinal plants as a source of medicine has been realized since the time of Rig-Veda (Kumar & Srivastava, 2002). *Catharanthus roseus* (L.) G. Don is known for its medicinal value in folklore of a number of countries, since 50 B.C. (Das & Alam, 2001). Its roots and leaves are useful in treating oliguria, haematuria, diabetic's mellitus, mental disorder and many other diseases (Purohit & Vyas, 2004). Disease is a regular phenomenon in the medicinal plants and the young plants are affected most (personal communication, M.A.U. Mridha). Leaf diseases of medicinal plants are frequent in the nurseries. The common nursery diseases are leaf blight, leaf spot, leaf rust, damping off, wilt, powdery mildew etc. There are several pathogens recorded all over the world, which caused different types of diseases of *C. roseus* (Holcomb, 1998; Burns & Benson, 2000; Holcomb, 2000; Montano *et al.,* 2001; Holcomb and Carling, 2002; Purohit & Vyas, 2004; Garibaldi *et al.,* 2006; Bhale *et al.,* 2009; Hao *et al.,* 2010; Mazidah *et al.,* 2012; Sharma *et al*., 2013). In Bangladesh, there is little work on the disease of medicinal plants (Basak & Mridha, 1987) and previously no work has been done on the leaf blight of *C. roseus.* Biological control is gaining world recognition as a primary and often essential component for successful integration of pest management. Biopesticidal control is less expensive and environmentally sound (Deacon, 1994; Whipps, 1997; Whipps & Davies, 2000). The main objectives of the present investigation are to determine the severity of leaf blight diseases of *C. roseus* and to identify the causal organism as well as to find out suitable *In vitro* biopesticidal control measure of the pathogen.

Materials and Methods

Survey: The samples for the present research were collected from Bangladesh. An observation was also made in the avenues and garden plants of *C. roseus* in King Saud University (KSU), Kingdom of Saud Arabia. An intensive survey was performed to study the symptoms and the severity of the leaf blight diseases of C. *roseus* at different nurseries of Chittagong Metropolitan areas namely Institute of Forestry and Environmental Sciences, University of Chittagong (IFESCU); Bangladesh Council of Industrial Research (BCSIR) and Bangladesh Forest Research Institute (BFRI). Following random sampling procedure, at first three sample plots (each sample plot comprising 100 plants) was taken and the total numbers of infected plant were counted. Then from the infected plants, ten plants were selected at random. To determine the infection percentage of leaves per plant, five randomly selected branches of one stem were taken. According to the percentage of infected area, infected leaves were classified into the following five categories. Descriptions of rating are as follows:

Numerical rating	Description of rating
0.	Healthy leaves
1.	1-10% infected area of leaf
2.	11-25% infected area of leaf
3.	26-40% infected area of leaf
4.	41-60% infected area of leaf
5.	Above 60% infected area of leaf

The severity of diseases in plants was determined by using the following formula:

$$\frac{\text{Total number of infected leaves}}{\text{Total number of leaves present}} \times 100$$

$$\text{Diseases index (\%)} = \frac{\text{Sum of all numerical ratings}}{\text{Total number of leaves per plant/branch x maximum rating}} \times 100$$

The data collected from the infected plants in the nurseries were recorded individually. Total number of leaves, healthy leaves and infected leaves of an individual plant were counted. The symptoms of the leaf spot disease were recorded carefully in the field as well as on detached infected leaves *In vitro*.

Isolation and identification of the associated organisms: Various types of infected leaves were collected from the nurseries for the isolation of associated organism. Three types of leaves (young, medium-aged and old leaves) were collected. The associated organism was isolated by placing the surface sterilized (0.01% Mercuric chloride solution for three minutes) diseased plant tissue aseptically on sterilized potato-dextrose-agar (PDA) medium. The inoculated Petri dishes were incubated at room temperature (25 ± 5°C) for 9 days. The pure culture was prepared in PDA plates and the causal organism was identified. The fungal pure culture was prepared and preserved at 4°C to avoid excessive growth and for further study. The fungus isolated from the infected tissues of *C. roseus* leaves was identified according to the developed colonies and sclerotial characters (Singh, 1998; Atiq *et al*., 2001).

Pathogenicity test: The pathogenicity test was carried out with the isolated pathogen on detached healthy leaves. The leaves were inoculated with mycelial block of 4-5 days old fungal culture under pricked and un-pricked condition. Un-pricked leaves were considered as control. The selected leaves were inoculated at two sites. The leaves were kept in a moist chamber within Petri dishes. Sterilized filter papers were placed under the leaves and sterilized water was used to moisten the chamber. After every 24 hours, the Petri dishes were sprayed with sterilized water. Observations were made up to 6 days for spot development and compared with those that occurred under natural condition. The pathogen was reisolated from the artificially inoculated leaves following the same procedure as isolation from the diseased field plants. The morphological characters of the reisolated fungus were compared with the original isolates by which the leaves were inoculated.

Effect of different plant extracts on mycelial growth: To determine the effect of different plant extracts on mycelial growth of the fungus was studied to find out the control of the pathogen *In vitro*. Different plant parts such as leaves, rhizome and bulbs were used to study the inhibition rate against the pathogen. Following plants and plant parts were used for the study.

Vernacular name	Scientific name	Plant parts used
Neem	*Azadirachta indica* A. Juss.	Leaf
Tulsi	*Ocimum sanctum* Linn.	Leaf
Nishinda	*Vitex negundo* Linn.	Leaf
Alkushi	*Mucuna pruriens* (L) DC.	Leaf
Akand	*Calotropis procera* (Aiton) W.T. Aiton	Leaf
Arjun	*Terminalia arjuna* (Roxb. ex DC.) Wight & Arn.	Leaf
Garlic	*Allium sativum* L.	Bulb
Zinger	*Zingiber officinalis* Roscoe 1807	Rhizome
Onion	*Allium cepa* L.	Bulb

To prepare the media with plant extract, the plant parts were cut into small pieces (2-3cm) and they were kept in a pan. Then sterilized distilled water was added with a ratio at 1:2 (w/v) and kept for twenty four hours. After that, the water soaked samples were crushed with a pestle and mortar. Then the extracts were passed through Whitman no. 1 filter paper. The extracts were used for screening their antifungal activities. Effect of different plant extract on mycelial growth was tested on PDA medium. Mycelial growth inhibition test was carried out following Miah *et al.* (1990). Plant extracts were mixed with PDA medium to have 25% concentration of the crude extract. After autoclaving at 121°C and 15 psi for 30 minutes, these were poured in sterilized Petri dishes. Extracts of plant species were tested following the poison food technique method (Bashar & Rai, 1991). From 7 days old cultures of the test fungus a 5mm mycelial disc was made by using sterilized cork borer and a long glass rod. One mycelial disc was placed in the center of each plate. The experiment was replicated five times. Radial mycelial growth was measured in mm. Incubation was done for 9 days at room temperature (25 ± 5°C). A control

set was maintained in each case using only PDA as growth medium.

The percentage inhibition of mycelial growth of test fungus was calculated by using the following formula:

$$I = \frac{C - T}{C} \times 100$$

where,

I = Percentage inhibition

C= Diameter of fungal colony on PDA (without extract)

T= Diameter of the fungal colony in treated plate

Statistical analysis: Statistical analysis was done by SPSS-21 version.

Results and Discussion

Symptoms and severity of leaf blight disease of *C. roseus*: Initial symptoms of leaf blight disease of *C. roseus* was as water soaked lesions on the leaves and gradually the disease appeared as small light brown spots

on the surface of the leaves. Lesion developed which later became necrotic, resulting in typical blight symptoms (Fig. 1). The mature leaves had more infection percentage of leaf surface area than the younger ones, may be because of proper nutrient availability in mature leaves than young leaves. The severity of leaf blight disease of *C. roseus*, was recorded from the three selected nurseries. It was found from table 1 that the infection percentage and disease index of infected leaves of *C. roseus* plant varied considerably in the three nurseries. The highest infection percentage and the highest disease index were found in BCSIR nursery, which was followed by BFRI and the lowest was recorded in IFESCU nursery. Average infection percentage was 43.65%, 40% and 41.78% in BCSIR, IFESCU and BFRI nurseries respectively. Again, it was observed that the average disease index of leaves was 35.68%, 31.27% and 32.8% in BCSIR, IFESCU and BFRI nurseries, respectively. The little differences in disease incidence may be related to plant canopy, plant age, availability of inoculum etc.

Among the nursery disease of medicinal plants leaf blight disease is a regular phenomenon to the young seedling. Not much work has been done in identifying and controlling nursery diseases in Bangladesh, especially the diseases of medicinal plants including *C. roseus* (Basak & Mridha, 1987). Several pathogens, which caused different types of diseases of *C. roseus* are recorded throughout the world (Bhale *et al.,* 2009; Burns & Benson, 2000; Garibaldi *et al.,* 2006; Hao *et al.,* 2010; Holcomb & Carling, 2002; Holcomb, 2000; Holcomb, 1998; Mazidah *et al.,* 2012; Montano *et al.,* 2001; Purohit & Vyas, 2004).

Very recently Sharma *et al*. (2013) from India recoded twig blight on *C. roseus* caused by *Colletotrichum gloeosporioides*. The present field investigation indicated that the disease incidence was severe during the dry seasons, which implied that drought may be one of the important factors. At the advent of rainy season the intensity becomes less (Purohit & Vyas, 2004). This indicates that under water stress condition the seedlings might be prone to fungal attack.

Fig. 1. Leaf blight disease of *Catharanthus roseus* caused by *Macrophomina phaseolina*.

Table 1. Infection percentage and disease index (percentage) of leaf blight of *Catharanthus roseus* in three nurseries.

Nursery	Sample plot	Infection percentage/plot	Average infection (%)	Disease index	Average disease index
BCSIR	1	43.06		36.74	
	2	44.30	43.65	34.07	35.68
	3	43.60		36.23	
IFESCU	4	39.81		30.95	
	5	42.52	40.00	32.94	31.27
	6	37.70		29.94	
BFRI	7	42.81		32.62	
	8	40.23	41.78	31.37	32.80
	9	42.32		34.41	

Table 2. Effect of different plant extracts on the radial growth and inhibition percentage of mycelium of *Macrophomina phaseolina*.

Plant extract	Radial mycelial growth (mm)/ day								Inhibition (%)
	2nd	3rd	4th	5th	6th	7th	8th	9th	
Neem	13.8	18.2	23.1	27.5	31.4	35.9	37.8	40.2g	55.72b
Tulsi	21.6	30.9	36.4	41.3	46.7	51.25	55.2	60.5c	33.37f
Akand	19.5	25.6	32.8	38.9	42.3	45.2	47.1	49.4e	45.61d
Garlic	12.2	16.8	22.5	28.3	35.6	39.2	41.1	43.5f	52.53c
Onion	25.6	32.4	37.8	42.7	46.4	49.2	51.7	53.9d	40.63e
Zinger	17.5	21.8	24.3	27.1	30.2	33.6	37.5	40.4g	55.50b
Alkushi	25.6	30.4	36.8	44.2	50.6	54.4	59.2	65.8a	27.47h
Arjun	11.8	15.5	20.2	24.5	27.8	31.2	34.5	38.5h	58.37a
Nishinda	17.8	25.4	32.8	39.2	45.9	50.75	56.8	61.5b	32.26g
PDA	29.4	36.6	45.9	58.6	69.2	75.4	83.6	90.8	

Different letters indicate the significant difference as shown by DMRT (p<0.05)

Identification and pathogenicity of the fungus: The mycelium was superficial, brown to dark brown and septate. The sclerotia were scattered on the surface of the mycelium and it was brown to dark black in color and the pycnidia were brown. The fungus isolated from the diseased samples was identified as *Macrophomina phaseolina* (Tassi) Goid. The fungus produced typical symptoms on leaves of *C. roseus* seedlings during pathogenicity test. The sign of infection was observed to start after three days of incubation. The highest percentage of infection was observed when pricked leaves were inoculated with mycelial blocks. Very low percentage of infection was obtained from the inoculation of unpricked leaves. All types of leaves were found to be susceptible to the fungus. Younger leaves were more susceptible than the middle aged and mature leaves. *M. phaseolina* was fast growing in nature. On the 9th day of incubation in PDA, it occupied the petri dish (90.8mm).At the initial stage the color of the colony of *M. phaseolina* in PDA was ashy, centrally blackish and finally it became dark in color. Mycelia were fluffy and aerial in nature. *M. phaseolina* is one of the more common root parasites in warmer lands and the causal organism of many different plant diseases. *M. phaseolina* from *C. roseus* was reported for the first time in the present study.

Effect of different plant extracts on mycelial growth of *M. phaseolina*: The effect of plant extracts on the mycelial growth of *M. phaseolina* was studied to find out the effective bio-pesticides. Table 2 represents the result of the increment and percent inhibition of mycelial growth of *M. phaseolina* treated with different plant extracts. It was found that the mycelial growth of the test fungus was lowest in *T. arjuna* (38.5mm) followed by *A. indica* (40.2mm), whereas PDA medium was 90.8mm after 9th day of incubation. *Z. officinalis* (40.4mm), *A. sativum* (43.5mm) *C. procera* (49.5mm) showed moderate inhibitory effect on the mycelial growth. The highest (65.85mm) mycelial growth was found in *M. pruriens* leaf extract. The rest of the leaf and bulb extracts showed more or less similar effect on the mycelial growth. Out of the nine plant extract medium, *T. arjuna* showed the highest (58.37%) inhibition percentage which was followed by *A. indica* (55.72%). The lowest (27.4%) inhibition percentage was obtained from the *V. negundo* medium whereas the rest of the plant extracts showed more or less same inhibitory effect (Table 2). Bio pesticidal control of different plant pathogenic fungi including *M. phaseolina by* using various plant extracts such as *A. indica, A. arabica, Cassia fistula, Lantana camara, Rhododendron arboreum, Acalypha indica, V. negundo, A. sativum. T. arjuna* etc. were studied in various countries (Kumar *et al.*, 1997; Bhowmic & Chowdhury, 1982; Ahmed & Sultan, 1984; Siddarmaiah & Hedge, 1990; Sundriyal, 1991; Srivastava & Lal, 1997). Srivastava & Lal (1997) found that the aqueous leaf extracts of *C. procera*, *A. indica*, *L. camara* and *O. basilicum* have fungicidal properties against *Curvularia lunata*. Kumar *et al.* (1997) reported complete inhibition of spore germination of *Fusarium oxysporum, Alernaria alternata* and *Corynespora cassicola* by aqueous extracts of onion, *A. sativum*, *Kalanchoe* and cotton. Bhowmic & Chowdhury (1982) carried out an experiment on antifungal activity of leaf extract of medicinal plants on

A. alternata (Fr) Keissler. In a test with ten plant species greatest inhibition under *In vitro* condition was obtained from *A. indica*, followed by camphor, *V. negundo* and *A. indica*. Siddarmaiah & Hedge (1990) reported that the leaf extracts of *A. sativum*, *O. sanctum* and *Pimenta officinalis*, flower extracts of *A. sativum, Acorus calamus* and *Z. officinalis* were inhibitory to conidial germination of *Cercospora moricola* at 1:2 concentrations. In our study we have also recorded positive results with nine plant extract to inhibit the growth of *M. phaseolina* causal organism of leaf blight disease of *C. roseus.*

Conclusions

M. phaseolina has a wide host range and is responsible for causing losses on more than 500 cultivated and wild plant species (Indera *et al.,* 1986). In this study we have also investigated the effect of plant extracts on the mycelial growth of *M. phaseolina* to find out the effective bio-pesticides. The result indicated that most of tested plants were effective in controlling the mycelial growth of *M. phaseolina* treated with different plant extracts. It is concluded that *C. roseus* is suffering from severe diseases caused by *M. phaseolina* and the pathogen may be controlled by using plant extracts of different plants species.

Acknowledgments

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