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# FIRST REPORTS ON FUNGAL PATHOGENS OF IMPROVED JUJUBE IN BANGLADESH

**M. Z. Hoque[1], M. A. Mannan Akanda[2], I. H. Mian[2] and M. K. A. Bhuiyan[2]**

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ABSTRACT

M. Z. Hoque, M. A. Mannan Akanda, I. H. Mian and M. K. A. Bhuiyan. 2012. First reports on fungal pathogens of improved jujube in Bangladesh. Bangladesh J. Plant Pathol. 28(1&2):1-8.

A survey was conducted in Bangladesh to identify the fungal diseases of jujube and their causal pathogens occur in commercial orchards, planted with high yield varieties. The identified diseases and their causal fungi were leaf spots (*Alternaria alternata*, *Cercospora ziziphi*, *Curvularia lunata*, *Fusarium semitectum*, *Lasiodiplodia theobromae*, *Mitteriella ziziphina*, *Pestalotiopsis palmarum*), fruit spots and pre-harvest fruit rot (*A. alternata*, *Colletotrichum gloeosporioides*, *C. lunata*, *F. semitectum*, *L. theobromae*, *M. ziziphina*, *Nigrospora oryzae*, *P. palmarum*, *Phomopsis* sp.), post-harvest fruit rot (*A. alternata*, *Aspergillus* spp., *C. gloeosporioides*, *C. lunata*, *F. semitectum*, *Nigrospora oryzae*, *Penicillium* spp., *Phomopsis* sp. *P. palmarum*, *Rhizopus* sp.), powdery mildew (*Oidium erysiphoidess* f. sp. *ziziphi*), sooty mould or black mold or *Isariopsis* mold (*Isariopsis indica* var. *ziziphi*, *Capnodium* sp.) and stem bleeding or gummosis (*P. palmarum*, *C. lunata*). Among the fungal pathogens causing jujube diseases, *P. palmarum*, *C. gloeosporioides*, *A. alternata*, *F. semitectum*, *C. lunata*, *L. theobromae*, *C. ziziphi*, *Phomopsis* sp., *I. indica* var. *ziziphi*, *M. ziziphina* and *N. oryzae* were noted as new records in Bangladesh.

**Keywords**: Diseases, fungal pathogen, jujube

## INTRODUCTION

Jujube (*Ziziphus mauritiana* Lam.) is a nutritious indigenous fruit of Bangladesh cultivated in homestead since time immemorial. The fruits contain high protein, phosphorus, calcium, carotene and vitamins than apple (Bakhshi and Singh 1974, Kuliev and Guseinova 1974). Available reports from China and India reveal that jujube is vulnerable to many diseases (Gupta and Madan 1977, Singh and Singh 1979, Rai *et al.* 1982, Sharma *et al.* 1993, Nallathambi *et al.* 1999, Quin and Tian 2004). *Alternaria alternata* causes pre-harvest infection on jujube fruits that result in 25% yield loss in India (Nallathambi 2001, Nallathambi *et al.* 2006). Moreover, jujube fruits are highly vulnerable to post-harvest color fading, browning, decay, and water loss (Tian 2000). Powdery mildew caused by *Oidium erysiphoides* f.sp. *ziziphi* is a major disease of jujube in India. Jamadar *et al*. (2009) found 50-60% loss in fruit yield due to powdery mildew of jujube and reduced market value of the produce. *Pseudocercospora jujubae* a fungal pathogen has been reported from Bangladesh by Khan *et al.* (1983).

In Bangladesh, many diseases appear on foliage and fruits of jujube. In many cases, disease occurrence is an important threat for commercial cultivation of jujube. However, reports on the occurrence of diseases in Bangladesh are scanty (Rahman *et al.* 2011).

Considering the above facts a study was undertaken to survey the occurrence of fungal diseases of jujube in Bangladesh and their causal fungi.

## MATERIALS AND METHODS

Commercial jujube orchards were selected in five jujube growing districts namely Gazipur, Pabna, Rajshahi, Rangpur and Mymensingh. The orchards were planted with three improved jujube varieties viz. apple Kul, BAU Kul and BARI Kul-1. A total of 405 diseased samples representing 150 fruits, 135 leaves, 30 trunks and 90 inflorescences of jujube were collected from the selected areas during 2007-08, 2008-09 and 2009-10. Among 150 fruit samples 73 were collected from markets of those locations. The collected samples were examined carefully and symptoms of individual diseases were recorded and photographs were taken.

Fungal pathogens associated with the diseased samples were isolated from infected plant samples following standard tissue planting methods using

potato dextrose agar (PDA) medium as described by Aneza (2003). When ever necessary, infected samples were placed in moist chambers and incubate at room (25 ±2 C) temperature for two days. Fungal mycelia grew from the samples were transferred directly to PDA plates.

Fungi isolated from the collected diseased specimens were purified following single spore culture and/or hyphal tip culture method (Aneza 2003). The isolated fungi were identified based on colony and morphological characters on PDA and consulting available literature (Barnett and Hunter 1972, Sutton 1980). Pathogenicity of different fungal pathogens isolated from diseased samples of jujube was performed following detached leaf and detached fruit inoculation method (Omar and Wahid 2001).

## RESULTS AND DISCUSSION

### Occurrence of jujube diseases in Bangladesh

In Bangladesh, at least seven diseases were found to attack jujube in commercial orchard planted with high yielding varieties. These were leaf spots (*Alternaria alternata, Cercospora ziziphi, Curvularia lunata, Fusarium semitectum, Lasiodiplodia theobromae, Mitteriella ziziphina, Pestalotiopsis palmarum*), fruit spots and pre-harvest fruit rot (*A. alternata, Colletotrichum gloeosporioides, C. lunata, F. semitectum, L. theobromae, M. ziziphina, Nigrospora oryzae, P. palmarum, Phomopsis* sp.), post-harvest fruit rot (*A. alternata, Aspergillus* spp. *C. gloeosporioides, C. lunata,., F. semitectum, Nigrospora oryzae, Penicillium* spp., *Phomopsis* sp. *P. palmarum, Rhizopus* sp.), powdery mildew (*Oidium erysiphoidess* f. sp. *ziziphi*), sooty mould or black mold or *Isariopsis* mold (*Isariopsis indica* var. *ziziphi*,*Capnodium* sp.) and stem bleeding or gummosis (*P. palmarum, C. lunata*). Harvested jujube fruits were found to be attacked by *A. alternata, Aspergillus* spp., *C. gloeosporioides, C. lunata, F. semitectum, L. theobromae, F. semitectum, Nigrospora sp., Penicillium* spp., *Phomopsis* sp., *P. palmarum, Rhizopus* sp. causing post harvest fruit rot (Table 1).

Table 1. Diseases of Jujube occur in Bangladesh and their causal fungi recorded during 2008 to 2010 crop seasons

| Diseases | Pathogens |
|---|---|
| Powdery mildew | *Oidium erysiphoidess* f.sp *ziziphi* |
| Leaf spots | *Alternaria alternata, Cercospora ziziphi, Curvularia lunata, Fusarium semitectum, Lasiodiplodia theobromae, Mitteriella ziziphina, Pestalotiopsis palmarum* |
| Sooty, black or *Isariopsis* mold | *Isariopsis indica* var. *ziziphi, Capnodium* sp. |
| Rust disease | *Phakopsora zizyphi-vulgaris* |
| Stem bleeding or gummosis | *P. palmarum, F. semitectum, C. lunata* |
| Fruit spots and Pre-harvest fruit rot | *A., Colletotrichum gloeosporioides, C. lunata, F. semitectum, L. theobromae, M. ziziphina, Nigrospora oryae, P. palmarum, Phomopsis* sp. |
| Post-harvest fruit rot | *A. alternata, Aspergillus* spp., *C. gloeosporioides, C. lunata, F. semitectum, L. theobromae, F. semitectum , N. oryzae, Penicillium* spp., *Phomopsis* sp. *P. palmarum, Rhizopus* sp. |

Altogether 16 fungal pathogens were associated with diseased samples of jujube plants during the study. Of them, association of 11 fungi with jujube is new records in Bangladesh. They are A. *alternata, C. ziziphi, C. gloeosporioides, C. lunata, F. semitectum, I. indica* var. *ziziphi, L. theobromae, M. ziziphina, Nigrospora* sp., *P. palmarum* and *Phomopsis* sp. Results of their pathogenicity test on inoculated leaves and fruits, and their prevalence in collected samples are described below:

***Alternaria alternata***: Among the tested samples, one hundred eighty five samples yielded *A. alternata*. Characteristic muriform spores in chain were observed on culture plate as well as on infected plant parts (Plate I A & B). The pathogen causes leaf spot (Plate I C), fruit spot (Plate I D & E) and fruit rot (Plate I F). Symptoms of leaf spots are observed on the upper surface of leaves as small irregular, brown lesions. Dark brown to black spots are found on the lower surface of leaves. Sometimes several spots coalesce together to form large lesions. On fruits, symptoms appear as slightly depressed, almost circular, brown to black lesions on the surface. Sometimes concentric rings are formed on some

spots. Ultimately, whole infected fruits become rotted (Plate 1 F).

***Cercospora ziziphi***: *Cercospora ziziphi* (Plate II A & B) was isolated from leaf samples. Sometimes it was found on leaves as mixed infection with other fungi like *Pestalotiopsis*. Symptoms appeared on leaves as circular to oval lesions with yellow halo. Initially, spots are yellow and later turn into brown. Finally, spots are surrounded by a dark brown margin. In course of time, spots become larger and visible on both sides of the leaves (Plate II C).

***Colletotrichum gloeosporioides:*** *Colletotrichum gloeosporioides* was isolated from fruit and leaf samples of jujube. The fungus produces disc-shaped or cushion-shaped and waxy acervuli (Plate III A) containing simple conidiophores bearing conidia. The conidia are hyaline, 1-celled (Plate III B). Under natural conditions it infects immature fruits causing anthracnose symptoms (Plate III C). Initial symptom of the disease appears as black sunken lesions. On inoculated fruits, the pathogen develops characteristic symptoms of anthracnose (Plate III D).

***Curvularia lunata****: Curvularia lunata* was isolated from fruit, leaf, stem and inflorescence samples (Plate IV A). It was found on fruits as mixed infection with other fungi. Due to its infection, dark brown to black irregular lesions are observed on lower surface of leaves (Plate IV B). The upper surface of infected leaves become yellow. Black depressed spots are found on fruits (Plate IV C).

***Fusarium semitectum*****:** *Fusarium semitectum* (Plate V A) was isolated from fruit, leaf, stem and inflorescence samples. The fungus causes fruit spot under natural conditions (Plate V B) as well as inoculated condition (Plate V C). Infected inflorescence becomes black and rotted. In the morning, sometimes white fungal mycelia appear on inflorescence. Infected stems become black and bark of plants becomes crack and grayish exudates come out from infected stem. Ultimately whole branch withered and die.

***Isariopsis indica* var. *ziziphi*:** The fungus was isolated from only leaf samples. It causes black leaf spot or *Isariopsis* mold on leaf (Plate VI A). The characteristic symptoms of the disease are sooty tuft-like circular to irregular black spots on ventral surface of leaves. Later, it covers the entire lower surface of leavesgiving a sooty appearance (Plate VI B). Septate, dark mycelium and dark synnemata bearing dark conidia form sooty mold (Plate VI C).

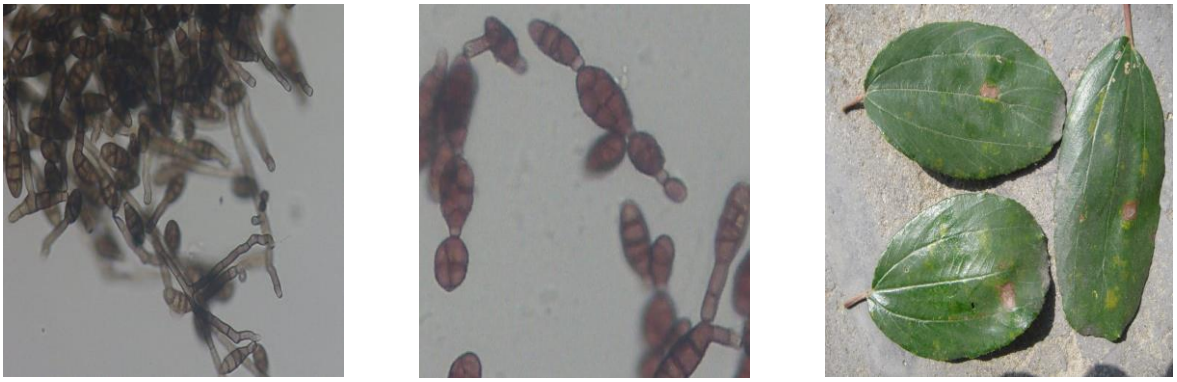
***Lasiodiplodia theobromae****:* The pathogen was isolated from fruit samples and leaf samples and identified based on characteristics of mycelia and spore (Plate VII A, B & C). This was found on infected fruits as mixed infection with *P. plamarum, C. gloeosporioides, F. semitectum* and *A. alternata*. *L. theobromae.* The pathogen causes spots on leaves and fruits (Plate VII D & E) under natural condition as well as inoculated condition (Plate VII F).

***Mitteriella ziziphina***: *Mitteriella ziziphina* (Plate VIII A & B) was isolated from fruit and leaf samples. The fungus develops almost round and black spots on leaves (Plate VIII C & C$_{(a)}$) and fruit under natural (Plate VIII D & E) as well as inoculated conditions (Plate VIII F). At advanced stage of infection, severely infected fruits become black and superficial black fungal growth is found leaves and fruits (Plate VIII C, D & E).

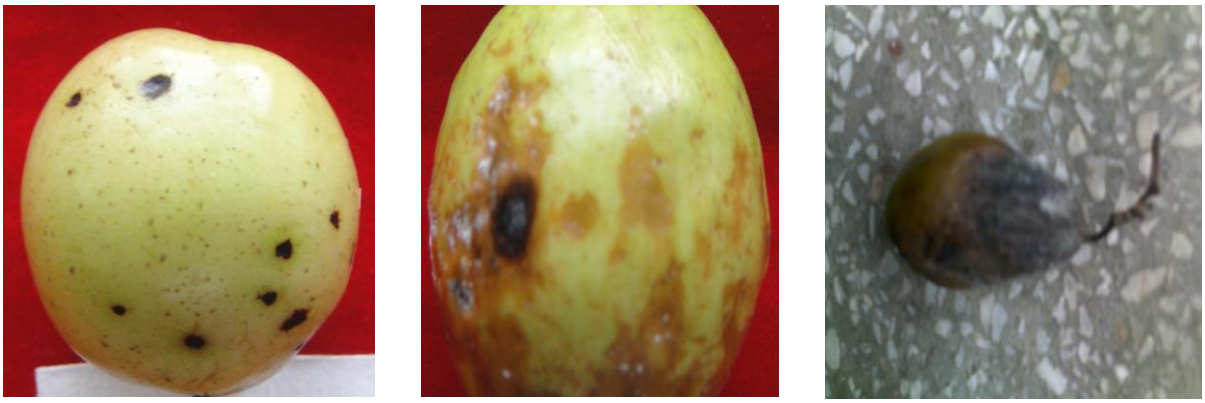
***Nigrospora oryzae***: The pathogen was isolated from infected fruits samples. It was found on fruits as mixed infection with *Alternaria, Lasiodiplodia, Fusarium* and *Colletotrichum.* The pathogen causes black discoloration on both young and mature fruits (Plate IX A & B). Apex of the immature fruits becomes shriveled, rotted and ultimately fruit dropping occurs.

***Pestalotiopsis palmarum*****:** *Pestalotiopsis palmarum* was isolated from infected leaf, fruit, and stem samples and it was identified based on conidia with appendages (Plate X B). The fungus causes leaf spot (Plate X C) and gummosis or stem bleeding diseases (Plate X D). Under natural conditions, irregular brown to dark brown lesions with black dot of acervuli were observed on infected leaves (Plate X C). Fruit spot and fruit rot were also observed (Plate XI D). Spots are brown to dark brown in color, raised and corky. Infected stem becomes rotted and gum like substance comes out from the infected area causing gummosis (Plate X E). Inoculated fruits also formed symptoms similar to natural symptoms (Plate XI C).

***Phomopsis* sp.**: *Phomosis* sp. was isolated from diseased fruit samples mixed infection with *P. palmarum, C. gloeosporioides* and *A. alternata*. Due to infection of *Phomosis*, initially black dot like water soaked small spots appear on fruits. Later, the spot size increase gradually and several spots coalesce together and form large lesion (Plate XI A). At later stage, fruits become soft and rotted and fruit color turns into pale brown. Pycnidia develop on PDA culture (Plate XI B). Sometimes dot like black pycnidia are found on lesions. Characteristic alpha and beta conidia were observed under microscope (Plate XI C).

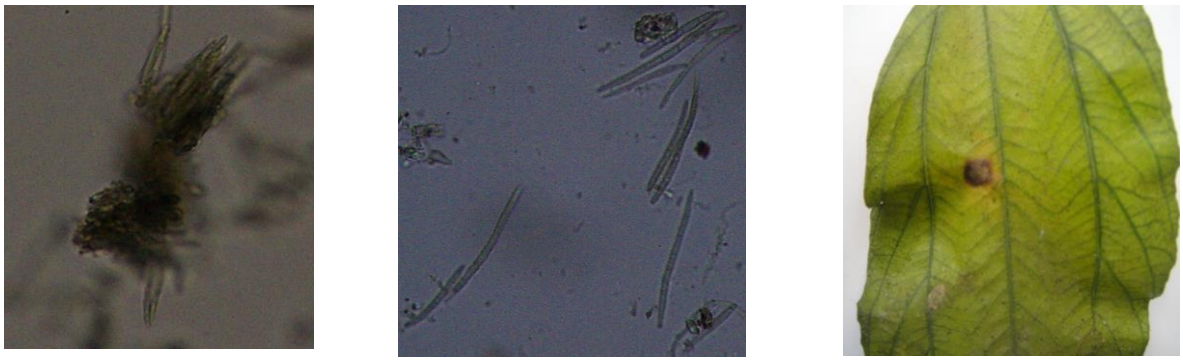


A B C



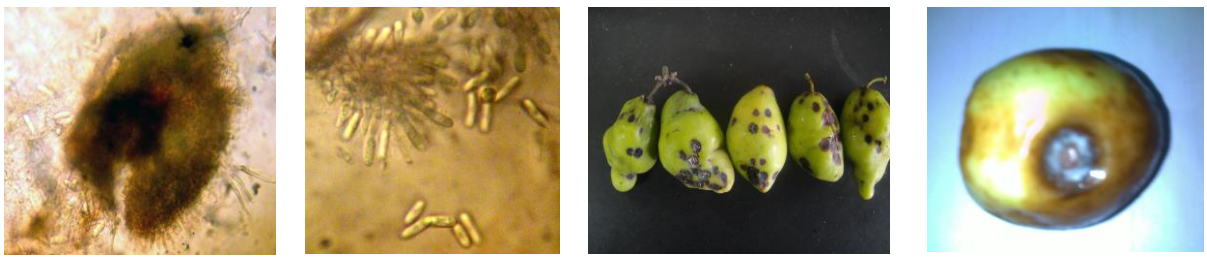
D E F

Plate I. Spore of *Alternaria alternata* (A&B) and *Alternaria* spots on naturally infected leaves (C) and naturally infected fruit (D) as well as inoculated (E) fruits and rotted and mummified fruits of jujube.



A B C

Plate II. Conidiophore (A) and conidia (B) of *Cercospora ziziphi* isolated from infected jujube plant samples and leaf spot symptoms developed on jujube leaf (A) after inoculation



A B C D

Plate III. Acervulus (A) and conidiophores bearing conidia (B) of *Colletotrichum gloeosporioides* isolated from jujube fruits infected with anthracnose (C) and fruits symptoms on inoculated fruits (D)

A B C

Plate IV. Mycelia and conidiophores bearing conidia of *Curvularia lunata* (A) isolated from diseased samples and symptoms of leaf spot (B) and fruit rot (C) of jujube

A B C

Plate V. Characteristic conidia (A) of *Fusarium semitectum* isolated from diseased samples of jujube and fruit rot under natural (B) and inoculated conditions (C)

A B C

Plate VI. Symptoms of black leaf spot or *Isariopsis* mould on jujube leaves and conidia of *I. indica* var. *ziziphi* [A. Black leaf spot symptoms, B. Severely infected leaves C. Conidia of *I. indica* var. *ziziphi*]

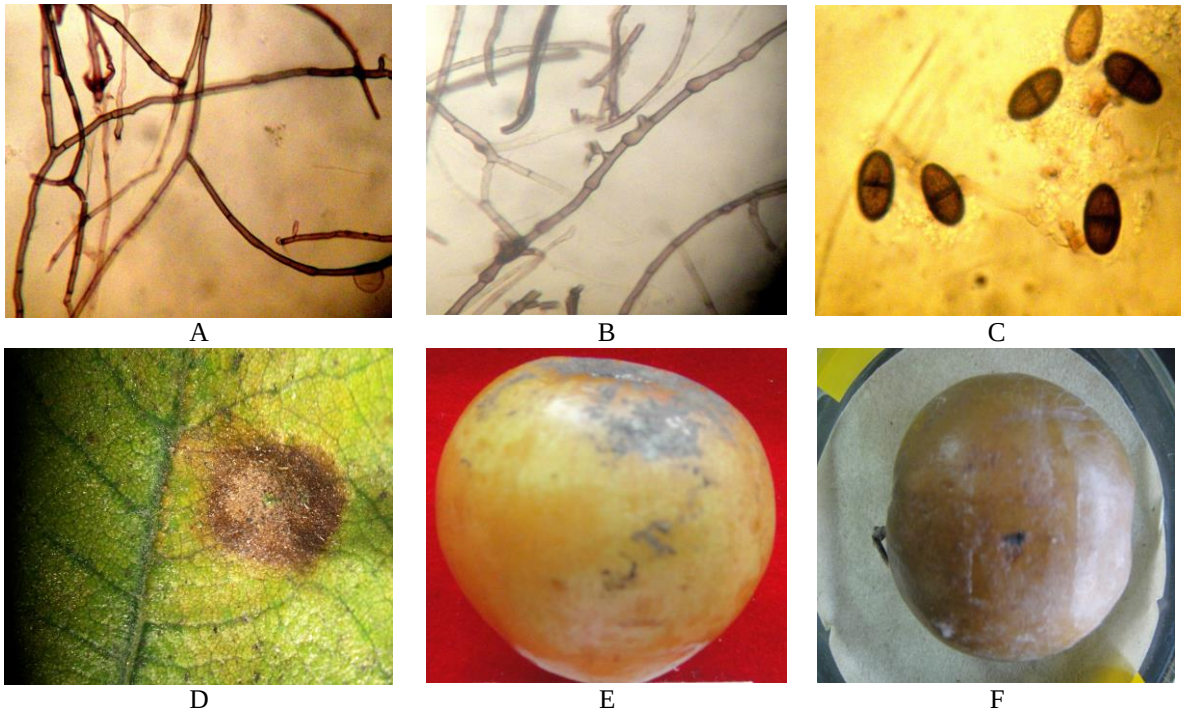



Plate VII. *Lasiodiplodia theobromae* isolated from diseased specimens of jujube [A. Characteristic mycelium, B. Enlarge view of mycelium, C. Characteristic 2-celled conidia, D. Leaf spot symptoms, E. Fruit spot symptoms under natural conditions and F. fruit spot symptoms under inoculated condition]

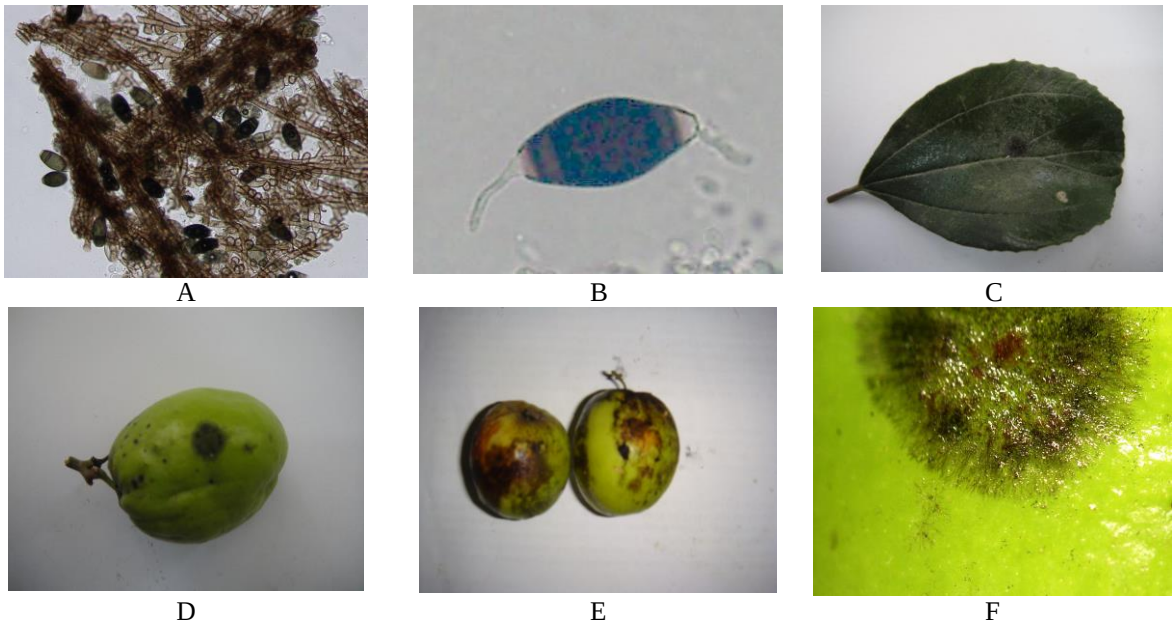
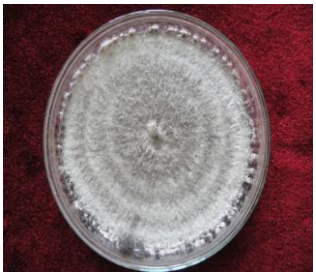
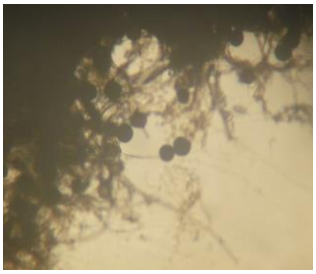



Plate VIII. Symptoms of leaf and fruit spots of Jujube caused by *Mitteriella ziziphina* [A. Dark mycelium B. Black conidia with two polar germ tubes, C. Leaf spot, D. Black spot on fruit, E. Severely infected fruits F. Enlarged view of spot on inoculated fruit.]



A



B

Plate IX. Mycelium, conidiophore and spore of *Nigrospora* sp. isolated from jujube fruits [A. Fungal colony on PDA plate, B. Mycelium, conidiophore bearing conidia]

A

B


C

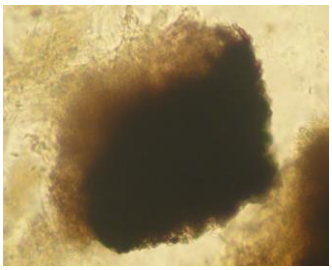
D

E

F

Plate X. *Pestalotiopsis palmarum* causing leaf spot, fruit spot, fruit rot and gummosis diseases of jujube [A. Acervuli on PDA, B. Conidia with appendages, C. Leaf lesion symptom, D. Fruit spot fruit rot, E. Stem gummosis diseases and F. Fruit spot on inoculated fruit].

A



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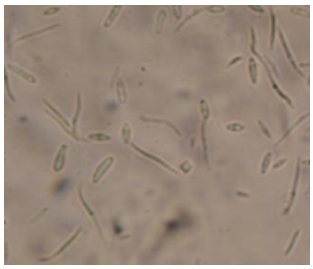

C

Plate XI. Symptoms of fruit rot of Jujube caused by *Phomopsis* sp. [A. fruit rot symptoms, B. Pycnidia and C. α and β conidia of *Phomopsis* sp.]

Findings of the present investigation reveal that leaf spots, fruit spots and pre-harvest and post-harvest fruit rot, sooty mould or black mold or *Isariopsis* mold and stem bleeding or gummosis are common diseases of high yielding jujube varieties. After harvest jujube fruits are attacked by *A. alternata, Aspergillus* spp., *C. gloeosporioides, C. lunata, F. semitectum, L. theobromae, F. semitectum, Nigrospora* sp., *Penicillium* spp., *Phomopsis* sp., *P. palmarum, Rhizopus* sp. causing post harvest fruit

rot. The diseases and their causal fungi recorded in Bangladesh also occur in other jujube growing countries of the world. In India, jujube plants are attacked by powdery mildew (*O. erysiphoides* var. *ziziphi*), sooty mold (*I. inidica* var. *ziziphi*), and leaf spots caused by *A. alternata, Cercospora, Septoria, Cladosporium, Pestalotiopsis* (Jamadar *et al*. 2009) and *P. palmarum* (Madan and Gupta 1976, Rai *et al*. 1982)**.** From China, Yuan *et al*. (2009) reported that jujube trees are attacked by powdery mildew, anthracnose (*C. gloeosporioides*), black spot (*A. alternata*) and brown spot (*P. mauritiana*). Morton (1987) reported that jujube fruits are attacked by *A. chartarum, A. nanus, A. parasiticus, H. atroolivaceum, P. hessarensis,* and *S. valparadisiacum.* Fruit rots are caused by *Fusarium* spp., *N. oryzae, Epicoccum nigrum,* and *Glomerella cingulata*. Of the diseases only anthracnose (*C. gloeosporioides*) has been reported earlier from Bangladesh (Rahman *et al.* (2011).

Based on findings of the present investigation, it may be concluded that 16 fungal plant pathogens are associated with improved and high yielding varieties of jujube diseases in Bangladesh. They cause leaf spots, rust, fruit spots and fruit rot, powdery mildew, sooty mold, stem bleeding and post-harvest deterioration of the fruits. Among the fungal pathogens of jujube A. *alternata, C. ziziphi*, *C. gloeosporioides, C. lunata, F. semitectum, I. indica* var. *ziziphi, L. theobromae, M. ziziphina, Nigrospora* sp. and *Phomopsis* sp. are recorded as new pathogens of the fruit in Bangladesh.

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# INFLUENCE OF SOME GROWTH FACTORS ON *IN-VITRO* GROWTH OF *FUSARIUM OXYSPORUM* F. SP. *PHASEOLI* CAUSING SEEDLING MORTALITY OF BUSH BEAN

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ABSTRACT

S. Sharmin Siddique, K. A.Bhuiyan, M. R. Uddin and M. B. Anwar. 2012. Influence of some growth factors on *in-vitro* growth of *Fusarium oxysporum* f. sp. *phaseoli* causing seedling mortality of bush bean. Bangladesh J. Plant Pathol. Vol. 28 (1&2): 9-14.

The present experiment was conducted to determine the influence of different temperature regimes (15, 20, 25, 30 and 35C), pH levels (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5), nitrogen sources (peptone, L-asparagines, $NaNO_3$, $NH_4NO_3$, $(NH_4)_2SO_4$,), carbon sources (dextrose, D-xylose, sucrose, glycerol and D-mannitol) and C/N ratio (5, 10, 20, 40, 80, and 100) on *in-vitro* growth of *Fusarium oxysporum* f.sp. *phaseoli* isolated from diseased bush bean (*Phaseolus vulgaris* L.) seedlings. The growth was measured in terms of radial colony diameter on semi solid medium and dry mycelial weight grown in liquid medium. The pathogen grew well at the temperature range of 15-35 C, pH range of 4.0-8.5 and C/N ratio range of 5-100. The maximum colony diameter of 85.47 mm and 272.89 mg mycelial dry weight per plate were found at $30^0$C. The highest colony diameter of 78.67 mm/plate was recorded from pH 6.0 and maximum dry mycelial weight of 246.06 mg/plate was found at pH 6.5. The highest colony diameter and dry mycelial weight were recorded when C/N ratio was maintained at 20. The best source of nitrogen was peptone and that of carbon was sucrose. Based on results of the present study it may be concluded that the optimum temperature, pH and C/N ratio for mycelial growth of the fungus are 30 C, pH 6.0-6.5 and C/N ratio 20, respectively. Peptone and sucrose are the best sources of nitrogen and carbon, respectively.

**Keywords**: Growth factors, in-vitro growth, *Fusarium oxysporum* f.sp. *phaseoli*.

## INTRODUCTION

*Fusarium oxysporum* f. sp. *phaseoli* is a major cause of seedling mortality of the vegetable including bush bean (*Phaseolus vulgaris* L.). It causes seed deterioration and root rot or wilt (Cavalacanti *et al.* 2002). Ellanskaia (1969) demonstrated that different sources of carbon had remarkable influence on the growth and conidia formation of fungi under the genus *Fusarium.* Warner (1990) studied the effect of temperature and medium composition on growth and sporulation of *F. oxysporum.* He found that the maximum growth and sporulation of the fungus occurred on potato dextrose agar and malt agar at 25-30C. Chlamydospore germination is influenced by the presence of exogenous sources of carbon and nitrogen (Ciotola *et al.* 2000). The effects of physiological and pathological factors on growth, development and Pathogenicity of *F. oxysporum* have been well documented by Ciotola *et al*. (2000). Systematic researches on physiological aspects of *F. oxysporum* f. sp. *phaseoli* causing seedling mortality of bush bean are not available in Bangladesh. But such information is essential to develop an appropriate control strategy against seedling mortality of vegetable crops caused by *F. oxysporum* f.sp. *phaseoli*.

The present study was undertaken to determine the influence of different growth factors on *in-vitro* growth of *F. oxysporum* f. sp. *phaseoli* infecting bush bean.

## MATERIALS AND METHODS

Influence of five important growth factors namely temperature during incubation, and pH, nitrogen source, carbon source and C/N ratio of the culture medium were tested following *in-vitro* method (Dhingra and Sinclair 1985). First two experiments were conducted to test five temperature regimes (15, 20, 25, 30 and 35 C) and ten levels of pH (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5). In both the experiments, potato dextrose agar (PDA) and potato dextrose broth (Tuite 1969) were used. Another three experiments were conducted to test the effect of five nitrogen sources ($NaNO_3$, $NH_4NO_3$, $(NH_4)_2SO_4$, peptone and L-Asparagine), five carbon sources (Dextrose, D-Xylose, Sucrose, Glycerol and D-

mannitol) and six levels of C/N ratio (5, 10, 20, 40, 80, and 100) on mycelium growth of *F. oxysporum* f.sp. *phaseoli*. Czapek's solution with agar (semisolid) and without agar (liquid) was used as basal media. The test fungus was *F. oxysporum* f.sp. *phaseoli* which was isolated from foot rot infected bush bean seedling following tissue planting method (Tuite 1969). To prepare its inocula, the fungus was grown on PDA medium in Petri dishes. Mycelial discs were cut from growing edge of 4 days old culture of the fungus with a flame sterilized 5 mm diameter cork borer. Except the experiment with pH levels, the pH of the culture medium was adjusted to 6 using 0.1N HCl and 0.1N NaOH and sterilized in an autoclave at 120C under 1kg/cm$^2$ pressure for 20 minutes. The liquid medium (broth) was prepared using all ingredients except agar.

To test carbon sources, Czapek's medium was prepared by mixing each carbon source at 30 g per 1 liter. Czapek's medium without any carbon source was used as control. To study the influence of different nitrogen sources, the Czapek's medium was prepared by mixing requisite quantity of each nitrogen source which is equivalent to the amount of nitrogen obtainable from 3g of $NaNO_3$. The Czapek's medium without any nitrogen source was used as a control. To test the effect of C/N ratio on growth of the pathogen, Czapek's medium was prepared by mixing requisite quantity of $NaNO_3$ as a source of nitrogen at two fixed levels of sucrose (2g and 5g) as a source of carbon. The levels of the C/N ratio were 5, 10, 20, 40, 80, and 100.

The growth of the fungus was measured in terms of colony diameter and mycelial dry weight. To measure colony diameter, agar media (semisolid) was used and the fungus was grown in 90 mm glass Petri dishes. For the measurement of mycelium dry weight, the fungus was grown in 100 ml conical flasks containing liquid medium (without agar). The agar media were dispensed into the Petri dishes and the liquid media were also poured into the conical flasks at 20 ml per dish or flask.

To measure colony diameter, Petri plates containing agar medium were inoculated after solidification with the mycelium discs (0.5 cm) of the test fungus. The inoculum was placed at the center of each plate. Except the experiment to test different temperature regimes, the inoculated Petri plates under all experiment were incubated at 25C in incubators. The plates were arranged in the incubators following completely randomized design with three replications. The colony growth was measured by averaging the two diameters taken at right angle for each colony after 4 days of incubation.

To measure mycelium dry weight, conical flaks containing potato dextrose broth or liquid Czapeck's medium (40 C) were inoculated with the inoculum at one disc per flask. The inoculated flasks were incubated at room temperature (25-28 C) for 14 days. The flasks were arranged on the laboratory desks following completely randomized design with three replications (flasks). At the end of incubation period, the cultures in all flasks were filtered through dry (at 70 C for 12 hr) and pre-weighed filter paper. Dry weight of mycelium was determined after drying the mycelium along with the filter paper in an oven at 70 C for constant weight. Dry weight of mycelium was obtained by subtracting weight of only filter paper from weight of filter paper plus mycelium.

Data collected from different experiments were analyzed for ANOVA using MSTAT-C program. Duncan's Multiple Range Test (DMRT) was performed to compare treatment means. Whenever necessary, data were transformed following appropriate method before statistical analysis.

## RESULTS AND DISCUSSION

### Effect of temperature on mycelial growth

Results on the effect of temperature regimes on radial colony diameter and mycelium dry weight of *F. oxysporum* f. sp. *phaseoli* are presented in Figure 1. Significantly the highest radial growth was observed at 30C followed by room temperature, 25 and 20C. The lowest radial growth was observed at 15C. The optimum temperature for the radial colony growth was 30C. An increase or decrease from the optimum temperature, the radial growth of the pathogen was significantly reduced. The relationship between temperature regimes and radial colony diameter was polynomial. Similar trend was observed in case of mycelium dry weight. The highest mycelium dry weight was found at 30C followed by room temperature (25-28 C), 25 and 20C. However, the mycelium dry weight at those levels was statistically similar but significantly higher compared to 35 and 15C. Relationship between mycelium dry weight and temperature level was also polynomial. Findings of the present study suggest that temperature regime play an important role on the growth of the *F. oxysporum* f.sp. *phaseoli*.

### Effect of pH on mycelium growth

The pH of the culture medium showed appreciable influence on radial colony diameter and mycelium dry weight of *F. oxysporum* f. sp. *phaseoli*. The highest radial growth was observed at pH 6.5,

which was statistically similar to the growth at pH 5.0, 5.5, 6.0 and 7.0. The lowest radial hyphal growth was recorded at pH 4.0 followed by pH 8.5. The highest mycelium dry weight was found at pH 6.5, which was statistically similar to pH 5.5, 6.0, 6.5 and 7.0. The lowest mycelium dry weight was observed at pH 4.0, which was statistically similar to pH 8.5. The relationship between radial colony diameter and pH level, and mycelium dry weight and pH level was polynomial. The results of the present experiment indicate that *F. oxysporum* f. sp. *phaseoli* can grow at a wide range (4.0-8.5) of pH levels of culture medium (Fig. 2).

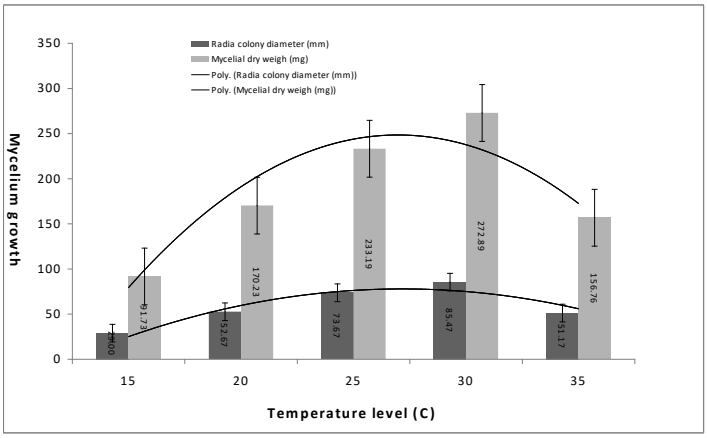


Fig 1. Effect of temperature on radial colony diameter and mycelial dry weight of *Fusarium oxysporum* f. sp. *phaseoli*.

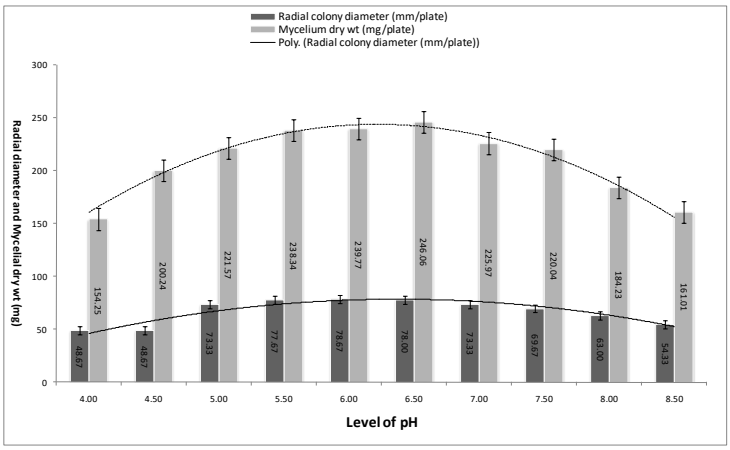


Fig 2. Effect of pH on the radial growth diameter and mycelial dry weight of *Fusarium oxysporum* f. sp. *phaseoli***.**

**Effect of nitrogen sources on mycelial growth**

The effect of five nitrogen sources on radial colony diameter and mycelium dry weight of *F. oxysporum* f. sp. *phaseoli* is shown in Figure 3. All the source of nitrogen increased radial growth significantly over control (Czapek's medium). The highest radial colony diameter of the pathogen was obtained from Czapek's basal medium supplemented with peptone as a nitrogen source. The radial growth obtained from the medium containing $NaNO_3$ and $NH_4NO_3$ was statistically similar but significantly higher compared to $(NH_4)_2SO_4$. The highest mycelial dry weight was observed in peptone medium followed by L-Asparagine. But their differences were not significantly different. Other nitrogen sources also gave higher mycelial dry weight compared to control.

**Effect of carbon sources**

Use of different carbon sources in the growth medium gave significant increase in colony growth of *F. oxysporum* f. sp. *phaseoli* compared to control. The highest radial growth was obtained with

Czapek's medium containing sucrose as a carbon source followed by glucose and dextrose. Among the carbon sources, glycerol appeared to be a poor source of carbon for *in vitro* the growth the pathogen. Similar growth trend was also observed incase of mycelium dry weight. Sucrose was appeared to be the best source of carbon for mycelium dry weight of *F. oxysporum* f. sp. *phaseoli* followed by glucose, dextrose, mannitol, glycerol and PDA (Fig. 4).

**Effect of C/N ratio on colony growth**

At 2 g sucrose, the highest radial colony diameter was found at C/N ratio 20, which was statistically similar to C/N ratio 10 but significantly higher compared to other level of C/N ratios. At C/N ratios 5, 10 and 20 the radial colony diameter was statistically similar but significantly higher compared to only two higher C/N ratios. At 5 g sucrose, the highest radial colony diameter was also observed at C/N ratio 20, which was statistically similar to C/N ratio of 10 but significantly higher compared to other level of C/N ratio. The second highest radial colony growth was recorded from C/N ratios 10 followed by C/N ratios 5, 40 and 80. The differences in colony diameter at those three levels of C/N ratios were not significant. At both 2 and 5 g sucrose, the lowest colony diameter was recorded from C/N ratio 100, which was statistically similar to C/N 80 (Fig. 5).

In presence of 2 g as well as 5 g sucrose, the mycelium dry weight of the fungus increased gradually with increased level of C/N ratios up to 20 and decrease thereafter. However, the mycelium dry weight at C/N ratios 5, 10 and 20 was statistically similar and significantly higher compared to all other of C/N ratios. The lowest dry weight was found at the highest C/N ratio followed by C/N ratio 80 (Fig. 6).

In general, colony diameter and mycelium dry weight was comparatively higher at 5 g sucrose. At both levels of sucrose, the colony diameter and mycelium dry weight increased gradually with the increase of C/N ratio up to 20 and decease thereafter. Relationship of two parameters with level of C/N ratio was polynomial (Fig. 5 and 6).

Results of the present study reveal that all growth factors tested have shown considerable influence on the radial colony diameter and mycelial dry weight of *F. oxysporum* f. sp. *phaseoli.* The pathogen can grow at a temperature range of 15-35 C, pH range 4.0-8.5 and C/N ratio 5-100. Influence of nitrogen and carbon sources on mycelial growth of the pathogen is also considerable. The fungus grows well in culture medium containing peptone, L-Asparagine, $NaNO_3$ and $NH_4NO_3$ as nitrogen sources, and manitol, dextrose and glucose as carbon sources. The best source of nitrogen is peptone and that of carbon is sucrose. More or less similar findings have been reported by many other researchers. Gaikwad and Pachpande (1992) and Osman *et al.* (1992) observed that the optimum temperature range for better growth of *F. oxysporum* is 20 to 35C with the highest growth at 30C. Other investigators also found optimum colony growth of *Fusarium* at a temperature rage of 24 -27C (Chen *et al*. 2003, Dwievedi and De 2003). Raghuwanshi (1995), Kumar *et al*. (2000) and Ragazzi (1992) found that various formae speciales of *F. oxysporum* grow well at a range of pH of 5.0-7.5.

The results on the effect of nitrogen sources on the growth of the pathogen recorded in the present study are in agreement with findings of other investigators (Rahman *et al*. 1993, Chen *et al*. 2003, Kumar *et al*. 2000, Ciotola *et al* 2003). The results on the effect of nitrogen sources on the growth of the pathogen recorded in the present study are in agreement with findings of other investigators (Rahman *et al*. 1993, Chen *et al*. 2003, Kumar *et al*. 2000, Ciotola *et al* 2003). Griffin (1970) demonstrated that carbon and nitrogen are the most vital nutrients for the growth of *F. oxysporum.* Kumar *et al*. (2000) and Desai *et al*. (1994) obtained maximum growth of *F. oxysporum* f. sp. *lentis* and *F. oxysporum* f. sp. *ciceri* with mannitol and maltose used as carbon sources in culture medium.

Based on findings of the present investigation it may be concluded that the optimum temperature and pH for mycelial growth of *F. oxysporum* f. sp. *phaseoli* is 30 C and pH 6.0-6.5, respectively. The optimum C/N ratio for its mycelial growth is 20. Peptone and sucrose are the best sources of nitrogen and carbon, respectively for mycelial growth of the fungus.

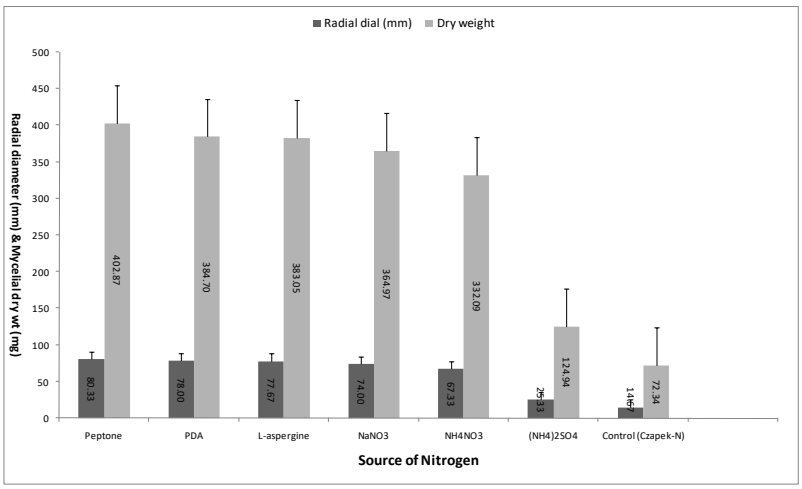


Fig 3. Effect of different nitrogen sources on colony diameter and mycelial dry weight of *Fusarium oxysporum* f. sp. *phaseoli*.

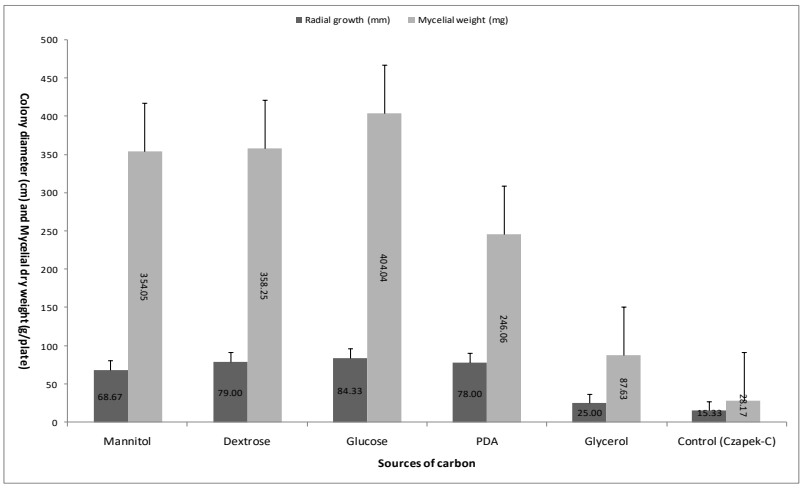


Fig 4. Effect of five carbon sources in culture medium on *in-vitro* growth of *Fusarium oxysporum* f. sp. *phaseoli*

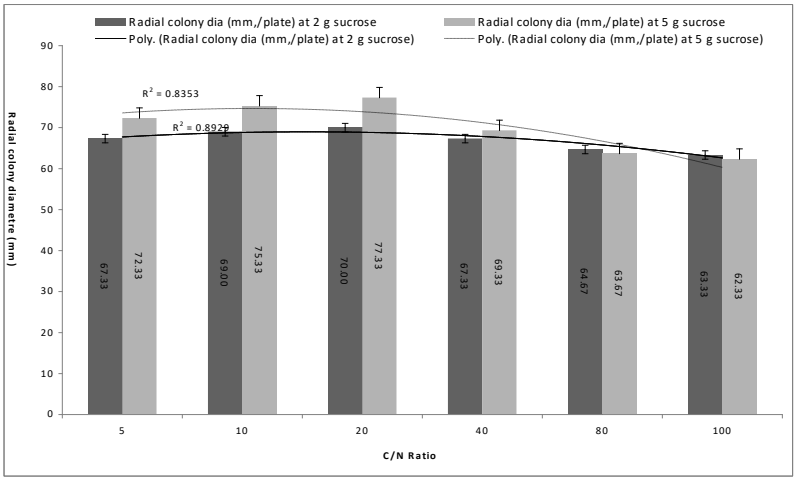


Fig. 5. Effect of C/N ratio on radial growth of *Fusarium oxysporum* f. sp. *phaseoli* at 2 and 5 g sucrose

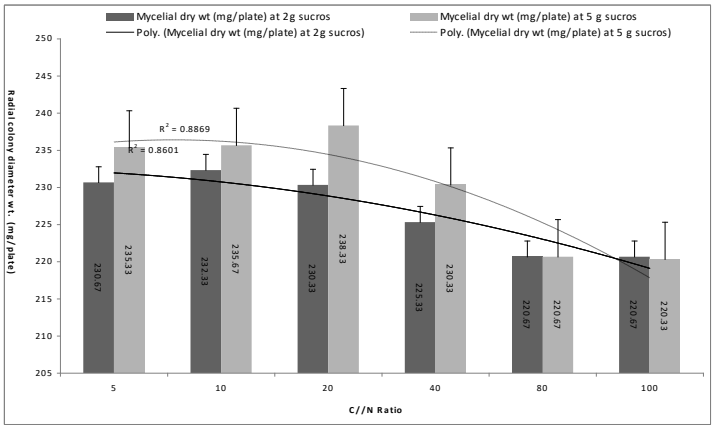


Fig 6. Effect of C/N ratio on mycelial dry weight *Fusarium oxysporum* f. sp. *phaseoli* in sol plate at 2 and 5 g sucrose

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# EFFICACY OF FUNGICIDES TO CONTROL WHITE RUST (*ALBUGO OCCIDENTALIS*) OF RED AMARANTH (*AMARANTHUS* SP.)

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ABSTRACT

M. M. R. Talukder, M. Riazuddin, M. M. Rahman, M. S. Uddin and M. S. I Khan. 2012. Efficacy of Fungicides to control white Rust (*Albugo occidentalis*) of red amaranth (*Amaranthus* Sp.). Bangladesh J. Plant Pathol. 28(1&2):15-17.

A field experiment was carried out to evaluate the efficacy of six fungicides to control white rust (*Albugo occidentalis*) of red amaranth (*Amaranthus* sp.). The fungicides were Sunvit @ 0.2%, Ridomil gold 68 WP (Chlorothalonil + Mefenoxam) @ 0.2%, Contaf 25 EC (Triazole) @ 0.1%, Orzim 50 WP (0.1%), Zoom 50 WP (0.1%), and X-tra care 300 EC (myclobutanil) % 0.05%. These were applied as foliar spray. The fungicides caused 8.18-70.28% reduction in severity in terms of percent disease index of white rust. On the contrary, the fungicides gave 22.31-110.50% increase in red amaranth fresh yield and 8.06-27.42% increase in 1000-seed weight. Among six fungicides tested, the most effective one to control white rust and to increase yield of red amaranth was Ridomil gold followed by Sunvit. Based on the findings of the present investigation Ridomil Gold 68 WP @ 0.2% foliar spray may be recommended to control white rust of red amaranth.

**Key words**: White rust, red Amaranthus, fungicides, Redomil gold, control

## INTRODUCTION

Red amaranth (*Amaranthus* sp.) is a widely grown leafy vegetable crop in Africa and Asia. It is an annual, fast growing plant and is easily cultivated in homestead gardens and fields. Amaranth is a rich source of calcium, iron, and vitamins A and C. Red amaranth is also one of the important leafy vegetables in Bangladesh. The crop is attacked by about a dozen of diseases in the country (Talukder 1974, Anon. 1984). Among them white rust caused by *Albugo occidentalis* is noted as the major one considering its high incidence, wide distribution and adverse effect on yield and quality of the crop. It causes defoliation and withering of whole plant and also reduces market value. The Oomycetous fungus, *A. occidentalis* is known to be present wherever red amaranth is grown (Nyvall 1989). When the infection is confined to leaves it may not result in any appreciable yield loss (Kolte 1985). However, the presence of conspicuous white pustules at the vegetative stage can be of serious concern to farmers. A number of chemicals have been suggested to control the disease in India (Verma and Petrie 1979), In Bangladesh, recommendation for effective control of the disease is not available.

The present experiment was conducted to test some available fungicides against white rust of red amaranth with a view to select an effective fungicide to control the disease.

## MATERIALS AND METHODS

Six fungicides namely Sunvit (0.2%), Ridomil gold 68 WP ((Chlorothalonil + Mefenoxam @ 0.2%), Contaf 25 EC (Triazole @ 0.01%), Orzim 50WP (0.01%), Zoom 50 WP (0.01%), X-Tra care 300 EC (0.05%) were tested against white rust of red amaranth under natural field condition. The experiment was conducted in the experimental farm of Regional Agricultural Research Station (RARS), Rahmatpur, Barisal during 2008-2009, 2009-2010, 2010-2011. The experimental plots were prepared following standard procedures and standard doses of manure and fertilizers were used. The experiment was laid out in a randomized complete block design with 3 replications, 2 m x 2 m unit plot and 1m distance between blocks and plots. Seeds of an amaranth variety BARI Lal shak-1 were sown in continuous lines maintaining 50 cm line to line distance. The fungicides were suspended in tap water and applied as foliar spray for 3 times starting from appearance of the disease with 15 days interval. Plants in control plots were sprayed with plain water. Fifteen days after last spray, the severity of white rust was indexed on a 0-5 rating scale (Mehta and Mondal

1978), where 0 = No infection, 1 = 10% leaf area infected, 2 = 11-30% leaf area infected, 3 = 31-50% leaf area infected, 4 = 51-70 % leaf area infected and 5 = 71% and above leaf area infected. The disease severity was expressed in percent disease index (PDI). The PDI was computed following a standard formula suggested by Krisna Prasad *et al.* (1979) as shown below:

$$PDI = \frac{\text{Sum of (Disease score} \times \text{Number of leafin that score)}}{\text{Total number of leaves checked} \times \text{the highest disease score}} \times 100$$

When amaranth plants were suitable for harvest as vegetable, an area of 1 $m^2$ were randomly selected from each unit plot and harvested. Fresh weight of harvested red amaranth was measured and the yield was expressed as $g/m^2$. After maturation, seeds were also harvested from an area of 1 $m^2$ per plot. The yield of amaranth vegetable and seeds were expressed in kg/ha. Collected data were analyzed following standard statistical procedure (Gomez and Gomez 1983). Treatment means were compared using DMRT (Zaman *et al.* 1982).

## RESULTS AND DISCUSSION

The highest PDI of 73.40, 77.74 and 82.40 was recorded from control plot respectively in first, second and third years. All treatments caused significant reduction in disease severity compared to control. Every year, significantly the lowest PDI was obtained with Ridomil gold followed by Sunvit. Efficacy of two fungicides to reduce the PDI was significantly different. The PDI under Contaf 25EC, Orzim 50WP, Zoom 50WP and X-Tra Care 300EC ranged 47.43-68.28 in first year, 46.03-67.91 in second year and 45.43-69.60 in third year. On an average the four fungicides gave 40.52, 30.34, 19.50 and 8.18% reduction in PDI over control. However, their efficacy was nor consistent in every year (Table 1).

Table 1. Effect of fungicidal spay on the severity of white rust (*Albugo occidentalis*) of red amaranth in three consecutive years

| Fungicide with dose of application | Percent Disease Index (PDI) | | | Average | % Reduction |
|---|---|---|---|---|---|
| | 2008-2009 | 2009-2010 | 2010-2011 | | |
| Sunvit (0.2%) | 43.60f | 40.93f | 38.60f | 41.04 | 47.23 |
| Contaf 25 EC (0.1%) | 53.93d | 46.03e | 45.43e | 46.30 | 40.52 |
| Orzim 50 WP (0.1%) | 47.43e | 51.83d | 56.93d | 54.23 | 30.34 |
| Zoom 50 WP (0.1%) | 59.60c | 58.80c | 78.27b | 62.67 | 19.50 |
| Ridomil gold 68 WP (0.2%) | 24.77g | 23.87g | 20.77g | 23.14 | 70.28 |
| X-Tra care 300 EC (0.05%) | 68.27b | 67.91b | 69.60c | 71.48 | 8.18 |
| Control | 73.40a | 77.74a | 82.40a | 77.85 | - |

Mean(s) within the same column with a common letter (s) do not differ significantly at 0.05 levels.

Average yield of red amaranth (vegetable) recorded from control plot was 195.66, 188.00 and 205.00 kg/ha during 2008-2009, 2009-2010 and 2010-2011, respectively. Due to foliar spray with the test fungicides, the yield was increased to 245.50-425.66, 233.50-410.45 and 241.00-403.00kg/ha, respectively. The increase in yield obtained with each of the fungicides was significant compared to control. The highest and the lowest yield were recorded from the treatments with Ridomil gold and X-Tra Care, respectively. The second highest yield was obtained with Sunvit, which was statistically similar to Contaf and Orzim. Efficacy Zoom to increase yield was not significantly different form X-Tra Care (Table 2).

Table 2. Effect of fungicidal spray against red rust (*Albugo occidentalis*) on yield of red amaranth in three consecutive years

| Fungicide with dose of application | Yield (kg/ha) | | | Average | % Reduction |
|---|---|---|---|---|---|
| | 2008-2009 | 2009-2010 | 2010-2011 | | |
| Sunvit (0.2%) | 405.0 b | 375.00 b | 385.00 b | 388.33 | 97.91 |
| Contaf 25 EC (0.1%) | 320.0 bc | 309.33 ab | 329.33 ab | 319.55 | 62.85 |
| Orzim 50 WP (0.1%) | 323.3 bc | 300.00 ab | 332.00 ab | 318.43 | 62.28 |
| Zoom 50 WP (0.1%) | 230.0 c | 270.84 bc | 270.84 bc | 257.23 | 31.09 |
| Ridomil gold 68 WP (0.2%) | 425.66 a | 410.45 a | 403.00 a | 413.04 | 110.50 |
| X-Tra care 300 EC (0.05%) | 245.50 c | 233.50 c | 241.00 c | 240.00 | 22.31 |
| Control | 195.66 d | 188.00 d | 205.0 d | 196.22 | - |

Mean(s) within the same column with a common letter(s) do not differ significantly at 0.05 level

In three consecutive years, seed size in terms of 1000-seed weight of red amaranth increased significantly over control due to foliar spray with different fungicides to control white rust disease of the crop. Significantly the highest 1000-seed weight was achieved with Ridomil gold. The second highest 1000-seed weight recorded from the treatment with Contaf, which was statistically similar to Orzim, Zoom and X-Tra care. The lowest yield increase was observed under the treatments with Sunvit, which was not significantly different from Orzim, Zoom and X-Tra care (Table 3).

Table 3. Effect of fungicidal spray against white rust (*Albugo occidentalis*) on 1000-seed weight of red amaranth in three consecutive years

| Fungicide with dose of application | 1000-seed weight (g) | | | % Reduction over control |
|---|---|---|---|---|
| | 2008-2009 | 2009-2010 | 2010-2011 | |
| Sunvit (0.2%) | 1.40 c | 1.30 c | 1.30 c | 7.26 |
| Contaf 25 EC (0.1%) | 1.47 b | 1.37 b | 1.37 b | 12.90 |
| Orzim 50 WP (0.1%) | 1.43 b c | 1.33 bc | 1.33 bc | 9.68 |
| Zoom 50 WP (0.1%) | 1.44 bc | 1.34 bc | 1.34 bc | 10.48 |
| Ridomil gold 68 WP (0.2%) | 1.58 a | 1.58 a | 1.58 a | 27.42 |
| X-Tra care 300 EC (0.05%) | 1.41 bc | 1.31 bc | 1.31 bc | 8.06 |
| Control | 1.28 d | 1.23 d | 1.23 d | - |

Mean(s) within the same column with a common letter (s) do not differ significantly at 0.05 level.

Results of the present study reveal that Ridomil gold and Sunvit gave an average of 70.28 and 47.23% decrease in white rust severity (PDI), 110.50 and 97.91% increase in red amaranth yield and 27.42 and 7.26% increase in 1000-seed weight. Other fungicides gave 8.18-40.52 decrease in PDI, 22.31-62.85 increase in red amaranth yield and 8.06-12.90% increase in 1000-seed weight. Among six fungicides tested, the most effective fungicide to control white rust and to increase yield of red amaranth was Ridomil gold followed by Sunvit. Other investigators also effectiveness of fungicides to control white rust of amaranth (Verma and Petrie 1979). Based on this results Ridomil Gold 68 WP @ 0.2% foliar spray may be recommended to control white rust of red amaranth.

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# APPLICATION OF ORGANIC AMENDMENT AND FURADAN 5G FOR THE MANAGEMENT OF ROOT-KNOT NEMATODE OF CUCUMBER

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ABSTRACT

R. Momotaz, R. Islam, M. E. Ali, and S. S. Siddique. 2012. Application of organic amendment and Furadan 5g for the management of root-knot nematode of cucumber. Bangladesh J. Plant Pathol. 28 (1&2):19-00.

Efficacy of seven different treatments with poultry refuse (PR), mustard oilcake (MOC), *Trichoderma harzianum* based compost (TZ) and Furadan 5G alone, and application of three organic materials combined with Furadan 5G were tested for the management of root-knot (*Meloidogyne incognita*) of cucumber. The experiments were conducted during three consecutive years. All treatments considerably reduced root-knot severity and increased plant growth as well as yield. The highest reduction in root-knot severity was obtained with Furadan+MOC (81.33%) followed by Furadan+PR (76.52%), Furadan 5G alone (54.60), PR alone (67.89) and MOC alone (63.08). The maximum shoot growth was obtained with Furadan+MOC (41.38%) followed by Furadan+PR (38.87%), Furadan 5G alone (27.54) and PR alone (21.32). The highest yield increase was obtained with Furadan+MOC followed by Furadan+PR, Furadan 5G alone, MOC alone, PR alone, Furadan 5G+TZ and TZ alone. The treatments gave 127.98, 103.40, 64.47, 43.06, 33.09, 27.00 and 22.62% yield increase over control, respectively. Considering control of root-knot and increase in plant growth and yield, the treatments, Furadan 5G+ mustard oilcake, Furadan 5G+ poultry refuse and Furadan alone may be recommended for the control of the disease.

**Key words :** Integrated management, root-knot nematode, cucumber.

## INTRODUCTION

In Bangladesh, cucumber (*Cucumber sativa*) is an important vegetable and salad crop. It is grown round the year. The crop is attacked by different diseases caused by viruses, bacteria, fungi and nematodes. Among the nemic diseases, root-knot caused by *Meloidogyne incognita* is widely distributed throughout the country and causes severe damage to the crop. Different researchers demonstrated that nematicides, organic amendments and plant extracts are effective to control root-knot disease (Hossain *et al*. 1989, 2003, Ahmed and Karim 1991, Faruk *et al*. 2003). Efficacy of organic amendments to soil against the nematode has been demonstrated by many investigators (Muller and Gooch 1982, Chiado and Khan 1990, Wahundeniya 1991). Furadan 5G (Carbofuran) is a common insecticide with nematicidal properties. The pesticide is effective to control root-knot nematodes and to increase plant growth as well as crop yield (Hossain *et al.* 1989, 2003, Khan 1996). Application of nematicidal chemicals in combination with organic amendments to soil enhances their effectiveness to reduce the nematode populations in soil and to improve soil physical properties and plant growth (Hussain and Khan 1988). Information regarding combined effect of nematicide and organic amendments to soil on the severity of root-knot nematode in cucumber is not available in the country. Considering the above facts, the present experiment was conducted to evaluate the efficacy of three organic amendments and a nematicide applied in different combinations in an integrated management approaches against root-knot nematode of cucumber.

## MATERIALS AND METHODS

The nematicide was Furadan 5 g and the organic amendments were Poultry refuse (PR), Mustard oilcake (MOC) and *Trichoderma harzianum* (TZ). The materials were applied @ 45 kg, and 10 ton, 800 kg, 10 ton per hectare, respectively in 7 different combinations representing 7 treatments. The treatments were Poultry refuse alone , Mustard oilcake alone Furadan 5G alone, *Trichoderma harzianum* alone, Furadan 5G + PR, Furadan 5G + MOC, and Furadan 5G + TZ. An additional treatment was maintained where no materials were applied, which served as control. In case of combined application, PR, MOC, Furadan 5G and TZ based compost were applied @ 5 ton/ha, 400 kg/ha, 22.5 kg/ha and 7 g/pits, respectively. The PR and MOC were incorporated with soil at 21 and 15 days, before transplanting of cucumber seedlings. Whereas Furadan 5 G and *T. harzianum* were added to the soil at the time of transplanting.

The experiment was conducted under field conditions during three consecutive years (2008-

2010) in the research farm of BARI, Joydebpur, Gazipur. Unit plot size was 3 m x 2.5 m. Four pits were dig out in each plot and 21 days old healthy cucumber seedlings of a local variety were transplanted in the pit at two seedlings per pit. There were 8 seedlings per plots. The experiment was laid out in a randomized complete block design with three replications. After establishment of seedlings, severely galled roots of cucumber were collected from a sick bed of nematode and were chopped into small pieces. Two grams of chopped roots was incorporated with soils near the base of transplanted seedling. Irrigation and intercultural operation were done as and when necessary.

The plants were allowed to grow for 20 days. At the end of the growing period, 50% plants were uprooted from the field carefully to minimize the damage of roots. The root systems were washed under running tap water and data on length and weight of shoot and root were recorded. The number of galls per gram of roots and yield contributing characters were also recorded. The degree of root galling was indexed on a 0-10 scale (Zeck 1971), where 0 represented roots free from gall and 10 represents severely galled root system. The data were analyzed statistically for ANOVA and means were compared following Duncan's Multiple Range Test.

## RESULTS AND DISCUSSION

### Gall index and Gall number

Gall index values were 6.46, 7.65 and 7.10 under control in 2007-08, 2008-09 and 2009-10, respectively. Application of different treatments reduced the parameter by 1.46-3.36, 1.21-3.45, 1.31-3.42% over control. The reduction was significant under all treatments. Every year, the maximum reduction was achieved with Furadan 5G+MOC, which was statistically similar to Furadan 5G+PR, MOC alone and PR alone. The least effective treatment was TZ based compost alone which was not significantly different from Furadan 5G+TZ, Furadan 5G alone, MOC alone and PR alone. Maximum of 88.76, 91.14 and 91.00 galls per gram roots were recorded from control in 2007-08, 2008-09 and 2009-10, respectively. Every year, all treatments gave significant reduction in that parameter. Treatments with PR alone, MOC alone, Furadan 5G alone, TZ alone, Furadan 5G+PR, Furadan 5G+MOC and Furadan 5G+TZ caused respectively 62.95, 61.28, 50.35, 39.44, 66.61, 67.51 and 47.88% reduction in gall number over control (Table 1).

### Shoot growth

Under different treatments, average shoot length of cucumber plants ranged 590.0-800.0, 520.0-750.0 and 560.0-810.0 cm per plant during 2007-08, 2008-09 and 2009-10, respectively. Every year, the lowest shoot length was recorded from plants grown in control plots, which was statistically similar to Furadan+TZ, TZ alone and MOC alone. Other treatments gave significant increase in shoot length over control. The highest shoot length was achieved with Furadan+MOC, which was statistically similar to Furadan+PR and Furadan alone (Table 1).

On 20th day of planting, average shoot weight of cucumber plants under control were 650.0, 753.3 and 615.0 g/plant during 2007-08, 2008-09 and 2009-10, respectively. Every year, the shoot weight increased over control due to different treatments. However, the increase was significant under the treatments with Furadan 5G+MOC, Furadan 5G+PR and PR alone in the first year and under all treatments in second and third years. In 2007-08 and 2008-09, the heaviest shoot was obtained with Furadan 5G+MOC, which was statistically similar to Furadan 5G+PR and PR alone. In third year, maximum shoot weight was also obtained with Furadan 5G+MOC followed by Furadan 5G+PR. Their difference was not significant (Table 2).

### Root growth

Every year, all of the treatments caused considerable increase in root length. In 2007-2008 and 2008-09, the root length recorded from the treatments with PR as well as MOC alone and their mixed application with Furadan 5G was statistically similar but significantly higher compared to control. In 2009-2010, Furadan 5G+PR and Furadan 5G+MOC gave significantly increase in the parameter (Table 3).

Every year, the lowest root weight was recorded from control, which was statistically similar to the treatments with Furadan+TZ, TZ alone and Furadan 5G alone. Root weight obtained with Furadan+PR and Furadan+MOC was statistically similar but significantly higher compared control and other treatments. During 2009-10, efficacy of PR and MOC alone to increase root weight was statistically similar but significantly higher compared to control and other treatments which received TZ (Table 3).

### Fruit yield

Number of fruit per plot ranged 26.00-43.33, 22.10-39.65 and 21.00-43.35 in first, second and third years, respectively. Every year the lowest number of fruits was recorded from control plot, which was statistically similar to the treatments with MOC and TZ alone. The highest fruit number per plot was obtained with Furadan 5G+MOC followed by Furadan 5G+PR. Efficacy of two treatments to increase fruit setting was statistically similar but significantly higher compared to control. Significant increase over control in fruit setting was also obtained with PR alone in first and second year and

with Furadan 5G alone and Furadan 5G + TZ in second year (Table 4).

Table 1. ffect of soil amendment with Furadan 5G, Mustard oilcake, Poultry refuse and *Trichoderma harzianum* in seven different combinations on gall formation in roots of cucumber infected with root-knot (*Meloidogyne incognita*)

| Organic amendment | Gall index (0-10)scale | | | Gall number/ gram root | | | Gall number decrease over control (%) |
|---|---|---|---|---|---|---|---|
| | 2007-08 | 2008-09 | 2009-10 | 2007-08 | 2008-09 | 2009-10 | |
| Poultry refuse (PR) alone | 2.36bc | 2.15bc | 2.29bc | 30.25d | 37.61d | 32.5cd | 62.95 |
| Mustard oilcake (MOC) alone | 2.43bc | 3.12b | 2.30bc | 32.25d | 32.54d | 40.1c | 61.28 |
| Furadan 5G alone | 3.20b | 3.24b | 3.21b | 42.5cd | 47.5cd | 44.5bc | 50.35 |
| *Trichoderma harzianum* (TZ) alone | 3.36b | 3.45b | 3.42b | 57.00bc | 55.64bc | 51.4b | 39.44 |
| Furadan 5G + PR | 1.96c | 1.35c | 1.67c | 29.25d | 31.00d | 30.21d | 66.61 |
| Furadan 5G + MOC | 1.46c | 1.21c | 1.31c | 28.56d | 30.21d | 29.22d | 67.51 |
| Furadan 5G +TZ | 3.33b | 3.01 | 3.21b | 42.5c | 48.6c | 50.10b | 47.88 |
| Control | 6.46a | 7.65a | 7.10a | 88.76a | 91.14a | 91.00a | - |

Means within the same column with a common letter(s) do not differ significantly (P=0.05).

Table 2. Effect of soil amendment with Furadan 5G, Mustard oilcake, Poultry refuse and *Trichoderma harzianum* in seven different combinations on shoot growth of cucumber infected with root-knot (*Meloidogyne incognita*)

| Organic amendments | Shoot length (cm/plant) | | | Shoot weight (g/plant) | | |
|---|---|---|---|---|---|---|
| | 2007-08 | 2008-09 | 2009-10 | 2007-08 | 2008-09 | 2009-10 |
| Poultry refuse (PR) alone | 760.0a | 606.0 b | 660.0 b | 1207.0 ab | 1104.0 a | 750.0 cd |
| Mustard oilcake (MOC) alone | 616.7c | 589.0 c | 590.0 bc | 916.7 c | 750.0 c | 810.0 c |
| Furadan 5G alone | 733.3ab | 699.0 ab | 698.0 ab | 850.0 c | 825.0 c | 825.0 c |
| *Trichoderma harzianum* (TZ) alone | 586.7c | 562.3c | 610.0 bc | 736.7 c | 730.0 c | 1101.0 bc |
| Furadan 5G + PR | 786.7a | 740.5a | 792.0 a | 1370.0 a | 1320.0 a | 1410.0 a |
| Furadan 5G + MOC | 800.0a | 750.0 a | 810.0 a | 1483.0 a | 1358.0 a | 1440.0 a |
| Furadan 5G +TZ | 643.3bc | 556.0 c | 609.0 bc | 970.0 bc | 865.0 bc | 1049.0 bc |
| Control | 590.0 c | 520.0 c | 560.0 c | 753.3 c | 615.0 d | 640.0 d |

Means within the same column with a common letter(s) do not differ significantly (P=0.05).

The fruit yield of 4.35, 3.65 and 4.35 tons per hectare was found in control plots during 2007-08, 2008-09 and 2009-10, respectively. The highest fruit yield of 9.55, 8.61 and 9.52 t/ha was obtained with Furadan 5G+MOC in those three consecutive years, respectively. However, the yield obtained with Furadan 5G+PR and Furadan+MOC was statistically similar but significantly higher compared to control. The highest average yield increase over control was obtained with Furadan+MOC followed by Furadan+PR, Furadan 5G alone, MOC alone, PR alone, Furadan 5G+TZ and TZ alone. The treatments gave 127.98, 103.40, 64.47, 43.06, 33.09, 27.00 and 22.62% yield increase over control, respectively (Table 4).

Table 3. Effect soil amendment with Furadan 5G, Mustard oilcake, Poultry refuse and *Trichoderma harzianum* in seven different combinations on root growth of cucumber infected with root-knot (*Meloidogyne incognita*)

| Organic amendments | Root length (cm/plant) | | | Root weight (g/plant) | | |
|---|---|---|---|---|---|---|
| | 2007-08 | 2008-09 | 2009-10 | 2007-08 | 2008-09 | 2009-10 |
| Poultry refuse (PR) alone | 162.0ab | 152.0ab | 172.0ab | 49.7c | 42.7c | 52.0b |
| Mustard oilcake (MOC) alone | 198.7a | 188.0a | 192.0ab | 57.5bc | 52.4b | 51.0b |
| Furadan 5G alone | 209.0a | 201.0a | 191.0ab | 49.6c | 49.0 bc | 49.0bc |
| *Trichoderma harzianum* (TZ) alone | 174.7ab | 168.0ab | 169.0ab | 46.9c | 42.5c | 45.5c |
| Furadan 5G + PR | 222.3a | 210.0a | 256.0a | 62.4a | 59.9a | 68.2a |
| Furadan 5G + MOC | 203.3a | 209.0a | 252.0a | 61.5a | 60.0a | 70.2a |
| Furadan 5G +TZ | 193.0ab | 184.0ab | 189.0ab | 50.5bc | 46.2c | 42.0c |
| Control | 134.0b | 115.0b | 150.0b | 40.9c | 35.21c | 40.51c |

Means within the same column with a common letter(s) do not differ significantly (P=0.05)

Table 4. Effect of soil amendment with Furadan 5G, Mustard oilcake, Poultry refuse and *Trichoderma harzianum* in seven different combinations on fruit setting and fruit yield of cucumber infected with root-knot (*Meloidogyne incognita*)

| Organic amendment | Number of fruit /plot | | | Fruit Yield (t/ha) | | | Yield increase over control (%) |
|---|---|---|---|---|---|---|---|
| | 2007-08 | 2008-09 | 2009-10 | 2007-08 | 2008-09 | 2009-10 | |
| Poultry refuse (PR) alone | 36.00bc | 31.25ab | 31.2bc | 5.5ab | 5.5cd | 5.5cd | 33.09 |
| Mustard oilcake (MOC) alone | 31.67bcde | 26.25bc | 29.9c | 5.4ab | 5.7cd | 6.6d | 43.06 |
| Furadan 5G alone | 33.00bcd | 29.31b | 30.1bc | 6.9ab | 6.5bc | 6.8b | 64.47 |
| *Trichoderma harzianum* (TZ) alone | 28.67e | 25.31bc | 26.7bc | 4.91b | 5.2cd | 5.0bc | 22.62 |
| Furadan 5G + PR | 37.33ab | 34.54a | 37.3ab | 8.7a | 7.6ab | 8.9a | 103.40 |
| Furadan 5G + MOC | 43.33a | 39.65a | 43.4a | 9.6a | 8.6a | 9.5a | 127.98 |
| Furadan 5G + TZ | 30.00cde | 31.21ab | 30.2bc | 5.6ab | 4.2de | 5.9c | 27.00 |
| Control | 26.00 de | 22.10 c | 21.0c | 4.4b | 3.7de | 4.4d | - |

Means within the same column with a common letter(s) do not differ significantly (P=0.05)

Results of the present study reveal that poultry refuse, mustard oilcake, *T. harzianum* based compost and Furadan 5G alone, and application of the organic materials mixed with Furadan 5G are effective to control root-knot disease and to increase plant growth and fruit yield of cucumber. The maximum decrease in root-knot severity and increase in plant growth were achieved with Furadan 5G+MOC followed by Furadan 5G+PR and Furadan 5G alone. The results indicate that combined application of PR and MOC with Furadan showed better performance than other treatments. Similar observations have been documented by many other researchers (Mian and Rodriguez-kabana 1982, Muller and Gooch 1982, Marull *et al* .1997, Faruk *et al.*2001, Hossain *et al*. 2003, Bari *et al.* 2004). Effectiveness of Furadan 5G and organic amendments to control root-knot has also been reported by Khan (1996), Hossain *et al.* (1989, 2003), Faruk *et al*. (2003), Bari *et al.* (2004) and Hossain *et al*. (2003, 2007).

Based on findings of the present investigation it may be concluded that pre plant soil treatment with mustard oilcake and poultry refuse may be recommended to control root-knot of cucumber. However, their efficacy can be improved considerably if two organic amendments are applied mixed with Furadan 5G.

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# EFFICACY OF FUNGICIDES FOR CONTROLLING EARLY BLIGHT OF TOMATO

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ABSTRACT

R. Islam, Kawser-E- Jahan and S. Ali. 2012. Efficacy of fungicides for controlling early blight of tomato. Bangladesh J. Plant Pathol. Vol. 28 (1&2): 25-27.

Eight fungicides namely Pipertax 50WP (Copper Oxychloride), Mancothane 80WP (Mancozeb), Meena 80WP (Mancozeb), Sazid 70WP (Mancozeb + Metalaxyl 7%), Media 80WP (Mancozeb), Win 77WP (Copper Hydroxide), Bicozeb 80WP (Mancozeb) and Rovral 50WP (Iprodion) were tested for their effectiveness against early blight (*Alternaria solani*) of tomato (*Lycopersicon esculentum*). The fungicides were applied as foliar spray at the rate 0.2% suspension in water for 4 times at 12 days interval starting from first appearance of symptoms. All the fungicides significantly reduced disease severity and increased fruit yield and yield components compared to control. Among the fungicides tested, the highest disease reduction with maximum fruit yield and yield components were obtained with Bicozeb, which was similar to Rovral as a standard check. Reduction of disease severity (PDI) and increase of fruit yield in different farms by Bicozeb ranged 58-70% and 27-49% respectively. Rovral also gave 58-70% disease reduction and 27-57% yield increase. Other fungicides reduced disease severity by 44-69% and increase in fruit yield by 4-44% over control.

**Keywords**: Fungicide, early blight, tomato, control.

## INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is a widely cultivated popular vegetable crop in Bangladesh. It is cultivated in about 18,800 hectares producing about 143,000 metric tons (Anon. 2008). The average yield of tomato is 7.6 t/ha, which is far below the average yields in other countries. Diseases are one of the major constraints for sustainable yield of crops causing about 30-40% losses annually (Rahman *et al.* 2001). A number of diseases attack tomato in Bangladesh. Of those, early blight caused by *Alternaria solani* is the most prevalent throughout the tomato growing areas of the country (Meah and Khan 1987). Crop disease control with chemicals is very popular because of its quick action, broad spectrum activity and easy availability to the growers. Different fungicides have been successfully used in controlling *Alternaria* blights of mustard, cabbage and cauliflower (Hossain and Mian 2004, Kudrati Khuda *et al.* 2003, Kohinoor *et al.* 2003, Rahman 2000). However, limited information is available about the fungicidal control of tomato early blight in Bangladesh (Meah 1994). An attempt was, therefore, made to evaluate the effectiveness of eight newly imported fungicides against early blight of tomato.

## MATERIALS AND METHODS

The fungicides tested in the present investigation were Pipertax 50WP (Copper Oxychloride), Mancothane 80WP (Mancozeb), Meena 80WP (Mancozeb), Sazid 70WP (Mancozeb + Metalaxyl 7%), Media 80WP (Mancozeb), Win 77WP (Copper Hydroxide), Bicozeb 80WP (Mancozeb) and Rovral 50WP (Iprodion). There were altogether nine treatments including a control (plain water). Rovral 50WP was included as a standard. The fungicides were applied as foliar spray at 0.2% suspension in water. Spraying was started immediately after the onset of disease and a total of four sprays were applied at an interval of 12 days.

The experiment was conducted at the Central Research Farms of Bangladesh Agricultural Research Institute (BARI), Gazipur and Regional Agricultural Research Station (RARS), BARI, Rangpur during the winter season of 2006-2007. Cultivation procedures of tomato and doses of manure and fertilizers were the same as recommended by BARI (Razzaque *et al.* 2000). The unit plot size was 2.0 m x 1.2 m and line-to-line and plant-to-plant distances were 60 cm and 40 cm, respectively. Distance between blocks and between plots was 1.0 m. The experiment was laid out in a randomized complete block design with 3 replications. Fifty-centimeter wide and 30-cm deep drain was made surrounding each block to facilitate irrigation and drainage. Four weeks old seedlings of variety BARI Tomato-2 (Ratan) were transplanted in the field on 9 November in Rangpur and 20 November in Gazipur. Ten seedlings were transplanted in each plot in two rows. Intercultural operations such as weeding, irrigation and mulching were done as and when necessary. Insect infestation

was controlled by spraying Malathion 57 EC @ 0.15%.

Blight severity was recorded on a 0-5 scale from five plants selected randomly in each plot after 7 days of last spray and percent disease index (PDI) was calculated according to Meah (1994). Mature fruits were picked once or twice in a week and fruit number and fruit weight0 per plant were recorded. Fruit yield was expressed in ton per hectare. Plant height (cm) was recorded at the end of the crop period. All data were analyzed for ANOVA and means of the treatments were compared using MSTAT-C computer program.

## RESULTS AND DISCUSSION

### Disease severity

Under control plots, the maximum percent disease index (PDI) of 79.07 was observed in Gazipur, and 45.34 in Rangpur. Foliar spray with all of the fungicides resulted in significant reduction in disease severity over control. The reduction ranged 65-70% in Gazipur and 44-58% in Rangpur. The highest reduction in PDI was achieved with Rovral and Bicozeb. However, effectiveness of all fungicides to reduce disease severity was statistically similar (Table 1).

### Plant height

In Gazipur, plant height ranged 31-43 cm under different treatments including control. The plant height was minimal under control and maximum under Bicozeb followed by Rovral. The parameter under these two fungicides was statistically similar but significantly higher compared to control and other treatments. In Rangpur, plant height ranged 24-36 cm but none of the fungicides could produce any significant effect on this parameter compared to control (Table 1).

Table 1. Efficacy of foliar spray with fungicides to control early blight (*Alternaria solani*) and increase plant height of tomato under field condition

| Fungicides (0.2%) | Percent disease index( PDI) | | % Reduction in PDI over control | | Plant height (cm) | |
|---|---|---|---|---|---|---|
| | Gazipur | Rangpur | Gazipur | Rangpur | Gazipur | Rangpur |
| Pipertax | 27.47 B | 23.03 B | 65.00 | 49.00 | 34.00 CD | 25.00 AB |
| Mancothane | 27.73 B | 25.55 B | 65.00 | 44.00 | 33.00 CD | 24.00 AB |
| Meena | 24.53 B | 20.25 B | 69.00 | 55.00 | 32.00 CD | 26.00 AB |
| Sazid | 24.80 B | 21.00 B | 69.00 | 54.00 | 34.00 CD | 28.00 AB |
| Media | 24.53 B | 19.75 B | 69.00 | 56.00 | 37.00 BC | 34.00 A |
| Win | 24.27 B | 19.60 B | 69.00 | 57.00 | 34.00 CD | 26.00 AB |
| Bicozeb | 23.73 B | 19.10 B | 70.00 | 58.00 | 43.00 A | 34.00 A |
| Rovral | 23.47 B | 19.00 B | 70.00 | 58.00 | 42.00 AB | 36.00 A |
| Control | 79.07 A | 45.34 A | - | - | 31.00 D | 27.00 AB |

### Fruit number

Under control, average fruit number per plant was 12.67 in Gazipur and 15.00 in Rangpur. Number of fruits per plant under different fungicide treatments ranged 14.34-34.05 in Gazipur and 15.05-35.95 in Rangpur. The fruit number was significantly higher under Rovral and Bicozeb compared to control in both the locations (Table 2).

### Fruit yield

In Gazipur, fruit yield under control was 65.83 t/ha, while those under different fungicide treatments ranged 71.25-83.75 t/ha. The yield was increased by 8-27% over control due to application of fungicides against early blight. The highest yield increase was obtained with Rovral and Bicozeb, which was followed by Pipertax and Media.

In Rangpur, the lowest yield of 50.03 t/ha was recorded from plots under control. Spraying with different fungicides increased the fruit yield to 52.10-78.75 t/ha. The increase in yield ranged 4 to 57% under different fungicides. The maximum yield increase was achieved with Rovral, which was followed by Bicozeb, Media and Pipertax (Table 2).

Results of the present study reveal that all the fungicides tested were effective to control early blight and improve plant growth and fruit yield of tomato. Among the fungicides, Rovral and Bicozeb provided better disease control and higher fruit yields compared to other fungicides.

Table 2. Effect of foliar spray with fungicides against early blight (*Alternaria solani*) on fruit number and fruit yield of tomato

| Fungicides (0.2%) | Fruit number/plant | | Fruit yield (t/ha) | | % Yield increase over control | |
|---|---|---|---|---|---|---|
| | Gazipur | Rangpur | Gazipur | Rangpur | Gazipur | Rangpur |
| Pipertax | 20.47 B | 22.67 BC | 77.92 | 68.53 | 18.00 | 36.00 |
| Mancothane | 14.34 BC | 15.05 C | 72.08 | 56.13 | 9.00 | 12.00 |
| Meena | 15.69 BC | 16.45 BC | 71.25 | 52.10 | 8.00 | 4.00 |
| Sazid | 16.95 BC | 20.38 BC | 74.17 | 61.30 | 13.00 | 22.00 |
| Media | 18.45 BC | 20.95 BC | 77.50 | 72.12 | 18.00 | 44.00 |
| Win | 15.33 BC | 18.38 BC | 71.25 | 52.10 | 8.00 | 4.00 |
| Bicozeb | 29.75 A | 30.48 A | 83.75 | 74.95 | 27.00 | 49.00 |
| Rovral | 34.05 A | 35.95 A | 83.61 | 78.75 | 27.00 | 57.00 |
| Control | 12.67 C | 15.00 C | 65.83 | 50.03 | - | - |

Many investigators reported effectiveness of foliar spray with fungicides to control early blight of tomato (Nene and Thapliyal 1979), and *Alternaria* blights of mustard (Rahman 2000), cabbage (Hossain and Mian 2004), cauliflower (Kohinoor *et al*. 2003, Kudrati Khoda *et al*. 2003) and sunflower (Meah 1994). Considering the efficacy of different fungicides evaluated in the present study, Bicozeb may be suggested as an alternative to Rovral in controlling early blight of tomato.

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# EFFICACY OF TWO FUNGICIDES AND TWO BOTANICALS TO CONTROL FOOT AND ROOT ROT DISEASE *(SCLEROTIUM ROLFSII)* OF COWPEA

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## ABSTRACT

M. Z. Rahman, A.H. M. Mafuzul Haque, M. A. Zaman, M. F. Amin, and A. K. Das. 2012. Efficacy of two fungicides and two botanicals to control foot and root rot disease *(Sclerotium rolfsii)* of cowpea. Bangladesh J. Plant Pathol. 28 (1&2): 29-32.

The effect of two fungicides Provax-200 (Carboxin + ThiraC) @ 0.25%, and Bavistin 50 WP (Carbedazim) @ 0.25 %, two botanicals, Neem leaf extract (1:1), and Garlic clove extract (1:1) along with Clean seed and Control (Untreated seed) were tested under field conditions to control foot and root rot disease (*Sclerotium rolfsii*) of cowpea. Among the treatments Provax-200 was the most effective followed by Bavistin 50 WP, Neem leaf extract and Garlic extract with respect to disease reduction and increase of seed yield. Maximum germination (85.53%), disease reduction (40.67%) and seed yield (1353 kg/ha) were obtained when seed was treated with Provax-200 @ 0.25 % followed by Bavistin 50 WP @ 0.25 %.

**Keywords**: Provax-200, Bavistin 50WP, Neem leaf extract, Garlic extract, Foot and root rot, control, cowpea.

## INTRODUCTION

Cowpea (*Vigna unguiculata*) is one of the important crops of coastal and hilly areas of Bangladesh where it is grown as pulse, fodder and green manure. People of hilly areas under Chittagong division use it as a staple pulse. The crop suffers from many diseases but foot and root rot caused by *Sclerotium rolfsii* is a major one (Talukder 1974, Ahmad and Hossain 1985). The disease is also known as wilt and stem rot (Dutta 1975). The fungus can attack the crop from seedling to flowering stage and are comparatively more destructive at seedling stage. The disease substantially reduces the crop yield annually (De Waard 1979). As the organism is soil - borne it is difficult and expensive to manage it. Good number fungicides inhibit the germination of sclerotia or mycelial growth of the fungus and effectively control the disease of various crops in the field (Tiwari 1987). Resistant variety of the crop is not yet available. Many researchers tried to control the pathogen by chemical and botanicals means on many other crops (Prasad *et al.* 1977, Arun *et al.* 1995, Sinha and Sinha 2004, Rana 2006, Fatema 2007). Seed treatment with fungicides or botanicals may be effective to control the disease.

The present experiment was conducted to evaluate the efficacy of seed treatment with two fungicides and two botanicals for control of foot and root rot disease caused by *S. rolfsii* of cowpea.

## MATERIALS AND METHODS

Two fungicides namely Provax-200 (Carboxin + Thiram) @ 0.25%, and Bavistin 50 WP (Carbedazim) @ 0.25%, and two botanicals namely Neem leaf extract (1:1), and Garlic clove extract (1:1) were tested in the experiment in six different treatments. The treatments (including control) were Provax-200, Bavistin -50 WP, Neem (*Azadirachta indica*) leaf extract (1:1), Garlic (*Allium sativum*) clove extract (1:1), seed cleaning and control (untreated seed).

The experiment was conducted at the Regional Agricultural Research Station, BARI, Hathazari, Chittagong during 2009-10 and 2010-11cowpea growing seasons. Cowpea variety BARI Felon-1 was used in the experiment. Size of unit plot was 4.0 m x 4.0 m and plant to plant spacing was 50 cm x 10 cm. The experiment was conducted under natural infection condition. Recommended doses of fertilizers and manure were used. Irrigation and weeding were done as and when necessary. Seeds of a cowpea variety (BARI Felon-1) were treated with the fungicides separately @ 2.5 g/kg (0.25%) before sowing. Required amount of seeds and individual fungicides were taken in a conical flask, few drops of sterilized water were added and shaken well with hands for 10 minutes. To prepare the standard plant extracts solution (100%), fresh leaves of Neem leaf and garlic cloves were collected, washed and crushed with sterilized distilled water at rate of one gram tissue in one milliliter of water (1:1w/v) separately as described by Shindhan *et al.* (1999). Seeds were uniformly coated with the plant extracts. Required amount of seeds and individual plant extract were taken in a conical flask and thoroughly mixed by shaking with hands for 10 min. In other two treatments clean seeds and seeds washed with

sterilized distilled water (control) were used. Treated seeds were air dried for 30 min and sown in the field.

The experiment was carried out in randomized complete block design with three replications. Data on germination, pre-emergence mortality and post-emergence mortality, yield and yield contributing character of the plant were recorded. The data were analyzed statistically and means per treatment were compared using least significant difference test (P=0.05).

## RESULTS AND DISCUSSION

During 2009-2010, pre and post emergence seedling mortality of cowpea ranged 5.65-17.11 and 5.75-17.07%, respectively. During 2009-2010, the ranges of two parameters were 5.65-17.11 and 5.75-17.07%, respectively. The lowest seedling mortality was observed in seeds treated with Provax-200 and the highest under control. All treatments with two fungicides, two botanicals and clean seeds significantly reduced seedling mortality compared to control during both the years. The highest reduction in pre and post emergence mortality was achieved with two fungicides followed by botanical and clean seed in both years. The reduction in disease severity was 8.46-37.83% during 2009-2010 and 11.00-40.67% during 2010-2011 (Table 1).

Germination and seed yield ranged 61.44-84.68% and 740-1327 kg/ha, respectively during 2009-2010, and 60.80-85.53% and 753-1353 kg/ha, respectively during 2010-2011. The highest germination and seed yield were observed when seeds were treated with Provax-200 and the lowest under control. All treatments with fungicides, botanicals and clean seed gave significant increase in germination as well as grain yield compared to control during both the years. Every year, the maximum increase in both the parameters was achieved with two fungicides followed by two botanicals and clean seed. Efficacy of Provax-200 was better than Bavistin 50 WP. The increase of germination was 24.79-59.56% and that of yield was 40.79-74.61% over control during 2009-201. The increase in both the parameters was slightly higher during 2010-2011 than 2009-2010 (Table 1).

Table 1. Effect of pre-sowing seed treatments with two fungicides and two botanicals on germination, seedling mortality and seed yield of cowpea during two consecutive years (2009-10 and 2010-2011)

| Treatments | Germination (%) | Seedling mortality % | | Yield (kg/ha) | Germination increase (%) | Disease reduction (%) | Yield increase (%) |
|---|---|---|---|---|---|---|---|
| | | Pre-emergence | Post-emergence | | | | |
| **Year 2009-2010** | | | | | | | |
| Provax-200 | 84.68 a | 5.65 e | 9.13 d | 1327 a | 59.56 | 37.83 | 74.61 |
| Bavistin 50 WP | 81.79 a | 7.66 d | 9.41 d | 1300 a | 53.32 | 33.12 | 71.05 |
| Neem leaf extract | 75.35 b | 9.22 c | 12.95 c | 1173 b | 39.34 | 22.64 | 54.34 |
| Garlic extract | 73.17 b | 9.29 c | 13.54 c | 1140 c | 37.54 | 19.03 | 50.00 |
| Clean seed | 66.64 c | 11.45 b | 16.04 b | 1070 c | 24.79 | 8.46 | 40.79 |
| Control | 61.44 d | 17.11 a | 19.44 a | 740 d | - | - | - |
| **Year 2010-2011** | | | | | | | |
| Provax-200 | 85.53 a | 5.75 d | 7.24 e | 1353 a | 64.25 | 40.67 | 79.61 |
| Bavistin 50 WP | 82.46 b | 7.00 d | 8.46 d | 1307 a | 57.39 | 35.63 | 73.50 |
| Neem leaf extract | 75.59 c | 9.33 c | 11.53 c | 1180 b | 42.50 | 24.33 | 56.64 |
| Garlic extract | 73.71 d | 9.39 c | 12.57 c | 1130 bc | 39.44 | 21.23 | 50.01 |
| Clean seed | 67.49 e | 11.70 b | 15.23 b | 1110 c | 25.77 | 11.00 | 47.35 |
| Control | 60.80 f | 17.07 a | 19.21 a | 753 d | - | - | - |

Values within the same column with a common letter(s) do not differ significantly (P=0.05)

The data on most important yield contributing parameters such as plant height, branch and pod number/plant, pod length, seed/pod and seed size recorded during 2009-2010 and 2010-2012 crop seasons were almost similar. Therefore, only second year's data on those yield attributes are presented in the Table 2. Plant height and branch number ranged 42.72-43.87 cm and 4.7-4.57/plant, respectively under different treatments including control. However, the variations in both parameters under different treatments were not significant. Seed treatment with fungicide as well as botanicals and use of clean seed caused significant increase over control in pod number per plant and seed number per pod, which are most important yield attributes of cowpea. All treatments with two fungicides and two botanicals gave significant increase in pod length and 1000-seed weight compared to control (Table 2). The

highest increase in pod number/plant, pod length, seed number/pod and 1000-seed weight was obtained with Provax-200 treatment followed by Bavistin 50 WP and Neem leaf extract. The lowest increase of those parameters was obtained with clean seeds followed by Garlic clove extract (Table 2).

Table 2. Effect of pre-sowing seed treatment with two fungicides and two botanicals on seed yield and yield attributes of cowpea during 2011-2012

| Treatments | Plant height (cm) | Branch per plant | Pod number per plant | Pod length (cm) | Seed per pod | 1000- seed weight (g) |
|---|---|---|---|---|---|---|
| Provax-200 | 43.87a | 4.57a | 13.74a | 17.42a | 15.94a | 110.50a |
| Bavistin 50 WP | 43.07a | 4.53a | 13.59b | 17.37a | 15.87ab | 110.30b |
| Neem leaf extract | 43.40a | 4.40a | 13.43c | 17.19b | 15.76bc | 110.30b |
| Garlic extract | 43.00a | 4.27a | 13.34cd | 17.13bc | 15.68c | 110.20bc |
| Clean seed | 42.72a | 4.13a | 13.27d | 17.09cd | 15.64c | 110.10cd |
| Control | 42.27a | 4.07a | 13.01e | 0.08 | 15.44d | 110.00d |

Values within the same column with a common letter(s) do not differ significantly (P=0.05)

Results of the present experiment reveal that seed treatment with vitavaxe-200, Bavistin 50WP, Neem leaf extract and Garlic clove extract cause appreciable reduction in foot and root rot severity and substantial increase in plant growth and grain yield of cowpea. Many other workers also reported that seed treatment with Vitavax-200 improved germination, yield contributing characters and yield of wheat (Deway and Albrechten 1977, Singh and Saksena 1986). In case of seed treatment, higher number of grain per spike, number of spikelet per spike and ultimately increased yield were obtained by Singh and Saksena (1986), Dey *et al*. (1992), Deway and Albrechtsen (1997).

Findings of the present study indicate that significant reduction of seedling mortality and thereby increased germination and crop yield of cowpea can be achieved with seed treatment with fungicides as well as botanicals. On the basis of the findings of two year's experiment, it can be noted that Provax-200 and Bavistin 50 WP are effective to control foot and root rot disease and to increase germination and seed yield of cowpea.

Based on results of the present investigation it may be concluded that pre sowing seed treatment with Provax-200 (0.25%) and Bavistin 50 WP (0.25%) is effective to control foot and root rot (*S. rolfsii*). Efficacy of Neem leaf and Garlic (1:1w/v) as seed treating botanicals is almost at par with the fungicides tested.

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# OCCURRENCE OF LEAF AND POD DISEASES OF *DALBERGIA SISSOO* IN BANGLADESH

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ABSTRACT

Shamim Shamsi, Razia Sultana and Rumana Azad. 2012. Occurrence of leaf and pod diseases of *Dalbergia sissoo* in Bangladesh. Bangladesh J. Plant Pathol. 28(1&2):33-40.

A study was conducted to identify fungi associated with diseased leaves and pods of sisso tree (*Dalbergia. sissoo* Roxb (Sisam). Associated fungi were isolated and identified following standard methods. The associated fungi with leaves were *Alternaria alternata* (Fries) Keissler, *Pseudocercospora dalbergiae* (Sun) Yen, *Chalara* sp., *Colletotrichum gloeosporioides* (Penz.) Sacc., *Cylindrocladium* sp., *Fusarium solani* (Mort.) Sacc., *Gebberella* sp., *Lasiodiplodia theobromae* (Pat.) Griffon and Maubol, *Memnoniella* sp., *Phyllactinia dalbergiae* Piroz. and its anamorph *Ovulariopsis sissoo* Shamsi, Sultana and Azad sp. nov, *Tetraploa* sp. and a rust fungus. From diseased pods, *Colletotrichum gloeosporioides* was isolated. This is the first report of association of *Chalara* sp., *Memnoniella* sp. and *Tetraploa* sp. with Sissoo from Bangladesh.

**Keywords**: Mycoflora**,** diseases, *Dalbergia sissoo*

## INTRODUCTION

*Dalbergia sissoo* Roxb. (Sisam) is an important timber tree with high timber value. The plants are attacked by a number of diseases such as powdery mildew, leaf rust, leaf blight, collar rot, wilt, die-back and *Ganoderma* root rot of the tree are reported by various researchers. The plant is susceptible to dieback, wilt and several other soil borne pathogens (Sah *et al.* 2003). Mukerji and Bhasin (1986) reported leaf spots caused by *Cercospora sissoo, Cochliobolus lunatus, Colletotrichum sissoo, Cylindro-cladium scoparium, Phomopsis dalbergiae, Phyllachora spissa, Phyllosticta sissoo*; leaf blight caused by *Colletotrichum gloeosporioides*; leaf rust caused by *Maravalia achora, Uredo sissoo* and powdery mildew caused by *Phyllactinia dalbergiae* from India. Pod was infected by *Catenulaster batistae, Glomerella cingulata* and *Septothyrella dalbergiae*. Bakshi (1974) isolated *Phellinus gilvus* from roots of trees affected by dieback. Richardson (1990) reported several species of *Aspergillus, Penicillium, Rhizopus, Alternaria, Fusarium, Chaetomium, Drechslera* and *Curvularia* from forest tree seeds. Parajuli *et al.* (1999) reported *Fusarium oxysporum* from *Dalbergia sissoo* on water-logged soils in Nepal. Manadhar *et al.* (2000) isolated *Botryodiplodia* sp. and *Fusarium solani* from five diseased samples of *D. sissoo.* Khan *et al.* (2001) detected *Aspergillus niger, A. flavus, A. terreus, Aspergillus* sp., *Alternaria alternata, Chaetomium* sp., *Drechslera australiensis, Fusarium pallidoroseum, F. solani, Fusarium* sp., *Penicillium* sp., *Rhizopus* sp., and *Geotrichum* sp. from seeds of shisham trees. Rajput *et al.* (2008) isolated *F. solani, Rhizoctonia solani* and *Curvularia lunata* as predominant fungi from shisham trees infected with dieback. From Bangladesh, Muehback *et al.* (2010) isolated *F. oxysporum* and *Lasiodiplodia theobromae* from dieback symptom of sissoo. Shamsi *et al*. (2008) reported *Phyllactinia dalbergiae* and *Ovulariopsis sissoo* from powdery mildew infected plant parts. Information about fungi associated with diseased leves and pods of Sisso tree is limited in Bangladesh (Shamsi *et al*. 2008). The present study was conducted to identify fungi associated with diseased leaves and pods of Sisso trees.

## MATERIALS AND METHODS

Diseased leaf and pod samples of Sisso were collected from Dhaka, Chittagong and Pabna districts during October 2008 to January 2010. Associated symptoms were recorded. The severity of disease on leaves was estimated visually using a 0-9 subjective scale, where 0= no infection, 1= up to 10% leaf area infected, 2= 10 – 20% leaf area infected, 3=20 – 30% leaf area infected, 4= 30 – 40% leaf area infected, 5= 40 – 50% leaf area infected, 6= 50 – 60% leaf area infected, 7= 60 – 70% leaf area infected 8= 70 – 80% leaf area infected and 9= 80% and above leaf area infected (Ghos *et al.* 2009).

Fungi associated with diseased samples were isolated following “tissue planting method" and "blotter method” (Anon. 1968). In case of “tissue planting method” fifty inocula each measuring 2 $mm^2$ was cut from a particular specimen. The inocula were washed in sterile water and surface sterilized by dipping in 10.0% Clorox for 3-5 minutes. Three inocula were placed in each Petri plate containing sterilized potato dextrose agar (PDA) medium and incubated for 5-7 days at 25±2C. In “blotter method” moist chambers were made by placing two layers of filter paper on the

bottom of the Petri plates. In each Petri plate, 5 surface sterilized inocula were placed and 10 plates were used. The inoculated plates were incubated at room temperature. The fungi growing out of the inocula were transferred to separate PDA plates and slants, and stored in a refrigerator for further studies. Prevalence of fungi associated with the specimens was expressed in percentage based on total number of leaf and pod samples checked. Identification of the isolated fungi was done using standard literature (Booth 1971, Ellis 1971, 1976, Ellis and Ellis 1982, Sutton 1980). All the specimens were preserved in the Herbarium, Mycology and Plant Pathology Section, Department of Botany, University of Dhaka, Bangladesh.

## RESULTS AND DISCUSSION

Different types of symptoms representing seven diseases were found on diseased leaves of *D. sissoo*. These were anthracnose, powdery mildew, angular leaf spot, leaf blight, leaf spot and leaf rust. Anthracnose symptom was also noticed on infected pods (Table 1 and Plate I-VI). Their severity in different months are shown in Figure 1.

**Anthracnose:** Anthracnose was found in all leaf samples (Plate I A) collected from Savar and Pabna. The disease also appeared on pod samples (Plate I B & C) collected from only Pabna (Table 1). The highest disease severity index of 8 was recorded in the month of March and the index value was 6 during April to December 2009 (Fig. 1). *Colletotrichum gloeosporioides* was associated with leaf and pod samples infected with anthracnose (Plate I D).

**Powdery mildew**: Symptoms of powdery mildew caused by imperfect stage of the pathogen (*Ovulariopsis sissoo* Shamsi, Sultana and Azad) (Plate II A & B) were recorded from five leaf samples collected from Dhaka (Table 1). Powdery mildew symptoms developed by perfect stage of the fungus (*Phyllactinia dalbergiae*) (Plate III A & B) were found on two leaf samples collected from Dhaka and two from Savar (Table 1). The infection starts in the middle of October 2008 with the formation of a white mycelial growth mostly on the lower surface of the leaves. With the age, the mycelium and conidiophores bearing conidia become grayish-white to pale yellow. By the end of December most of the leaves on the trees are covered with white colony growth of the fungus, perithecia start to form, which were initially orange in colour. The ascocarps turn into brown with the progress of the disease and ultimately become black at maturity. The powdery mildew causes severe defoliation but never kills the tree. New leaves develop with the advent of spring. The highest severity index of 8 was recorded in the month of January and 7 in November and December, 2009. Perithecia formed during February to March. Perithecia were found in the samples collected from Dhaka (Fig. 1 and Table 1).

**Angular leaf spot:** Angular leaf spot symptoms (Plate IV A) were frequently noticed on leaves collected from Chittagong (Table 1) was found in the samples. The causal fungus of angular leaf spot was identified as *Gibberella* sp. (Plate IV B).

**Leaf spot**: The disease appears as small circular spots on leaves. The size of the spots increases with the progress of times. Larger spots develop due to coalescence of closure spots (Plate IV C). The causal pathogen, *Pseudo-cercospora dalbergiae* (Plate IV D) attacks the leaves mostly on the lower surface of leaves producing yellowish to grayish-green discoloration. The mycelium is brown and intra-epidermal. The fungus produces asexual fruiting structure in July and August 2009. In the month of January, its severity index was 4. The highest disease index of 7 was recorded in the month of October and 5 in the month of November 2009 (Fig. 1 and 2 and Table 1).

**Leaf blight:** The leaf blight symptoms was associated with leaf samples collected from Savar (Plate IV A and Table 1). Several fungi namely *Alternaria alternata*, *Chalara* sp., *Cylindrocladium* sp., *Fusarium solani*, *Lasiodiplodia theobromae*, *Memnoniella* sp. and *Tetraploa* sp. were found to be associated with leaf blight infected samples (Plate V B-H). The fungi were associated with leaf samples during June to October 2009 and their prevalence ranged 5.88-16.67% (Fig. 1).

**Rust**: The rust disease appeared during January to March on leaves (Plate VI) and young twigs. Uredinial sori are yellowish and formed on the lower surface of the leaves (Plate V). The fungus was found in the samples collected from Savar (Table 1) It severity varied with months (Fig. 1). Present report is slightly differing from the observation of Bakshi (1967). The author reported that the rust disease appeared in January to March on leaves and juvenile twigs and continued attacking the foliage and young twigs up to July and August. The infection declines following monsoon rains. The affected parts are killed resulting in die-back and subsequent death of affected seedlings. *Maravalia achroa* is recorded on seedling in nurseries from Uttar Pradesh, Bihar, Maharashtra and Assam. The disease also occurs on young plantations but not in as severe form as in the nurseries.

Table 1. Fungi associated with leaf and pod samples of *Dalbergia sissoo* having different types of symptoms collected from various locations of Bangladesh during December 2008 to January 2010

| Sample No | Date of collectio | Locality | Diseases | Plant parts | Identified fungi |
|---|---|---|---|---|---|
| SS 2136 | 08-12-2008 | Pabna | Anthracnose | Leaf | *Colletotrichum gloeosporioides* |
| SS 2138 | 27-01-2009 | Pabna | Anthracnose | Pod | *Colletotrichum gloeosporioides* |
| SS 2164 | 10-03-2009 | Savar | Anthracnose | Leaf | *Colletotrichum gloeosporioides* |
| SS 2171 | 17-06-2009 | Savar | Anthracnose | Leaf | *Colletotrichum gloeosporioides* |
| SS 2174 | 22-07-2009 | Savar | Anthracnose | Leaf | *Colletotrichum gloeosporioides* |
| SS 2184 | 04-07-2009 | Savar | Anthracnose | Leaf | *Colletotrichum gloeosporioides* |
| SS 2152 | 22-01-2009 | Dhaka | Anthracnose | Leaf | *Ovulariopsis sissoo* |
| SS 2157 | 17-02-2009 | Dhaka | Powdery mildew | Leaf | *Ovulariopsis sissoo* |
| SS 2183 | 15-11-2009 | Dhaka | Powdery mildew | Leaf | *Ovulariopsis sissoo* |
| SS 2185 | 06-12-2009 | Dhaka | Powdery mildew | Leaf | *Ovulariopsis sissoo* |
| SS 2158 | 19-02-2009 | Dhaka | Powdery mildew | Leaf | *Ovulariopsis sissoo* |
| SS 2160 | 02-03-2009 | Dhaka | Powdery mildew | Leaf | *P. dalbergiae* |
| SS 2163 | 05-03-2009 | Dhaka | Powdery mildiew with perithicia | Leaf | *Phyllactinia dalbergiae* |
| SS 2184 | 22-11-2009 | Savar | Powdery mildiew with perithicia | Leaf | *P. dalbergiae* |
| SS 2172 | 08-07-2009 | Savar | Powdery mildiew with perithicia | Leaf | *P. dalbergiae* |
| SS 2170 | 17-06-2009 | Savar | Blight | Leaf | *Fusarium solani* |
| SS 2175 | 22-07-2009 | Savar | Blight | Leaf | *Alternaria alternata* |
| SS 2177 | 05-08-2009 | Savar | Blight | Leaf | *Tetraploa* sp. |
| SS 2179 | 19-08-2009 | Savar | Blight | Leaf | *L. theobromae* |
| SS 2180 | 26-08-2009 | Savar | Blight | Leaf | *Memnoniella* sp. |
| SS 2182 | 07-10-2009 | Savar | Blight | Leaf | *Cylindrocladium* sp. |
| SS 2181 | 23-11-2009 | Chittagong | Angular spot | Leaf | *Gibberella* sp. |
| SS 2186 | 12-12-2009 | Savar | Rust | Leaf | Urediospor |
| SS 2207 | 12-01-2010 | Savar | Rust | Leaf | Urediospor |

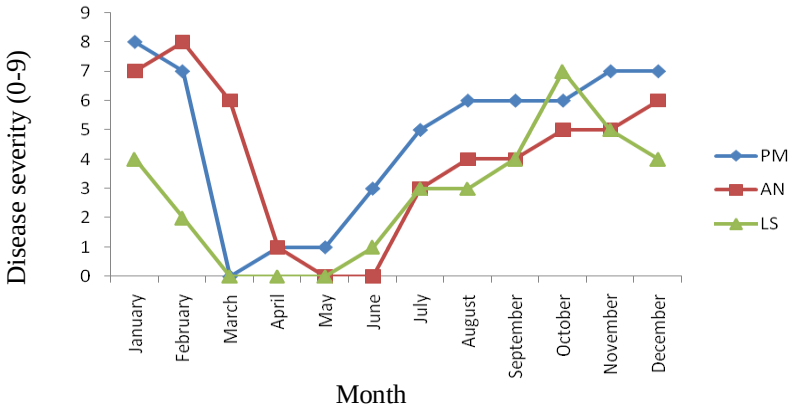



Fig. 1. Disease progress curve of powdery mildew (PM), anthracnose (AN) and leaf spot (LS) symptoms recorded on *Dalbergia sissoo* from January to December 2009.

**Fungi associated with diseased leaves and fruits**: A total of 13 fungal species namely, *Alternaria alternata, Pseudocercospora dalbergiae, Chalara* sp., *Colleto-trichum gloeosporioides, Cylindrocladium* sp., *Fusarium solani, Gibberella* sp., *Lasiodiplodia theobromae, Memnoniella* sp., *Ovulariopsis sissoo, Phyllactinia dalbergiae, Tetraploa* sp. and an unidentified rust fungus were found to be associated with infected leaves of *D. sissoo*. Prevalenc of *P. dalbergiae* was the highest followed by *C. gloeosporioides, O. sissoo,* and *A. alternata* showing the prevalence of 52.94, 46.67 and 16.67%. Prevalence of *F solani, P. dalbergiae* and *Gibberella* sp. was 11.76, 11.76 and 11.11%,

respectively. The most prevalence of 5.88 was recorded in *Chalara* sp., *Cylindrocladium* sp., *Memnoniella* sp., rust fungus and *Tetraploa* sp. *Colletotrichum gloeosporioides* was isolated from infected fruits (Fig. 2).

Results of the present study reveal that at least 13 species of fungi are associated with diseased leves of Sisso tree. The leaf spot, anthracnose and powdery mildew are the major diseases of *D. sissoo* in Bangladesh. The environment of Savar is favorable for growth and development of most the fungi than Chittagong, Dhaka and Pabna. Association of *Chalara* sp., *Memnoniella* sp. and *Tetraploa* sp. with *D. sissoo* is new records in Bangladesh.

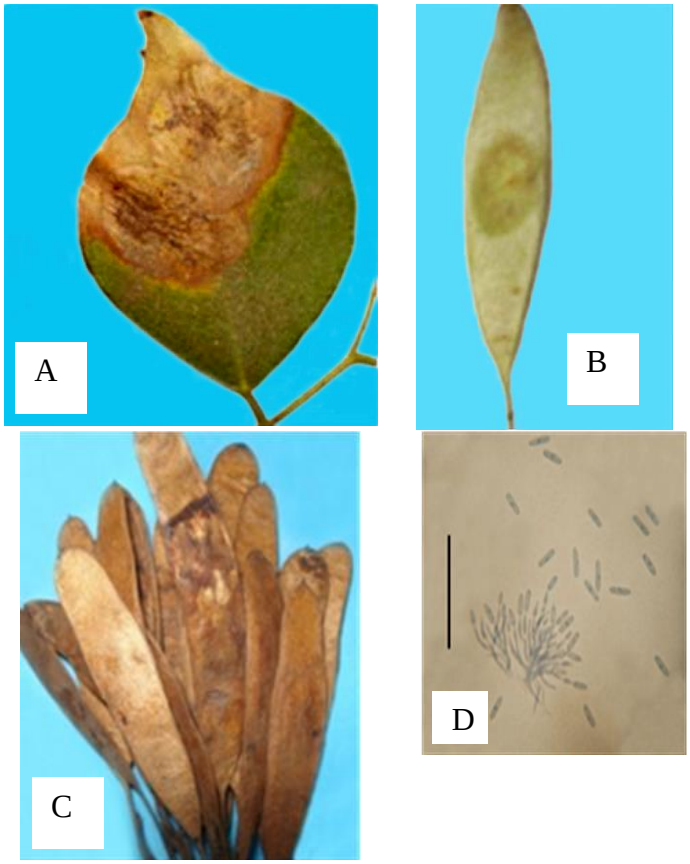



Plate I. Photographs showing symptoms of anthracnose on leaf (A) pods (B&C) of *Dalbergia sissoo* caused by *Colletotric gloeosporioides* (D)

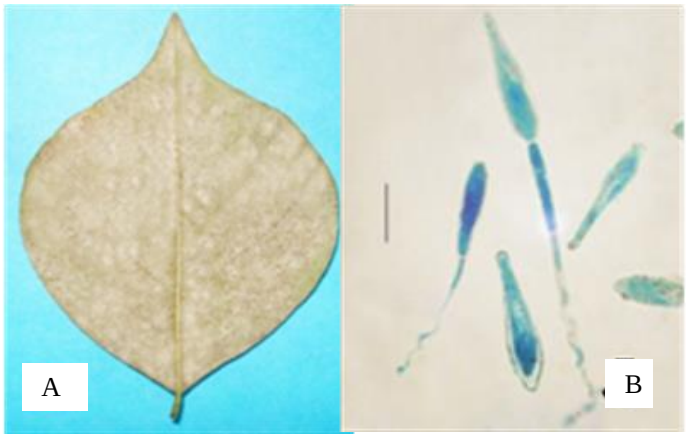



Plate II. Photographs showing symptoms of powdery mildew on leaf of *Dalbergia sissoo* caused by *Ovulariopsis sissoo* ((A & B) [Bar = 50 µm].

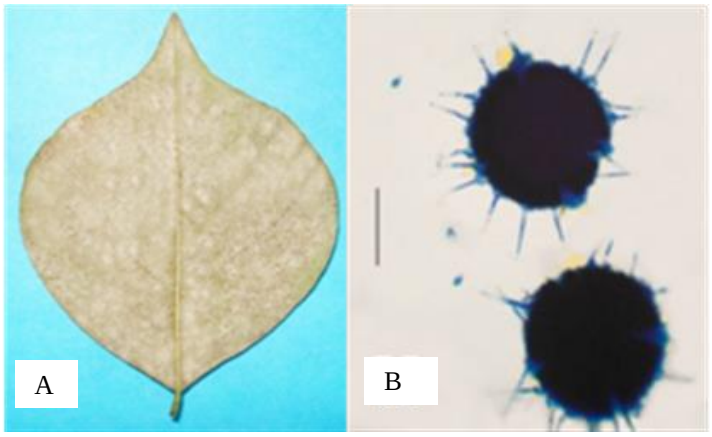



Plate III. Photographs showing symptoms of powdery mildew on leaf of *Dalbergia sissoo* caused by *Phyllactinia dalbergiae* (A & B) [Bar = 50 µm].

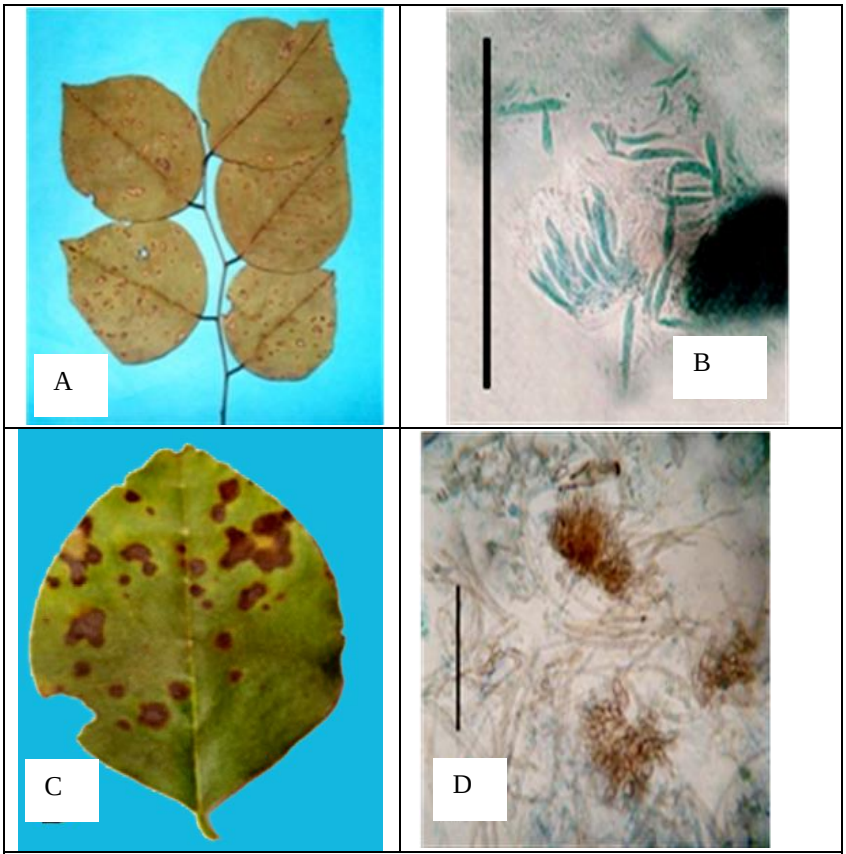
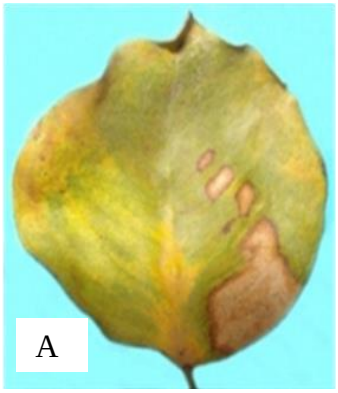
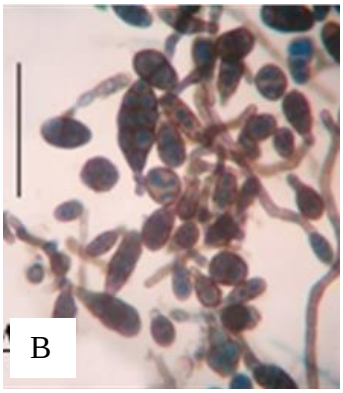


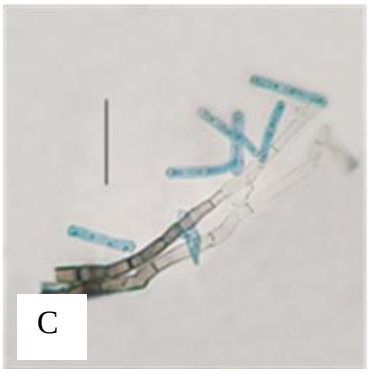

Plate IV. Photographs showing symptoms of angular leaf spot (A) caused by *Gibberella* sp. (B) and leaf spot (C) caused by *Pseudo-cercospora dalbergiae* (D).




Leaf blight symptoms



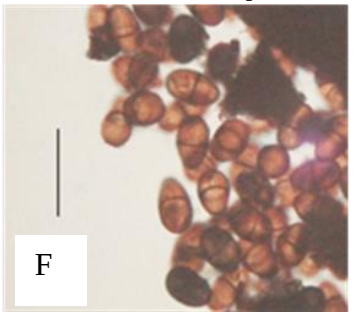

*Alternaria alternata*



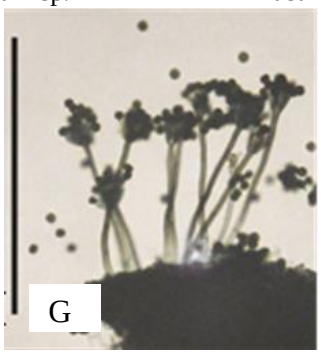

*Chalara* sp.


*Cylindrocladium* sp.

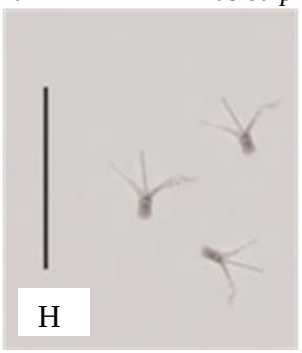

*Fusarium solani*




*Lasiodiplodia theobromae*.

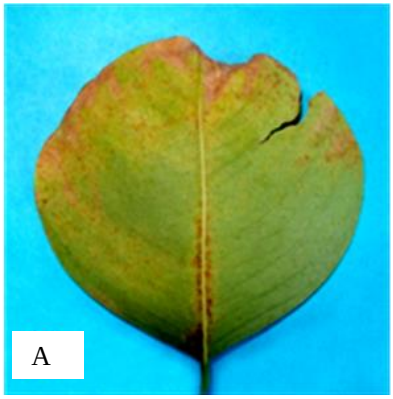



*Memnoniella* sp.

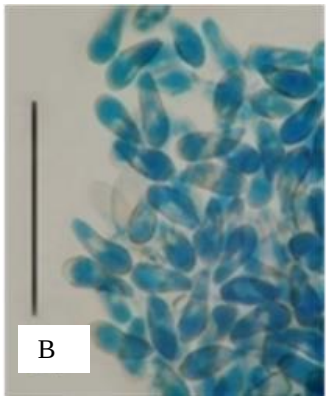



*Tetraploa* sp.

Plate V. Photographs showing symptoms of leaf blight symptoms (A) of *Dalbergia sissoo* and fungi associated with the blighted leaves(B-H) (Bar = 50 µm)




Rust disease




An unidentified species of rust fungus

Plate VI. Symptoms of rust disease on leaf (A) of *Dalbergia sissoo* caused by an unknown species of rust fungus (B) (Bar = 50 µm)

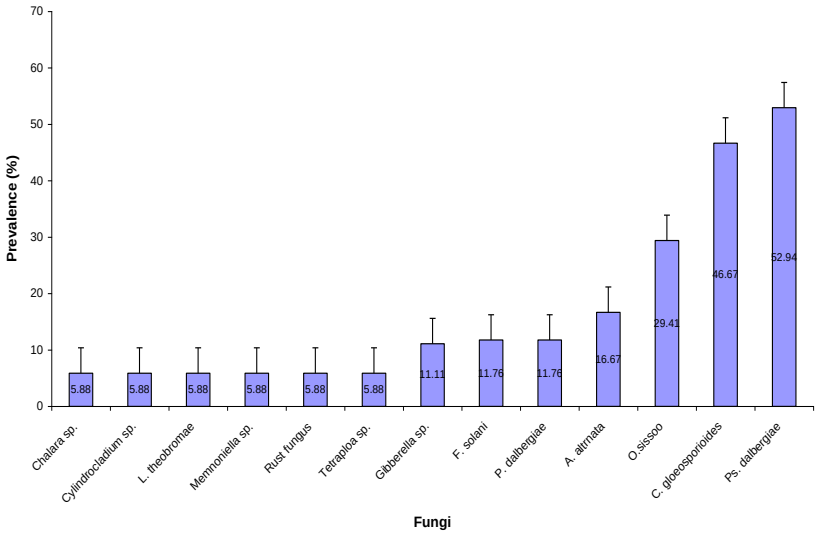



Fig. 1. Prevalence of fungi association with infected leaf samples of *Dalbergia sisso* grown in Bangladesh

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# EFFICACY OF GRANULAR FORMULATIONS OF CARBOFURAN TO CONTROL UFRA DISEASE (*DITYLENCHUS ANGUSTUS*) OF RICE

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ABSTRACT

I. H. Mian and Nurjahan Khatun. 2012. Efficacy of granular formulations of carbofuran to control ufra disease (*Ditylenchus angustus*) of rice. Bangladesh J. Plant Pathol. 28 (1&2): 41-45.

A series of experiments was conducted to determine the efficacy of five granular formulations of Carbofuran namely Brifuran 5G, Cemifuran 5G, Curatarr 5G, Edfuran 5G and Furadan 5G against Ufra disease of rice caused by *Ditylenchus angustus* under inoculated conditions. An Ufra susceptible rice variety (BR-3) was used in the experiments. Ufra infected stem pieces of rice were used as inocula. In first experiment, Brifuran 5G, Cemifuran 5G, Curatarr 5G and Furadan 5G (@ 15 kg/ha) were tested against Ufra disease of rice seedlings and found that the nematicides gave respectively 36.23, 41.71, 52.33 and 66.99% reduction in Ufra infestation of rice seedlings over control. In subsequent experiment, Brifuran was dropped because of its lowest efficacy and Edfuran 5G was included. In second and third experiments, Cemifuran 5G, Curatarr 5G, Edfuran 5G and Furadan 5G were tested against Ufra disease of rice under pot and field conditions. In pot experiment, the nematicides caused 35.25-76.76, 11.16 - 62.29 and 64.19 – 76.63% reduction in incidence of UI, UII and UIII over control. They also gave significant control of the disease under field conditions. Among the nematicides, Furadan 5G was the most effective one. Two more experiments were conducted under pot and field conditions to find out the influence of different levels of Ufra infected rice seedlings (0, 25, 50, 75 and 100%) on the efficacy of Furadan 5G against Ufra. It was found that treatment of soil with Furadan 5G caused significant reduction in incidence of Ufra and increase in grain yield as compared to control in both pot and field experiments. The efficacy of the nematicide was better when the crop was planted with lower levels of infected seedlings and even at 100% infected seedlings the nematicide was effective to control the disease.

**Keywords**: Carbofuran, nematicide, Ufra, *Ditylenchus angustus*, control

## INTRODUCTION

Ufra disease of rice caused by *Ditylenchus angustus* was first reported from Naokhali district of Bangladesh (the then East Bengal) over 100 years ago (Butler 1913). Locally, the disease was referred as 'Dak pora', as symptoms of damage look like burn due to thunder strike. The disease first appeared in deep water rice of a farmer named 'Uftur Rahman'. So, the disease was named as Ufra after the name of the farmer. Ufra is a major disease of rice in Bangladesh causing severe crop damage. Earlier, it was found to attack only deep water rice. Now-a-days it attacks rice plant in any growing seasons ('Aus', 'Aman' and 'Boro'), when rice is cultivated under standing water (Bakr 1977, Rahman *et al.* 1981). The disease was reported to cause 1.26 t/ha yield loss but total crop failure is not unlikely (Miah and Bakr 1977, Mondal *et al.* 1989).

If farmers go for planting a second rice crop without taking any protective measure, nematode population builds up and destroys the second crop. Resistant varieties of rice against Ufra are not available. Application of chemical nematicides is a dependable and effective method to control of the disease. Formulated products of Carbofuran, Phanamiphos and Isazophos are recommended for control Ufra (Rahman *et al*. 1992, Salim Miah *et al*. 2003, Latif *et al*. 2013). Rahman and Miah (1991) found that application of Carbofuran reduced Ufra infestation by 37% in transplanted rice and by 58% in broadcast Aman rice. Application of two sprays with Carbosulfan 40 EC at 0.2% followed by 2 spraying with Triazophos 40 EC at 0.2% reduced Ufra infestation appreciably (Deb Anand Das, 2004). Use of Furadan 5G, Arodhan 5G and Biestern 5G @ 1.0 kg a.i/ha was effective to control Ufra and increase rice yield (Latif *et al*. 2013). Application of nematicides was effective to produce Ufra free rice seedlings and to reduce 99% disease incidence. The efficacy of nematicide may be influenced by the levels of Ufra infestation in the field at the time of application. Considering the above facts the present investigation was conducted to find out the efficacy of five formulated products of Carbofuran to control Ufra disease of rice.

## MATERIALS AND METHODS

**Materials used**: Five granular formulations of Carbofuran namely Brifuran 5G, Cemifuran 5G, Curatarr 5G, Edfuran 3G and Furadan 5G were evaluated for their nematicidal value to control Ufra disease (*D. angustus*) of rice under inoculated conditions. Pieces of Ufra infected rice stems were used as inocula and base inoculation technique (Rahman 1993) was followed. Three pot and two field experiments were conducted and an Ufra susceptible rice variety BR 11 was used. In pot experiment, earthen pots (25 cm diameter and 25 cm height) were filled with steam sterilized sandy loam soil at 8 kg soil per pot. Recommended doses of fertilizers were added to the pots and thoroughly mixed with the soil.

**Experiment-1**: The first experiment was conducted in pots to find out the efficacy of Cemifuran 5G, Curatarr 5G, Edfuran 3G and Furadan 5G to control Ufra disease of rice seedling. Rice seeds (BR 11) were planted in the pots. From 3rd day of germination 2-3 cm water was maintained. Nine days after germination the seedlings were inoculated with Ufra infected rice stem pieces (Rahman 1980). After ten days of inoculation, the pots were treated with each of the selected nematicides at 15 kg formulated product per hectare. Thirty days after application of nematicides, incidence of Ufra infected seedlings was recorded and was expressed in percentage based on total number of seedlings checked.

**Experiment-2 & 3**: Second and third experiments were conducted under pot and field conditions, respectively to find out the efficacy of Cemifuran 5G, Curatarr 5G, Edfuran 3G and Furadan 5G to control Ufra disease of rice. In pot experiment, pot soil was treated with the nematicides at the rate of 15 kg formulated product per hectare just before transplanting. In field experiment, the experimental field was divided into 3 blocks with 1m wide drain between the blocks. Each block was divided into 5 unit plots (2 m X 2 m) maintaining 1m space between plots. Both pot and field soils were treated with the nematicides @ 15kg/ha. Thirty days old rice seedling infected with Ufra were transplanted just after application of nematicides. Each pot received 4 seedlings and the seedlings were transplanted in the field at one seedling per hill maintaining 20 cm X 20 cm plant to plant and row to row spacing. In the field experiment, appropriate measures were taken to restrict the movement of nematode from one plot to another with water flow. The plants were allowed to grow providing recommended doses of fertilizers, cultural and intercultural operations. At ripening stage, data on the severity of Ufra disease were recorded following standard procedures (Cox 1980).

**Experiment-4 & 5**: Fourth and fifth experiments were conducted to find out the influence of level of Ufra incidence in rice seedlings on the efficacy of nematicides to control the disease under pot and field conditions, respectively. Based on findings of the present study and considering its availability in Bangladesh Furadan 5G was selected as test nematicide. Soils, pots, experimental field and procedures of experimentation were more or less similar as mentioned under Experiments 2 and 3. Ufra infected and Ufra free rice seedlings were grown under controlled conditions. Soils of the pots as well as experimental plots were treated with Furadan 5G @ 15 kg/ha. Healthy and Ufra infected rice seedlings were mixed to have 0, 25, 50, 75, 100% level of infected seedlings. Just after treatment of soil, the seedlings were transplanted in the pots at 4 seedlings per pot and in unit plot. The plants were allowed to grow providing necessary fertilizers, cultural and intercultural operations.

**Design of experiment and data collection**: Design of pot experiment was completely randomized (CRD) and that of field experiment was randomized complete block (RCBD) with four replications. Control treatments received no nematicide (Control-1). One additional control (Control-2) was maintained in second and third experiments where 100% healthy seedlings were transplanted without any nematicide. At ripening stage, data on Ufra incidence were recorded following a standard method (Cox 1980). The panicles were grouped into three categories viz. Ufra I = fully enclosed panicles within the leaf sheaths (UI), Ufra II – partially enclosed panicles with few grains (UII), and Ufra III – complete emergence of panicles (UIII) (Cox 1980). Grain yield per pot was determined after harvest, threshing and adjustment of moisture content at 14% by sun drying. The data were analyzed using MSTAT-C.

## RESULTS AND DISCUSSION

### Experimet-1: Efficacy of nematicide to control Ufra disease of rice seedling

Maximum of 6.21% Ufra infected seedlings were recorded from control pots, where inoculum of *D. angustus* was used but no nematicide was applied. Application of all nematicides gave significant reduction in the incidence of Ufra disease of rice seedlings compared to control. The reduction was 36.23, 41.71, 52.33 and 66.99% under Cemifuran 5G, Furadan 5G, Curatarr 5G and Brifuran 5G, respectively compared to control (Table 1).

**Experiment-2&3: Efficacy of nematicide to control Ufra of rice under pot culture and field conditions**

In pots planted with healthy seedlings (control-2) were free from Ufra disease. In pots planted with seedlings infected with *D. angustus* and in which no nematicide was applied (control-1), the incidence of UI, UII and UIII was 20.21, 21.23 and 55.23%, respectively. Application of Cemifuran 5G, Curatarr 5G, Edfuran 3G and Furadan 5G caused 35.25-76.76, 11.16 - 62.29 and 64.19 – 76.63% reduction in UI, UII and UIII, respectively over control-1. The rates of reduction of UI over control achieved with four nematicides were not significant. Significant reduction of UII was achieved with only Cemifuran 5G compared to control-1. All nematicides gave significant reduction in incidence of UIII. The highest reduction was obtained with Cemifuran 5G. However, efficacy of all nematicides to reduce Ufra incidence was statistically similar (Table 2).

Maximum tiller height of 57.40 cm was recorded from the pots planted with healthy seedlings and no nematicide was applied (contrl-2). The lowest tiller height of 38.20 cm was recorded from pots planted with infected seedlings but not treated with any nematicide (control-1). The tiller height increased to 47.25-56.50 cm in pots received infected seedling but treated with four nematicides. Maximum increase in tiller height was achieved with Cemifuran 5G followed by Curatarr 5G. The effectiveness of all nematicides tested was similar to the control-2, where 100% healthy seedlings (0% infected seedlings) were used (Table 2). The incidence of Ufra disease was not found in plots where 100% healthy seedlings were transplanted. In plots transplanted with infected seedlings but no nematicide was applied (control-1), the incidence of UI, UII and UIII was 40.16, 29.17 and 18.99%, respectively. Treatment of soil with four nematicides caused appreciable reduction in the incidence of all three categories of Ufra. Furadan 5G caused significant reduction in UI and UII, whereas Curatarr 5G and Edfuran 5G gave significant reduction in UII and UIII as compared to control-1. Only Cemifuran 5G significantly reduced the incidence of UI, UII, UIII compared to control-1 (Table 3).

Table 1. Efficacy of four nematicides to control Ufra disease (*Ditylenchus angustus*) of rice seedling

| Nematicide (15 kg/ha) | Disease incidence (% infected seedling) | Reduction over control (%) |
|---|---|---|
| Control | 6.21A | - |
| Brifun 5G | 3.96 B | 36.23 |
| Curatarr 5G | 3.62 BC | 41.71 |
| Furadan 5G | 2.96 CD | 52.33 |
| Cemifuran 5G | 2.05 D | 66.99 |

Mean values within a column having a common letter do not differ significantly (P=0.05)

Table 2. Efficacy of four common nematicides to control Ufra disease (*Ditylenchus angustus*) of rice under pot culture conditions

| Nematicide (15 kg/ha) | Incidence of Ufra (% infected plants) | | | Tiller height (cm) |
|---|---|---|---|---|
| | Ufra-I | Ufra-II | Ufra-III | |
| Edfuran 5G | 13.48A | 15.91AB | 19.78B | 47.75A |
| Furadan 5G | 8.12A | 18.86AB | 15.90B | 47.25A |
| Cemifuran 5G | 9.29A | 7.87BC | 14.01B | 56.40A |
| Curatarr 5G | 4.84A | 10.62ABC | 19.38B | 52.75A |
| Control -1 (infected seedling) | 20.82A | 21.23A | 55.23A | 38.20C |
| Control -2 (100% healthy seedling) | 0.00B | 0.00C | 0.00B | 57.40A |

Values within the same column with a common letter(s) do not differ significantly (P=0.05)

The incidence of Ufra disease was not found in plots where 100% healthy seedlings were transplanted. In plots transplanted with infected seedlings but no nematicide was applied (control-1), the incidence of UI, UII and UIII was 40.16, 29.17 and 18.99%, respectively. Treatment of soil with four nematicides caused appreciable reduction in the incidence of all three categories of Ufra. Furadan 5G

caused significant reduction in UI and UII, whereas Curatarr 5G and Edfuran 5G gave significant reduction in UII and UIII as compared to control-1. Only Cemifuran 5G significantly reduced the incidence of UI, UII, UIII compared to control-1 (Table 3).

Grain yield per plot was 1233.0 g when 100% healthy seedlings were planted. The yield was reduced to 53.3 g/plot when infected seedlings were planted without application of any nematicides. Application of Furadan 5G, Curatarr 5G and Cemifuran 5G gave significant increase in grain yield over control-1. Per plot grain yield under three nematicides was 216.7, 350.00 and 566.7 g, respectively. The efficacy of Curatarr and Cemifuran to reduce Ufra incidence was not significantly different. The highest yield increase was achieved with Cemifuran, which was followed by Curatarr, Furadan and Edfuran (Table 3).

**Experiment-4&5: Influence of level of infected seedling on the efficacy of Furadan 5G to control Ufra disease of rice under pot and field conditions**

Under pot culture conditions, incidence of Ufra disease of rice was not found in pots planted with 100% healthy seedlings and received no nematicide. In pots planted with 100% infected seedlings without Furadan 5G (control) showed 63.10, 21.51 and 13.03% incidence of UI, UII and UIII. Application of Furadan 5G at 15 kg/ha reduced incidence of UI, UII and UIII to 9.79-3.74 in UI, 15.50-14.35% in UII and 17.40-25.74% respectively at 25, 50, 75 and 100% infected seedling. The reduction was significant in case of UI and UIII. The highest reduction was observed at 75% of infected seedlings, which was followed by 50, 25 and 100% infected seedlings. The reason of such results may be due to high levels of incidence of UI and UII and there was a few plants left to develop symptoms of UIII. The highest grain yield of 245 g/pot was recorded from control where 100% healthy seedling was planted and Furadan 5G was applied. On the other hand, the lowest yield of 121 g/pot was harvested when 100% infected seedlings were planted but nematicide was not applied. The yield increased significantly over control when 25-75% infected seedlings were planted and treated with Furadan 5G (Table 4).

Under field conditions, the incidence of UI, UII and UIII was 76.10, 14.90 and 9.00%, respectively when 100% Ufra infected seedlings were planted but the nematicide was not applied (control). Application of Furadan 5G gave significant reduction in only UI in plots planted with 25-100% infected seedlings compared to control. The differences in UI reduction at different levels of Ufra infected seedlings were not significant. Incidence of UII was reduced due to nematicide application only when 0 and 25% infected seedlings were planted. The incidence of UIII was statistically similar at all levels of infected seedlings and when Furadan 5G was applied. The highest grain yield of 510 g/plot was obtained when 100% healthy seedlings were planted and plots were treated with Furadan 5G. The lowest yield of 52 g/plot was recorded from plots planted with 100% infected seedling in spite of application of Furadan 5G. The grain yield in plots planted with 25-100% infected seedlings was statistically similar. The grain yield was reduced gradually with the increase in incidence of Ufra (Table 4).

Table 3. Efficacy of four common nematicides to control Ufra disease (*Ditylenchus angustus*) of rice under field conditions

| Nematicide (15 kg/ha) | Incidence of Ufra (% infected plants) | | | Grain yield (g/plot) |
|---|---|---|---|---|
| | Ufra-I | Ufra-II | Ufra-III | |
| Furadan 5G | 14.37 B | 19.50 B | 26.94 A | 216.7C |
| Edfuran 5G | 38.69 A | 17.93 B | 14.O9 B | 201.7 CD |
| Curatarr 5G | 27.45 AB | 19.55 B | 11.46 B | 350.0 BC |
| Cemifuran 5G | 18.55 B | 19.13 B | 12.92 B | 566.7 B |
| Control-1(Infected seedling & no nematicide) | 40.16 A | 29.17 A | 18.99 AB | 53.3 D |
| Control-2 ( 100% Healthy & no nematicide) | 0.00 C | 0.00 C | 0.00 C | 1233.0 A |

Mean values within a column having a common letter(s) do not differ significantly (P=005)

Table 4. Influence of level of infected seedling on the efficacy of Furadan 5G to control Ufra disease (*Ditylenchus angustus*) of rice under pot culture and field conditions

| Level of infected seedling (%) | Under pot condition | | | | Under field condition | | | |
|---|---|---|---|---|---|---|---|---|
| | Three types of Ufra incidence (%) | | | Grain yield (g/plot) | Three types of Ufra incidence (%) | | | Grain yield (g/plot) |
| | Ufra-1 | Ufra-II | Ufra-III | | Ufra-1 | Ufra-II | Ufra-III | |
| 0 | 0.00B[b] | 0.00B | 0.00B | 245 A | 0.37 B | 3.28 C | 4.91 A | 510 A |
| 25 | 9.79B | 15.50A | 17.40A | 182 C | 11.26 B | 8.01 BC | 9.17 A | 340 AB |
| 50 | 6.16B | 16.03A | 19.26A | 200 B | 25.86 B | 12.09 ABC | 10.22 A | 271 B |
| 75 | 6.08B | 14.48A | 18.05A | 192 BC | 21.65 B | 19.36 A | 8.59 A | 238 BC |
| 100 | 3.74B | 14.35A | 25.74A | 186 C | 32.77 B | 19.82 A | 9.82 A | 203 BC |
| Control[a] | 63.10A | 21.51A | 13.03AB | 121 D | 76.10 A | 14.90 AB | 9.00 A | 52 C |

[a]Use of 100% infected & no nematicide was used.
[b]Values within the same column with a common letter(s) do not differ significantly (P=0.05)

Results of five experiments conducted under the present study reveal that Ufra disease of rice caused by *D. angustus* can be controlled effectively by applying nematicides just before or after transplanting of seedlings. Four nematicides namely Brifun 5g, Curatarr 5G, Cemifuran 5G, and Furadan 5g were evaluated against Ufra disease of rice seedling and found that application of the nematicides caused significant reduction in the incidence of Ufra of rice seedlings. The highest reduction was obtained with Cemifuran, which was followed by Furadan 5G. In other experiments the nematicides Edfuran 5G, Curatarr 5G, Cemifuran 5G, and Furadan 5g were tested against Ufra disease of rice under field and pot conditions and similar results were recorded (Rahman and Miah 1991). Cemifuran 5G was noted as the best nematicide followed by Furadan 5G. The nematicide is more effective at low level of infected seedling. The nematicide is also effective if it is applied at early stage of infection causing UI. Appearance of Ufra in plots having 100% healthy seedlings might be due to transmission of nematodes from infected plot due to rain splash. Effectiveness of nematicides including some of them tested in the present study was also evaluated by many other researchers and found similar results. Salim Miah *et al*. (2003), Deb Anand Das (2004) and Latif *et al*. (2013) reported that application of formulated products of Carbofuran, Phanamiphos, Isazopos (Brifur5G, Curatarr 5G; Furadan 5G) are effective to control Ufra disease of rice. Soil incorporation of Carbofuran at 30 kg a.i. /ha has been reported to recover the Ufra infested plants or to reduce the disease incidence (Sein 1977). Field application of Furadan 3G is effective to control Ufra disease by 82%. Its application in soil at 1.0 kg a.i. /ha in an infested field increased yield by 105% (Rahman *et al*. 1981). Rahman and Miah (1985, 1991, and 1992) observed that application of Carbofuran gave reduction in Ufra of rice. It was observed that soil application of Carbofuran at 0.5 and 0.75 a.i. /ha caused effective and economic control of Ufra (Rahman *et al*. 1992).

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# REDUCTION IN SEEDLING GROWTH OF SOME VEGETABLES DUE TO INFECTION WITH ROOT- KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*)

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ABSTACT

T. Rukshshana and I. H. Mian. 2012. Reduction in seedling growth of some vegetables due to infection with root- knot nematode (*Meloidogyne incognita*). Bangladesh J. Plant Pathol. 28(1&2):47-52.

A pot experiment was conducted to find out the effect of root-knot nematode infection at seedling stage on plant growth of eleven vegetable crops commonly grown in Bangladesh. It was found that shoot and root growth of all eleven vegetables were significantly reduced due to inoculation with root-knot nematode at seedling stage. The reduction of height and fresh weight of shoot, and length and fresh weight of root of eleven crops varied from 16.83 to 63.65%, 15.50% to 46.652%, 23.30 to 57.53% and 22.18 to 74.00%, respectively. Number of galls and eggs per 20 g roots of eleven vegetables ranged 2.88-69.82 and 120.85-3482.00, respectively in vegetable eleven crops. The highest gall number was found in roots of tomato and the lowest in roots of Indian spinach. The lowest egg number was found in roots of cucumber and the highest in tomato roots. Among eleven vegetables, the highest population of $L_3$ and $L_4$ larvae, and immature as well as mature females were recorded from roots of tomato followed by cauliflower and eggplant. Based on findings of the present investigation it may be concluded infection of root-knot nematodes at seedling stage causes severe reduction in plant growth of vegetable seedlings commonly grown in Bangladesh.

**Keywords:** Root-knot nematode, vegetable crops, growth reduction

## INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) are common pests of vegetable crops in Bangladesh. At least four species of root-knot nematodes occur in Bangladesh. They are *M. incognita, M. javanica, M. graminicola* and *M. arenaria*. Among them the most frequently occurring species is *M. incognita* (Mian 1986). The nematode larvae infect plant roots causing the development of giant cells, root galls through hypertrophy and hyperplasia. The giant cells and galls disrupt uptake of nutrients and water from soil and interfere with plant growth (Sasser 1980, Sasser and Carter 1985).

About 2000 plant species including vegetables, small grains, fruits, field crops, nursery crops, ornamentals, forages and turf grasses are attacked by root-knot nematodes. They are major pests of vegetables, impacting both the quantity and quality of marketable yields (Taylor and Sasser 1978, Widmer *et al*. 2011). They are common pests of black pepper (Koshy *et al*. 1979), cowpea (Duncan and Ferris 1983), cucumber (Dropkin 1954), eggplant (Anon. 1986), okra (Bhatti and Jain 1977), potato (Mian 1987), tomato (Dareker and Mhase 1988). A report from India reveals that root-knot nematodes can cause 27% loss in eggplant (Reddy 1986). In that country 47% yield loss in tomato due to the disease has been recorded (Bhatti and Jain 1977, Darekar and Mhase 1988).

Root-knot nematodes are the most important plant-parasitic nematodes in Bangladesh (Timm and Ameen 1960, Talukder 1974). In a field survey throughout the country, Timm and Ameen (1960), Sam (1979), Chowdhury (1985), Mian (1986), Mian and Ali (1986) and Mian (1987) reported that root-knot nematodes are the most prevalent plant-parasitic nematodes in Bangladesh and the pests attack commonly grown vegetables such as amaranth, carrot, bottle gourd, white gourd, bitter gourd, beet, chili, coriander, cowpea, cucumber, garlic, Indian spinach, lettuce, okra, pea, potato, pumpkin, radish, ribbed gourd, sponge gourd, sugar beet and tomato. Stirling *et al*. (1992) reported that young plants are highly vulnerable to root-knot nematodes causing severe growth retardation.

Report on the effect of root-knot nematode infection on vegetable crop is scanty in Bangladesh. Choudhury (1985) reported from Bangladesh that root-knot disease reduced shoot and root growth of tomato. However, comprehensive reports on the effect of root-knot nematodes on the growth of common vegetables of the country are not available. Under the above circumstances the present piece of research was undertaken to find out the effect of root-

knot nematode infection at seedling stage on plant growth of eleven vegetable crops commonly grown in Bangladesh.

## MATERIALS AND METHODS

Eleven vegetable crops namely bottle gourd (*Lagenaria siceraria*), sweet gourd (*Cucurbita pepo*), white gourd (*Benincasa hispada*), cucumber (*Cucumis sativus*), eggplant (*Solanum melongena*), tomato (*Lycopersicon esculentum*), okra (*Abelmoschus eslentus*), Indian spinach (*Basella alba*), cauliflower (*Brassica oleracea* var. *botrytis*), green amaranth (*Amaranthus viridus*) and red amaranth (*Amaranthus tricolor*) were selected for the experiment.

The pot experiment was conducted in a pot house of Plant Pathology Department, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur during Kharif season (June to August) of 2010. Sandy loam soil was collected and steam sterilized for 4 hours. Urea, TSP and MOP were mixed with the soil at recommended doses (Anon. 1985). The prepared soil was poured into plastic pot (15 cm diameter) at 4 kg/pot. The pots were placed in the pot house following completely randomized design and kept for 48 hours for cooling of the soil.

The seeds of the selected crops were surface sterilized with 1.0% chlorox for 30 minutes and rinsed in sterilized water for three times. The surface sterilized seeds were planted in the pots. Each pot received 10-20 seeds depending on seed size. After germination the seedlings were thinned to have five healthy plants per pot. Eight pots (replication) were used for each crop.

Seven days old seedlings of each vegertable raised in 4 pots were inoculated with freshly hatched second stage active larvae of *Meloidoyne incognita* suspended in tap water at 2500 larvae per milliliter of water. Each pot received 1 ml of larval suspension. For inoculation, soil was removed from the base of the seedling and the larval suspension was spread uniformly near the exposed roots. After application of the inoculum suspension, the bases of the seedlings were covered with the soil. Immediately after inoculation water was sprayed over the pot soil. Seedlings in another 4 pots were not inoculated, which served as control for comparison. The seedlings were allowed to grow for 20 days providing necessary water and nutrients.

After 20 days of inoculation, both inoculated and uninoculated seedlings of each crop were removed carefully from the soil. At the time of uprooting precaution was taken to minimize root damage. The root systems were washed with running tap water to remove the adhering soil, wiped with tissue paper and number of galls per root system, height and fresh weight of shoot, and length and fresh weight of roots were recorded.

Populations of 2nd, 3rd, 4th stage larvae, immature and mature females in each root system of inoculated seedling were also recorded. To make the nematode in the root easily visible, the root system was stained following lactophenol cotton blue method (Mian 1994). The populations of $L_2$, $L_3$, $L_4$, immature female, mature female and eggs per root system were counted and their populations were expressed in number/20 g roots.

Collected data on all parameters related to plant growth, gall development and nematode population were analyzed using MSTAT-C software. Paired-t test was performed to compare growth of inoculated and uninoculated seedlings. Means were compared following Duncan's Multiple Range Test using the same computer program. Whenever, necessary data were transformed following suitable method. Relationships among some selected variables were also determined using MSTAT-C program.

## RESULTS AND DISCUSSION

### Reduction in height and weight of shoot

Infection of seedlings of all crops with *M. incognita* caused significant (P=0.01) reduction in shoot height within the range of 16.83 to 63.65% over uninoculated control. The reduction in okra, eggplant, tomato, Indian spinach and cauliflower was statistically similar and significantly lower compared to bottle gourd, sweet gourd and white gourd. Reduction in shoot height of latter three crops was also ot significantly different (Table 1).

Fresh shoot weight of eleven vegetable crops was reduced by 15.50% to 46.652% over uninfected seedlings under control. The reduction was highly significant (P=0.01). The lowest reduction was recorded from cucumber followed by tomato, eggplant, bottle gourd, okra sweet gourd and red amaranth. Differences in their shoot weight were not significantly different. The maximum reduction was found in cauliflower followed by white gourd and Indian spinach. The differences in the parameter among three crops were also not significant (Table 2).

### Reduction in length and weight of root

Infection with *M. incognita* at seedling stage caused reduction in root length of all crops

significantly over control within the range of 23.30 to 57.53%. Difference in root length of inoculated and uninoculated plants of every crop was highly significant. The maximum decrease in root length was recorded from cucumber followed by sweet gourd, red amaranth, bottle gourd, white gourd, Indian spinach and green amaranth. Differences in root length reduction of those six crops were statistically similar and significantly higher compared to only okra, tomato and eggplant (Table 3).

**Reduction in root weight**

Difference in root weight of uninoculated and inoculated plants of each crop tested was highly significant (P=0.01). The reduction ranged 22.18-74.00% in different crop species. The lowest reduction of root weight over control was observed in cauliflower and cucumber, which were statistically similar to bottle gourd, sweet gourd, eggplant, tomato and red amaranth. The reduction in root weight of seedling of Indian spinach, green amaranth and okra was also statistically similar but significantly higher compared to other crops (Table 4). Significantly the highest egg number was found in roots of bottle gourd. The second highest gall number was found in roots of sweet gourd, which was statistically similar to white gourd. The egg number in roots of cucumber, cauliflower and tomato was statistically similar but significantly lower compared to all other vegetable seedlings. The gall number in roots of red amaranth, green amaranth and okra was also statistically similar and significantly lower compared to three gourds. Gall number in roots of eggplant was also significantly lower compared to three gourds (Table 5).

Table 1. Influence of root-knot nematode (*M. incognita*) infection on shoot height of seedlings of eleven vegetable crops

| Crop | Shoot height (cm) | | % Reduction[a] | Paired-t value |
|---|---|---|---|---|
| | Uninoculated control | Inoculated | | |
| Bottle gourd | 44.25 | 17.46 | 60.55 AB | 24.09** |
| Sweet gourd | 60.67 | 22.05 | 63.65 A | 11.49** |
| White gourd | 39.68 | 20.53 | 48.25 BC | 4.25** |
| Cucumber | 80.05 | 48.57 | 39.33 CD | 7.17** |
| Eggplant | 28.42 | 23.35 | 17.83 F | 7.81** |
| Tomato | 47.53 | 33.25 | 30.05 DEF | 7.23** |
| Okra | 65.60 | 54.56 | 16.83 F | 9.00** |
| Indian spinach | 36.90 | 27.35 | 25.88 DEF | 6.60** |
| Cauliflower | 17.55 | 13.81 | 21.33 EF | 7.25** |
| Green amaranth | 44.80 | 28.12 | 37.23 CD | 7.25* |
| Red amaranth | 45.30 | 29.52 | 34.83 CDE | 6.53** |

[a]Values within the same column with a common letter(s) do not differ significantly.
**Paired-t is highly significant (P=0.01).

Table 2. Influence of root-knot nematode (*M. incognita*) infection on fresh shoot weight of seedlings of eleven vegetable crops

| Crop | Shoot weight (g) | | % Reduction[a] | Paired-t value |
|---|---|---|---|---|
| | Uninoculated control | Inoculated | | |
| Bottle gourd | 33.08 | 23.81 | 28.03 B-E | 7.06** |
| Sweet gourd | 40.02 | 27.33 | 31.70 A-E | 7.55** |
| White gourd | 18.03 | 9.83 | 45.48 ABC | 7.44** |
| Cucumber | 34.74 | 29.36 | 15.50 E | 6.40** |
| Eggplant | 6.48 | 4.74 | 26.80 CDE | 5.83** |
| Tomato | 6.51 | 4.82 | 25.98 DE | 4.64** |
| Okra | 20.41 | 14.20 | 30.43 A-E | 6.47** |
| Indian spinach | 26.65 | 15.36 | 42.35 A-D | 6.90** |
| Cauliflower | 1.00 | 0.53 | 46.65 AB | 5.06** |
| Green amaranth | 10.12 | 5.93 | 41.38 ABC | 6.51** |
| Red amaranth | 1.20 | 0.82 | 31.85 A-E | 14.12** |

[a]Values within the same column with a common letter(s) do not differ significantly.
**Paired-t is highly significant (P=0.01).

Table 3. Influence of root-knot nematode (*M. incognita*) infection at seedling ages on the reduction over control of root length of eleven vegetable crops

| Crop | Root length (cm) | | % Reduction[a] | Paired-t value[b] |
|---|---|---|---|---|
| | Uninoculated control | Inoculated | | |
| Bottle gourd | 43.90 | 21.59 | 50.83 AB | 8.76** |
| Sweet gourd | 34.45 | 16.59 | 51.83 A | 8.24** |
| White gourd | 24.43 | 12.25 | 49.85 AB | 7.01** |
| Cucumber | 57.06 | 24.23 | 57.53 A | 5.12** |
| Eggplant | 19.88 | 13.56 | 31.80 CD | 4.98** |
| Tomato | 19.96 | 15.31 | 23.30 D | 6.90** |
| Okra | 42.77 | 26.63 | 37.73 BC | 7.31** |
| Indian spinach | 25.72 | 13.86 | 46.13 AB | 8.76** |
| Cauliflower | 12.69 | 7.90 | 37.75 BC | 7.47** |
| Green amaranth | 16.56 | 8.92 | 46.13 AB | 4.46** |
| Red amaranth | 17.62 | 13.59 | 50.83 AB | 5.87** |

[a]Values within the same column with a common letter(s) do not differ significantly.

**=Paired-t is highly significant (P=0.01).

Table 4. Influence of root-knot nematode (*M. incognita*) infection at seedling ages on the reduction of root weight over control of eleven vegetable crops

| Crop | Root weight (g) | | % Reduction[a] | Paired-t value[b] |
|---|---|---|---|---|
| | Uninoculated control | Inoculated | | |
| Bottle gourd | 12.98 | 8.05 | 38.00 BC | 9.25** |
| Sweet gourd | 7.92 | 4.85 | 38.80 BC | 5.42** |
| White gourd | 5.58 | 3.22 | 42.28 B | 9.94** |
| Cucumber | 12.06 | 9.39 | 22.18 C | 13.47** |
| Eggplant | 1.6 | 0.99 | 37.88 BC | 7.62** |
| Tomato | 1.21 | 0.91 | 24.70 C | 7.34** |
| Okra | 10.09 | 3.93 | 61.03 A | 9.08** |
| Indian spinach | 6.23 | 1.62 | 74.00 A | 17.15** |
| Cauliflower | 0.79 | 0.61 | 22.18 C | 6.28** |
| Green amaranth | 1.58 | 0.61 | 61.53 A | 8.88** |
| Red amaranth | 1.20 | 0.84 | 38.00 BC | 14.12** |

[a]Values within the same column with a common letter(s) do not differ significantly.

**=Paired-t is highly significant (P=0.01).

**Population of 2nd, 3rd and 4th stage larvae, immature female and mature female**

The population second stage larvae ($L_2$) in different vegetables ranged 0.27-4.99/20 g of root. The maximum number $L_2$ was found in roots of cauliflower followed by tomato and eggplant. Their population in those three crops was not significantly different. The lowest number of $L_2$ was recorded from the roots of Indian spinach, which was statistically similar to green amaranth, red amaranth, bottle gourd, sweet gourd, white gourd, cucumber and Okra (Table 6). The maximum of 12.08 third stage larvae per 20 g of root was found in tomato, which was statistically similar to only cauliflower. The third highest $L_3$ population was observed in roots of eggplant, which statistically similar to cauliflower. Their lowest population of 0.42/20 g root was recorded from roots of Indian spinach followed by green amaranth, red amaranth, white gourd, okra, bottle gourd and sweet gourd. Differences in their populations in roots of those seven vegetables were not significant. The trends in population dynamics of the fourth stage larvae ($L_4$) developed in roots of eleven vegetable seedlings were almost similar to the population dynamics of $L_3$ (Table 6).

**Population of immature and mature females**

In case of immature and mature females, significantly the highest populations of were found in roots of tomato. The second highest populations of the nematodes were found in roots of cauliflower, which was statistically similar to cucumber and eggplant. Their lowest populations were found in roots of Indian spinach, which was statistically similar to red amaranth, green amaranth, okra, and three gourds (Table 6).

Table 5. Number of galls and eggs in roots of eleven vegetable seedlings inoculated with root-knot nematode.

| Crop | Gall number/20 g root | Egg number/20 g root |
|---|---|---|
| Bottle gourd | 15.30 D | 3482.00 A |
| Sweet gourd | 14.97 D | 2039.73 B |
| White gourd | 11.69 DE | 1860.55 B |
| Cucumber | 29.27 C | 120.85 G |
| Eggplant | 40.80 B | 1161.82 C |
| Tomato | 69.82 A | 318.06 FG |
| Okra | 16.89 D | 847.60 DE |
| Indian spinach | 2.88 F | 985.85 CD |
| Cauliflower | 42.36 B | 198.88 G |
| Green amaranth | 3.86 F | 733.32 E |
| Red amaranth | 8.12 EF | 585.64 EF |

Values within the same column with a common letter(s) do not differ significantly (P=0.05)

Table 6. Populations of nematodes at different stages recorded in the roots of eleven vegetable crops inoculated with root-knot nematode at seedling stage

| Crop | Population per 20g root | | | | |
|---|---|---|---|---|---|
| | $L_2$ | $L_3$ | $L_4$ | Immature female | Mature female |
| Bottle gourd | 1.40CD | 3.26CDE | 5.37CD | 9.57 CDE | 9.05BCDE |
| Sweet gourd | 1.31CD | 3.30CDE | 5.10CD | 9.30CDE | 7.81CDE |
| White gourd | 1.19CD | 2.50DE | 3.91CD | 9.84CDE | 8.39CDE |
| Cucumber | 2.35BCD | 4.13CD | 8.34C | 15.76BCD | 14.04BCD |
| Eggplant | 2.69ABC | 6.87BC | 9.28BC | 17.88 BC | 16.41BC |
| Tomato | 4.32AB | 12.08A | 16.43A | 35.46A | 33.14A |
| Okra | 1.11CD | 2.85D | 4.04CD | 7.35DE | 6.65DE |
| Indian spinach | 0.27D | 0.42E | 0.57D | 2.67E | 2.20E |
| Cauliflower | 4.99A | 9.20AB | 14.65AB | 20.63B | 17.95B |
| Green amaranth | 0.35CD | 0.43DE | 0.74D | 3.86E | 3.31E |
| Red amaranth | 0.91CD | 0.99DE | 1.56D | 9.22CDE | 7.44CDE |

Values within the same column with a common letter(s) do not differ significantly (P=0.05)

Results of the experiment show that shoot and root growth of all eleven vegetables were significantly reduced due to inoculation with root-knot nematode at seedling stage. Reduction of height and fresh weight of shoot, and length and fresh weight of root varied from 16.83 to 63.65%, 15.50% to 46.652%, 23.30 to 57.53% and 22.18 to 74.00%, respectively among different crop species. Number of galls and eggs per 20 g roots ranged 2.88-69.82 and 120.85-3482.00, respectively. The highest gall number was found in roots of tomato and the lowest in roots of Indian spinach. The lowest egg number was found in roots of cucumber and the highest in tomato roots. Among eleven vegetables, the highest population of $L_3$ and $L_4$ larvae, and immature as well as mature females were recorded from roots of tomato followed by cauliflower and eggplant. On the other hand, the maximum number of $L_2$ was found in roots of cauliflower followed by tomato and eggplant. It indicates that tomato is highly vulnerable to the nematode followed by eggplant and cauliflower.

The findings of the present investigation are in agreements with the findings of many other investigators worked with different crops including most of them tested in the present experiment. They reported appreciable reduction in plant growth and crop yield of cucumber (Dropkin 1954), eggplant (Anon. 1986), tomato (Hanounik *et al.* 1975, Chowdhury 1985, Dareker and Mhase 1988) and okra (Bhatti and Jain 1977). A report from India, reveals that root-knot nematodes can cause yield loss in eggplant (Reddy 1986) and 47% in tomato (Zahid and Ahmed 1986, Darekar and Mhase 1988). Based on findings of the present investigation it may be concluded infection of root-knot nematodes at seedling stage causes severe reduction in plant growth of vegetable seedlings commonly grown in Bangladesh.

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# CHEMICAL CONTROL OF *PSEUDOCERCOSPORA* LEAF SPOT (*P. ABELMOSCHI*) OF OKRA SEED CROP

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ABSTRACT

M. G. Kibria and I. H. Mian. 2012. Chemical control of *Pseudocercospora* leaf spot (*P. abelmoschi*) of okra seed crop. Bangladesh J. Plant Pathol. 28 (1&2): 53-60.

Results of a field experiment conducted to evaluate the efficacy of five fungicides against *Pseudocercospora* (*P. abelmoschi*) leaf spot (PLS) or black mould of okra seed crops showed that foliar spray with Aimcozim 200 EW (Carbendazim) @ 0.10%, Emivit 50 WP (Copper Oxychloride) @ 0.35%, Indofil M-45 (Mancozeb) @ 0.20%, Tilt 250 EC (Propiconazole) @ 0.05% and Folicur 250 EC (Tebuconazole) @ 0.10% decreased percent disease index (PDI) by 485.74-2465.77, 240.74-970.68, 124.39-537.50, 92.81-366.50 and 72.80-209.40% reduction in PDI, respectively over control recorded at 65, 72, 79, 86 days after sowing. On the other hand, the fungicides increased plant height, fruit yield, seed yield, seed germination and seed vigor index by 7.50-30.78% in 18.88-46.70% in 29.02-94.02% in 15.03-19.79% in 21.71-58.07%, respectively over control. The maximum reduction in disease severity and increase in plant growth, yield and yield attributes were achieved with Emivit followed by Aimcozim, Folicur and Tilt. Based on findings of the study Emivit 50 WP and Folicur 250 EC may be recommended to control PLS of okra seed crop.

**Key words:** Okra, *Pseudocercospora* leaf spot, Chemical control.

## INTRODUCTION

Okra also known as lady's finger (*Abelmoschus esculentus*) is widely cultivated and popular vegetable crop in Bangladesh. It can be grown round the year in the country except few cool months (Mid December to late February). However, summer (Kharif) is the best season for growing okra for good fruit. In Kharif season, humidity and temperature are high and okra plants are vulnerable to many fungal pathogens causing various diseases on plants and fruits. In Bangladesh, *Pseudocercospora* leaf spot (PLS) or black mould caused by *Pseudocercospora abelmoschi* (*Cercospora abelmoschi*) is a common disease of okra especially okra seed crop grown during late winter and early summer (Pabitra 2009, Anon. 2011, Jiskani 2011). The disease causes tremendous yield loss of okra seed crop (Pabitra 2009).

The most effective and easily available method to control the disease is application of chemical fungicides (Pant and Mukhopadhayay 2001, Singh *et al*. 2003). Report from India reveals that *Cercospora* causes sooty black, angular spots which are responsible for severe defoliation. Spraying Mancozeb or Zineb 2 g or Carbendazim 1 g per liter of water is recommended to control the disease (Anon. 2011).

To grow healthy seed crop of okra it is necessary to develop suitable methods of PLS control. Generally, fungicides are recommended to control foliar okra diseases including PLS (Marium *et al*. 2000). A study was conducted in Bangladesh to evaluate the efficacy of Thiovit (0.45%) and Bavistin (0.1%) to control PLS (*P. abelmoschi*). Bavistin applied alone at 15 days interval showed the lowest disease incidence followed by Thiovit+Bavistin at 30 days interval. The number of fruits/plant, number of seeds/fruits, and seed yield/plot increased due to those treatment. Bavistin and Thiovit were recommended to control PLS (Rahman *et al*. 2000). However, it is not wise to depend on only two fungicides to such an economically important disease of okra. Considering the above facts, the present study was undertaken to evaluate the efficacy of five fungicides to control PLS of okra seed crop.

## MATERIALS AND METHODS

Five fungicides namely Aimcozim 200 EW (Carbendazim), Emivit 50 WP (Copper Oxychloride), Indofil M-45 (Mancozeb), Tilt 250 EC (Propiconazole) and Folicur 250 EC (Tebuconazole) were tested against PLS of okra seed crop under natural field conditions. The fungicides were selected based on their effectiveness against foliar diseases of okra as reported by many other investigators (Pant and Mukhopadhayay 2001, Singh *et al.* 2003).

The experiment was conducted in the experimental farm of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh during March to June 2009. The experiment was laid out following a randomized complete block design with three replications. The unit plot size was 4.5 m × 1.2 m. Drains of 50 cm width and 30 cm depth were dug around each unit plot for facilitating irrigation and drainage of excess water. The experimental field was prepared properly for good tilth following standard practices (Razzaque *et al.* 2000). Fertilizers were applied @ 50-24-30-10-1-0.5 kg of N-P-K-S-Zn-B per hectare, respectively. Manure was applied as cowdung at the rate of 4 t/ha (Anon. 2005). The entire quantity of cowdung, P, K, S, Zn and B and half of N were applied at the time of final land preparation. Remaining N was applied around the base of the plant as top dress and incorporated with soil at 3rd and 5th week after sowing. Genotype BD 1911 was used in this experiment. It is susceptible to *Pseudocercospora* leaf spot. Seeds of the genotype were soaked in tap water for 12 hours. Water soaked seeds were dibbled in the field at 3-4 seeds/hill on 15 March 2008. Seeds of okra were sown at the rate of 3-4 seeds per pit maintaining row to row and plant to plant spacing of 60 and 50 cm, respectively. After 6 to 7 days of germination, the seedlings were thinned and only one apparently healthy seedling was allowed to grow per pot or per pit. If any seedling died within three weeks of germination it was replaced by seedling of same age raised in polyethylene bags (9 x 15 cm). The growth medium used in polyethylene bags was prepared by mixing with decomposed cowdung and soil in equal proportion. Irrigation and other necessary intercultural operations were done throughout the cropping season for proper growth and fruit production. Irrigation was applied after each top dressing of urea and whenever necessary. Every time, irrigation was followed by mulching and weeding.

Suspensions of Aimcozim, Emivit 50 WP, Indofil M-45, Tilt 250 EC and Folicur 250 EC were prepared in water at 0.1, 0.35, 0.20, 0.05 and 0.10% concentration, respectively and were applied as foliar spray using a knapsack sprayer starting from the onset of PLS (78 days after sowing) and continued for four weeks with 7 days interval.

Data on severity of PLS was recorded at 65, 72, 79 and 86 days after sowing (DAS) based on a 0-8 scale (Rahman and Nahar 1990). Disease severity was expressed in Percent Disease Index (PDI) for the leaf spot, which was computed according to the following a standard formula as described by Wheeler, (1969) and Mian (1995):

$$\text{Percent disease Index} \frac{\text{Sum of numerical rating *}}{\text{Total No. ofleaves observed} \times \text{Max. grade}} \times 100$$

**The numerical ratings were obtained by multiplying the number of leaves with their respective grades of indexing scale*

Data on plant growth, seed yield, selected yield contributing parameters, and seed quality were also recorded. Quality of seeds in terms of germination, seedling growth and vigor index of okra seeds was determined following methods of International Seed Testing Association (ISTA) (Anon. 1996). Seedling vigour was determined using a standard formula (Baki and Anderson, 1972) viz. Vigour Index = (mean root length + mean shoot length) x percent emergence. Fruits were collected from each of the unit plot after their ripening and seeds were separated and processed. Recorded data were subjected to statistical analysis following standard procedure (Gomez and Gomez 1984) using MSTAT-C statistical software. ANOVA was performed and means were compared following DMRT. Whenever necessary, data were transformed using ArcSin method before performing ANOVA.

## RESULTS

### Effect on PLS severity

Maximum PDI of 35.13, 42.31, 51.21 and 56.96 was observed under control at 65, 72, 79 and 86 DAS, respectively. The disease severity was reduced to 6.21- 20.33 at 65 DAS, 5.92-23.93 at 72 DAS, 3.01-21.20 at 79 DAS and 2.22-18.41 at 86 DAS due to application of Indofil M-45, Tilt 250 EC, Folicur 250 EC, Aimcozim 200 EW and Emivit 50 WP, respectively. The reduction was significant (P=0.05) compared to control (Table 1). The maximum reduction was achieved with Emivit 50 WP followed by Aimcozim, Folicur 250 EC, Tilt 250 EC and Indofil M-45. The fungicides gave 485.74-2465.77, 240.74-970.68, 124.39-537.50, 92.81-366.50 and 72.80-209.40% reduction in PDI, respectively (Fig. 2). Differences in rates of reduction of PLS severity under different fungicides was significant.

### Relationship of PDI with Days after sowing

At all stages of data collection (65, 72, 79, 86 DAS), the PDI of PLS were maximal under control, which was followed by Indofil, Tilt, Folicur,

Aimcozim and Emivit. Severity of PLS (PDI) was positively and linearly correlated with DAS and their relationship was highly significant under control. Under all fungicidal treatments the relationship between PDI and DAS was also linear and significant but negative (Fig. 1). The results indicate that application of the fungicides reduces severity of PLS gradually with the plant age due to decrease in inoculum potential after fungicidal sprays.

**Effect on plant height**

Foliar spray with all fungicides increased plant height significantly over control except Indofil M-45. The maximum increase was achieved with Emivit 50 WP followed by Aimcozim 200 EW, Folicur 200 EW and Tilt 250 EC. Their effectiveness to increase plant height was not significantly different with an exception (Table 2).

**Effect on Yield number per plant and fruit size**

Application of each fungicide increased the fruit number per plant and fruit length over control. The highest number of fruits was achieved with Emivit 50 WP, which was statistically similar to Aimcozim 200 EW and Tilt 250 EC. Significant increase in fruit length was achieved with only Emivit 50 WP, Aimcozim 200 EW and Folicur 250 EC. Differences in diameter of fruits under different treatments including control were not significant at any stage of data collection (Table 2).

Table 1. Severity of *Pseudocercospora* leaf spot of okra seed crop sprayed with five fungicides against the disease recorded at different day after sowing (DAS)

| Fungicide | Percent disease index (PDI) | | | |
|---|---|---|---|---|
| | 65 DAS | 72 DAS | 79 DAS | 86 DAS |
| Control | 35.13 a (36.35) | 42.31 a (40.57) | 51.21 a (45.69) | 56.96 a (49.00) |
| Indofil M-45 | 20.33 b (26.80) | 23.93 b (29.28) | 21.20 b (27.41) | 18.41 b (25.40) |
| Tilt 250 EC | 18.22 c (25.26) | 16.03 c (23.60) | 14.29 c (22.21) | 12.21 c (20.45) |
| Folicur EW 200 | 15.94 d (23.53) | 13.20 d (21.30) | 11.93 d (20.20) | 8.93 d (17.38) |
| Aimcozim | 10.31 e (18.72) | 9.01 e (17.46) | 7.11 e (15.46) | 5.32 e (13.33) |
| Emivit 50 WP | 6.21 f (14.42) | 5.92 f (14.08) | 3.01 f (9.99) | 2.22 f (8.57) |

Figures within parentheses are ArcSin(x+1) transformed values.

Means within each column followed by a common letter(s) do not differ significantly (P=0.05) by DMRT.

**Effect on seed yield and seed size**

Number of seeds per fruit ranged 40.38-47.74 under different treatments including control. Their differences were not significant. All fungicides gave significant increase in seed yield per plant over control. The maximum seed yield was obtained with Emivit 50 WP followed by Aimcozim 200 EW, Folicur 250 EC and Tilt 250 WP. Seed size in terms of 1000-seed weight varied from 56.32-63.00 g. The highest 1000-seed weight was observed under the treatment with Emivit 50 WP and the lowest under control. However, the differences were not significant (Table 3).

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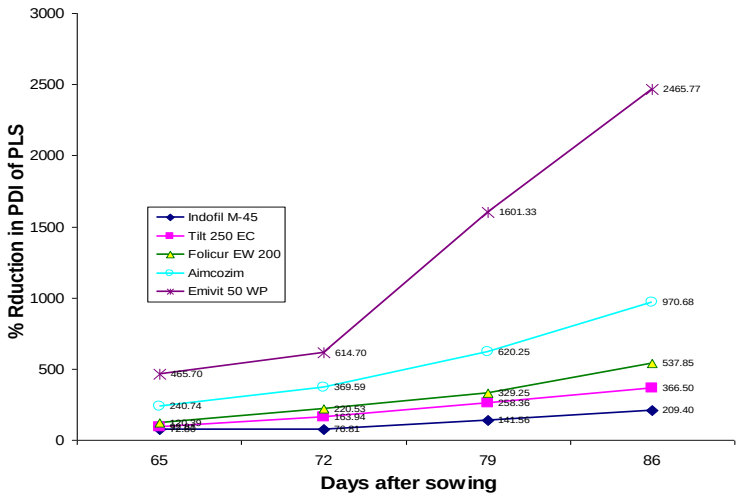


Fig. 1. Reduction in severity of *Pseudocercospora* leaf spot of okra due to spray with five fungicides recorded at different days after sowing

Table 2. Increase in plant height and yield and size of fruit due to spray with fungicides to control *Pseudocercospora* leaf spot of okra seed crop

| Fungicide | Plant height (cm) | Fruits number per plant | Fruit size (cm) | |
|---|---|---|---|---|
| | | | Length | Diameter |
| Control | 70.59 c | 9.85 d | 13.61 c | 1.98 a |
| Indofil M-45 | 75.89 bc | 11.71 c | 14.32 bc | 1.95 a |
| Tilt 250 EC | 83.71 ab | 13.61 ab | 14.59 bc | 2.01 a |
| Folicur EW 200 | 84.86 ab | 12.82 bc | 15.56 abc | 1.97 a |
| Aimcozim 200 EW | 89.21 a | 13.93 ab | 16.42 ab | 2.5 a |
| Emivit 50 WP | 92.32 a | 14.45 a | 17.01 a | 2.04 a |

Means within the same column with a common letter(s) are not significantly (P=0.05) different by DMRT.

Table 3. Increase in seed yield and yield contributing parameters of okra seed crops due to foliar spray with five fungicides to control *Pseudocercospora* leaf spot

| Fungicide | No. of seeds per fruit | No. of seeds per plant | 1000 seed weight (g) | Seed yield per plant (g) |
|---|---|---|---|---|
| Control | 40.38 | 397.74 d | 56.32 | 22.40 d |
| Indofil M-45 | 41.82 | 489.71 c | 59.01 | 28.90 c |
| Tilt 250 EC | 42.79 | 582.37 b | 60.23 | 35.08 b |
| Folicur EW 200 | 46.51 | 596.26 b | 59.29 | 35.35 b |
| Aimcozim 200 EW | 45.42 | 632.70 ab | 61.21 | 38.73 b |
| Emivit 50 WP | 47.74 | 689.78a | 63.00 | 43.46 a |

Means within the same column with a common letter(s) are not significantly (P=0.05) different by DMRT.

**Quality of seeds harvested from seed crop sprayed with different fungicides**

**Germination:** Germination of seeds harvested from the seed crop increased significantly over control due to spray with five fungicides. The highest increase was achieved with Emivit 50 WP followed by Aimcozim 200 EW and Tilt 250 EC. Efficacy of these three fungicides to increase germination was not significantly different (Table 4).

**Shoot and root length of seedlings:** Seeds harvest from okra seed crop received foliar sprays with five fungicides against PLS gave considerable increase in shoot length of seedling compared to control. The increase was significant when the seed crop was sprayed with Folicur 250 EC and Emivit 50 WP. The root length of seedling was the highest where seeds were harvested from plants sprayed with Emivit 50 WP followed the Aimcozim 200 EW. The efficacy of the two fungicides to increase seedling root length was significant compared to control. Other three fungicides also because increase root length of seedling but the increased was not significant compared to control (Table 4).

**Seed vigor index:** The seed vigor index was 900.00 under control. It was increased to 1492.88, 1422.60, 1342.00, 1205.43 and 1095.42 when the okra plants were sprayed with Emivit 50 WP, Folicur 250 EC, Aimcozim 200 EW, Tilt 250 EC and Indofil M-45, respectively. The increase was significant under all fungicide compared to control (Table 4).

**Increase in plant growth, fruit and seed yield, seed germination and seed vigor**

Spray of okra seed crop with Emivit 50 WP, Aimcozim 200 EW, Folicur 250 EC, Tlt 250 WP and Indofil M-45 increased plant height, fruit yield, seed yield, germination and seed vigor index by 7.50-30.78%, 18.88-46.70% in 29.02-94.02% in 15.03-19.79% in 21.71-58.07%, respectively over control (Table 4 and Fig. 2).

Results of the present experiment showed that PDI values of PLS was maximal under control. Foliar spray of okra seed crops with five fungicides namely Emivit 50 WP, Aimcozim 200 EW, Folicur 250 EC, Tilt 250 EC and Indofil caused considerable reduction in PDI of the disease. Maximum reduction in PDI of PLS was achieved by applying Emivit followed by Aimcozim, Folicur, Tilt and Indofil. The rate of reduction increased gradually with the progress of age of okra plants. The treatments with Emivit 50 WP, Aimcozim 200 EW, Folicur 250 EC, Tlt 250 WP and Indofil M-45 gave 7.50-30.78% in plant height, 18.88-46.70% in fruit yield, 29.02-94.02% in seed yield, 15.03-19.79% in germination and 21.71-58.07% in seed vigor index increase over control, respectively.

Table 4. Effect of foliar spray on okra seed crop with five fungicides to control *Pseudocercospora* leaf spot influencing seed quality characters

| Fungicide | % Germination | Shoot length (cm) | Root length (cm) | Seed vigour index |
|---|---|---|---|---|
| Control | 65.54 c (54.07) | 8.91 c | 4.82 c | 900.00 d |
| Indofil M-45 | 75.39 b (60.32) | 9.32 bc | 5.21 c | 1095.42 c |
| Tilt 250 EC | 77.42 ab (61.69) | 10.04 bc | 5.53 bc | 1205.43 bc |
| Aimcozim 200 EW | 81.63 ab (64.73) | 10.32 bc | 6.12 b | 1342.00 ab |
| Emivit 50 WP | 85.21 a (67.63) | 10.51 b | 7.01 a | 1492.88 a |
| Folicur 250 EC | 78.51 ab (62.45) | 12.50 a | 5.62 bc | 1422.60 a |

Figures within parentheses are ArcSin(x+1) transformed values.

Means within each column followed by a common letter(s) do not differ significantly (P=0.05) by DMRT.

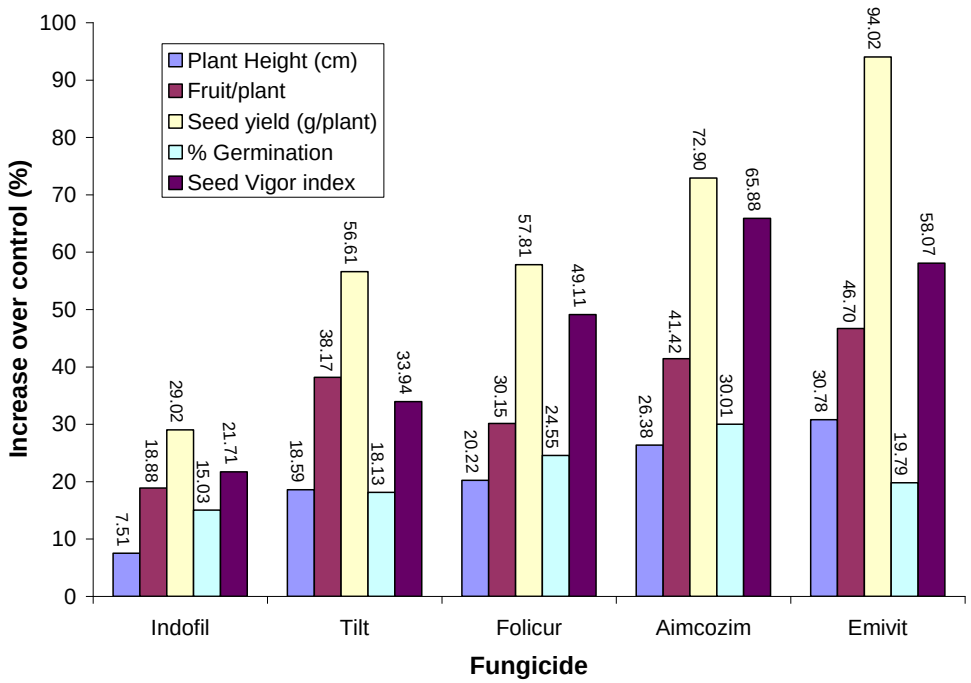


Fig. 2. Increase in plant growth, fruit and seed yield, seed germination and seed vigor index over control due to spray on okra seed crop with five fungicides to control *Pseudocercospora* leaf spot

Findings of the present study are in agreement with the findings of many other investigators worked in Bangladesh and abroad. They also achieved satisfactory control PLS using foliar spray with fungicides. Srivastava *et al.* (1992) found the best control of *Pseudocercospora* on okra in field trials with Copper oxychloride followed by Carbendazim. Anon. (2011) mentioned the recommendation of spraying Mancozeb or Zineb 2 g or Carbendazim 1 g per liter of water to control the disease.

In Philippines, Benlate 50 WP (Benomyl), Daconil 75 WP (Chlorothanil) and Funguran-OH (Copper hydroxide) are recommended to control PLS (Anon 2011). In Bangladesh, Rahman *et al.* (2000) evaluated the efficacy of Thiovit (0.45%) and Bavistin (0.1%) to control of *Pseudocercospora* leaf spot of okra. They found that Bavistin applied alone at 15 days interval showed the lowest incidence of the disease followed by Thiovin+Bavistin applied at 30 days interval. The yield and yield attributes of okra increased due to those treatment compared to control. They recommended Bavistin and Thiovit against PLS. Pabitra (2009) reported that Bavistin and Proud are effective to control PLS of okra and to improve plant growth, yield and seed quality and seed health. Based on findings of the present investigation Emivit 50 WP and Folicur 250 EC may be recommended to control PLS of okra.

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# COMPATIBILITY OF AN ISOLATE OF *TRICHODERMA HARZIANUM* WITH FUNGICIDES AND ORGANIC AMENDMENTS

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## ABSTRACT

Tanvir Rubaet and M. K. Alam Bhuiyan. 2012. Compatibility of an Isolate of *Trichoderma harzianum* with fungicides and organic amendments. Bangladesh J. Plant Pathol. 28 (1&2): 61-64.

An *in-vitro* study was undertaken to determine the effect of three fungicides namely Provax-200 (Carboxin), Rovral 50 WP (Iprodione) and Bavistin 50 WP (Carbendazim), and four organic materials namely mustrard oilcake, wheat meal, chickpea meal, rice bran and tea waste on mycelium growth and sporulation of an isolate of *Trichderma harzianum* (T-10) following "poison food technique". The fungicides were used at 50, 100, 250 and 500 ppm concentrations. At different concentrations, inhibition in colony diameter ranged 2.22-18.89%, 7.78-22.22% and 90.00-100.00% under Provax-200, Rovral 50 WP and Bavistin 50 WP, respectively. The inhibition in sporulation was 4.52-11.76, 10.07-21.04% and 90.50-100.00%, respectively. Inhibition of mycelium diameter due to amendment of PDA with mustard oilcake, tea waste, rice bran, chickpea meal and wheat meal ranged 0.00-36.30, 2.22-54.07 and 6.30-72.96%, respectively. The organic amendments caused respectively 6.02-53.01, 15.66-71.08 and 25.30-79.52% inhibition in sporulation. The rate of inhibition was corroborated with concentrations of fungicides. The findings of the study indicate that *T. harzianum* isolate T-10 was compatible with Provax-200 and Rovral 50 WP only at lower concentration. The biocontrol fungus showed insensitivity to all organic amendments at their lower concentrations except tea waste.

**Keywords**: *Trichoderma harzianum,* fungicide, organic amendment, compatibility.

Due to their excellent effectiveness, generally species of *Trichoderma* are used as biocontrol agents for against plant diseases. Insensitivity characters of biocontrol agents to other disease control measures especially fungicides are also desirable because such biocontrol agents are usable in integrated disease management program. Therefore, many researchers test sensitivity of selected biocontrol agents to fungicidal chemicals before recommendation to use. Available reports reveal that some species of *Trichoderma* are resistant to several chemical fungicides such as methyl bromide, PCNB (Pentrachloronitrobenzene), Captan, Chlorable-M and metalaxyl (Elad *et al.* 1980). Such research reports are scanty in Bangladesh (Raihan *et al* 2003, Khandakar *et al.* 2010).

An *in-vitro* experiment was conducted to determine the effect of three fungicides namely Provax-200 (Carboxin), Rovral 50 WP (Iprodione) and Bavistin 50 WP (Carbendazim) on mycelium growth and sporulation of an isolate of *T. harzianum* (T-10) following "poison food technique" (Dhingra and Sinclair 1985). Each of the fungicides was tested at 50, 100, 250 and 500 ppm concentrations. Potato dextrose agar (PDA) was prepared and poured into conical flasks at 100 ml/flask. Before solidification requisite quantity of individual fungicide was thoroughly mixed with the freshly prepared PDA to have desired concentrations of individual fungicide. The amended PDA was sterilized in an autoclave at 120C under 1 kg pressure per 1 $cm^2$ for 20 minutes. Sterilized medium was poured into 90 mm Petri dishes at 20 ml/dish. After solidification of amended PDA, the plates were inoculated with wheat grains colonized with *T. harzianum*. One wheat grain was placed in the center of each plate. Plates under control received unamended PDA. The radial diameter of the fungal colony and sporulation was recorded 7 days after incubation when the control plates were covered with the growth of test isolate of *T. harzianum.* Percent inhibition of the radial growth and sporulation was computed following standard procedures (Sundar *et al.* 1995).

Another *in-vitro* experiment was conducted to determine the effect of mustard oil cake, tea waste, rice bran, chickpea meal and wheat meal on the growth and sporulation of the isolate of *T. harzianum (*T-10) following the some technique mentioned earlier (Dhingra and Sinclair 1985). All of the organic amendments were tested @ 10, 20 and 30%. Freshly prepared PDA was poured into 250 ml conical flasks

at 100 ml/flask. Requisite amount of individual organic material was poured into the conical flasks and thoroughly mixed with the PDA. The amended PDA was autoclaved for sterilization. Sterilized PDA was poured into 90 mm Petri dishes at 20 ml per plate. Petri dishes under control received fresh PDA without amendment. The procedures of inoculation and data collection were the same as mentioned earlier.

The experiments were conducted in the plant pathology laboratory, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur following completely randomized design. Data were analyzed using MSTAT-C computer program. Differences in means of the treatments were evaluated for significant following Duncan's multiple range tests.

Effect of fungicides on growth and sporulation of *T. harzianum* is shown in Table 1. At different concentrations of Provax-200, inhibition in colony diameter and sporulation of *T. harzianum* isolate ranged 2.22-18.89% and 4.52-11.76%, respectively. In case of Rovral 50 WP, inhibition in two parameters was 7.78-22.22% and 10.07-21.04%, respectively. Complete inhibition of colony growth was obtained with Bavistin at 250 and 500 ppm. At 50 and 100 ppm, the fungicide caused 90.00 and 95.56% inhibition of colony growth, respectively. The sporulation of *T. harzianum* was inhibited by 90.50% at 50 ppm and 100% at three higher concentrations of Bavistin 50 WP. The rate of inhibition was corroborated with concentrations of two fungicides (Table 1).

The findings indicate that Bavistin 50 WP is highly effective fungicide to inhibit growth and sporulation of *T. harzianum*. In other words, the fungicide is highly incompatible with the biocontrol agent. Inhibition in growth and sporulation of the fungus obtained with Provax-200 and Rovral 50 WP was poor, which indicates compatibility of two fungicides with *T. harzianum* at least at their lower concentrations. Several investigators (Mondal *et al.* 1995, Khandakar 2004, Nahar *et al.* 2007) also reported similar findings. They found that *T. harzianum* is highly compatibility with Provax-200 (Carboxin).

Effect of organic amendment on growth and sporulation of *T. harzianum* is shown in Table 2. Inhibition of colony diameter due to amendment of PDA with mustard oilcake, tea waste, rice bran, chickpea meal and wheat meal ranged 0.00-36.30, 2.22-54.07 and 6.30-72.96%, respectively. The organic amendments caused respectively 6.02-53.01, 15.66-71.08 and 25.30-79.52% inhibition in sporulation (Table 2).

Table 1. Effect of Provax-200, Rovral 50 WP and Bavistin 50 WP on *in-vitro* mycelium growth and sporulation of *T. hrzianum*

| Fungicide | Concentration of fungicides (ppm) | | | |
|---|---|---|---|---|
| | 50 | 100 | 250 | 500 |
| | % Inhibition over control in colony diameter | | | |
| Provax-200 | 2.22 g | 5.56 g | 14.44 de | 18.89 cd |
| Rovral 50 WP | 7.78 fg | 12.22 ef | 16.67 cde | 22.22 c |
| Bavistin 50 WP | 90.00 b | 95.56 ab | 100.00 a | 100.00 a |
| | % Inhibition over control in spore production | | | |
| Provax-200 | 4.52 d | 8.48 cd | 9.16 bcd | 11.76 bcd |
| Rovral 50 WP | 10.07 bcd | 14.03 bcd | 17.42 bc | 21.06 b |
| Bavistin 50 WP | 95.50 a | 100.00 a | 100.00 a | 100.00a |

Values within the same column and row under a parameter having common letter(s) are significantly different.

Table 2. Effect of organic amendments to culture medium (PDA) on *in-vitro* mycelium growth and sporulation of *T. hrzianum*

| Organic amendment | Concentration of organic amendment (%) | | |
|---|---|---|---|
| | 10 | 20 | 30 |
| | % Inhibition over control in colony diameter | | |
| Mustrard oilcake | 0.00 k | 2.22 | 6.30 ij |
| Wheat meal | 4.07 hi | 9.26 b | 21.48 f |
| Chickpea meal | 12.96 g | 20.37 f | 32.22 d |
| Rice bran | 18.15 f | 25.93 e | 39.26 c |
| Tea waste | 36.30 c | 54.07 b | 72.96 a |
| | % Inhibition over control in spore production | | |
| Mustrard oilcake | 6.02 g | 15.66 f | 25.30 ef |
| Wheat meal | 21.69 f | 32.53 de | 48.19 bc |
| Chickpea meal | 34.94 de | 42.17 cd | 50.60 bc |
| Rice bran | 40.96 cd | 46.99 bc | 55.42 b |
| Tea waste | 53.01 b | 71.08 a | 79.52 a |

Values within the same column and row under a parameter having common letter(s) are significantly not different.

Among the five organic amendments, the lowest inhibition of radial growth and sporulation was observed under mustard oilcake at the lowest concentration of 10% while the highest inhibition was observed on tea waste. In inhibiting radial growth and sporulation, wheat meal was appeared to be second lowest followed by chickpea and rice bran. The percent inhibition of mycelium growth and sporulation *T. harzianum* increased with increasing concentration of all the tested organic matters. Findings of the present study suggest that mustard oilcake at the lowest concentration appeared to be compatible with *T. harzianum*. The findings are in agreement with other investigators (Dutta and Das 1999, Islam *et al*. 2002, Nahar and Bhuiyan 2003). Sivan *et al*. (1984) reported that *T. harzianum* can grow on different agricultural waste products.

One of the most desirable characteristics of an antagonist is its insensitivity to the fungicides and organic amendments, which are used to control plant pathogens. Until and unless antagonists are insensitive to fungicides and organic amendments, they can not be integrated successfully for the purpose of integrated disease management tactic.

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*Phytopathological note*

# *CHOANEPHORA* BLIGHT: A NEW DISEASE OF LABLAB BEAN (*LABLAB PUPUREUS*) IN BANGLADESH

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ABSTRACT

M. M. E. Rahman, M. M. Islam, M. E. K. Chowdhury, M. M. Rahman and T. K. Dey. 2012. *Choanephora* blight: a new disease of lablab bean (*Lablab pupureus*) in Bangladesh. Bangladesh J. Plant Pathol. Vol. 28 (1&2): 65-66.

A new disease of country bean (*Lablab pupureus*) caused by *Choanephora cucurbitarum* appeared in the districts of Gazipur, Pabna, Bogra and Jamalpur districts in Bangladesh. Initial symptoms of the disease were recognized as small water soaked lesion on leaves. The lesions enlarged gradually covering large areas, infected parts dried up with age showing blight symptoms. Blossoms and twigs were also infected. Dieback of vine and black soft lesions on young pods appeared with the progress of time. Mycelia and sporangiola of the causal fungus appeared on infected leaves, blossoms and pods as numerous black spore-bearing structures, sporangia. The causal pathogen of the disease was isolated and its morphological characters were studied and it was identified as *C. cucurbitarum*.

**Keywords**: Choanophora blight, *Choanephora cucurbitarum*, lablab bean

Country bean (*Lablab pupureus*) is a widely grown leguminous vegetable crop in Bangladesh. The crop is attacked by at least six plant pathogens in the country (Talukder 1974, Ahmed 1985). A new disease appeared on this vegetable in the districts of Gazipur, Pabna, Bogra and Jamalpur in Bangladesh. The disease was characterized by the appearance of blighted leaves and blossoms, dieback of vine tips, and black and soft rot lesions on fruit (Plate-I). Initially the symptoms appear as water soaked lesions, which enlarge, become dark and the infected parts dry up with age. Infected plant with die-back symptoms (Plate-I-A) and black and dry lesion were observed on young branch (Plate-I-B). Structures of the causal fungus appear on both surfaces of the leaves, blossoms and pods as numerous black spore-bearing structures, giving the appearance of "whiskers"(Plate-I C & D). Whiskers of the fungus, which are fungal strands consists of dark colored knobby sporangiola.

Infected leaf and fruit samples were collected and associated fungal pathogen was isolated following tissue planting method on potato dextrose agar (PDA) medium (Mian 1994). Off-white to yellowish white and fuzzy growth of the fungus appeared on PDA (Plate-I E). Morphological characters (Plate-I F) of the associated fungus were studied and it was identified as *Choanephora cucurbitarum* (Berk. & Rav.). The available literature (Ahmed 1986, Bakr, 2007, Talukder 1974) reveal that the fungus has not been reported earlier on this host from Bangladesh. Therefore, it is a new record of the disease of country bean in the country.

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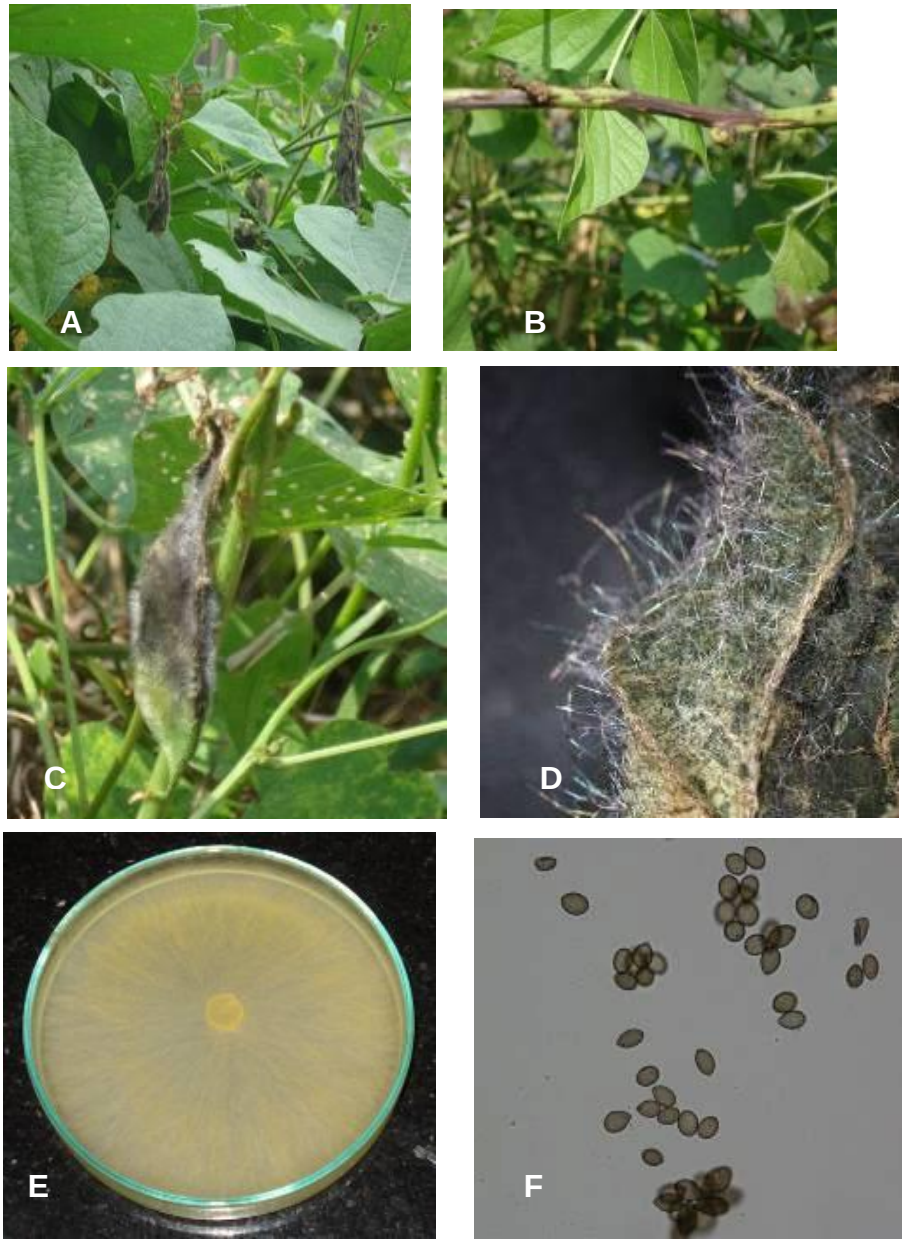


Plate 1. Symptoms of *Choanophora* blight (*Choanephora cucurbitarum*) of lablab bean [A: Blighted plant shoot with die-back; B: Black and dry lesion on young branch; C: Infected and rotten fruits; D: Mycelium and sporangiophore bearing sporangia of the causal fungus; E: Off-white to yellowish fungal colony on PDA; F: Microphotograph of dark colored knobby sporangiola of the fungus].

# PUMMELO CANKER: A NEW DISEASE OF PUMMELO IN BANGLADESH

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ABSTRACT

R. Islam and T. K. Dey. 2012. Pummelo canker: a new disease of pummelo in Bangladesh. Bangladesh J. Plant Pathol. 28(1&2): 67-68

Citrus canker caused by *Xanthomonas axonopodis* was first time recorded from pummelo (*Citrus grandis*) in Bangladesh. Initially the disease appeared as small, slightly raised, round and light green spots on young leaves, twigs, and fruits. The size of lesion increased gradually with the progress of time. At advanced stage, the lesions become grayish white. Leaf surface as well as fruit skins become ruptured forming corky appearance with brown and sunken centers. Similar symptoms also appeared on inoculated fruits

Keywords: Citrust canker, pummelo, *Xanthomonas axonopodis,* infection

Pummelo (*Citrus grandis*) is a common citrus fruit in Bangladesh. It is grown in backyard orchards as well as small holdings. A total of 56,000 metric tons of pummelo is produced in the country (Mondol *et al.* 2011). Canker caused by the phytopathogenic bacterium, *Xanthomonas axonopodis* pv. *citri)* is a common disease of pummelo and other citrus crops grown in Bangladesh. The disease is responsible for reducing yield, quality and market values of citrus fruits (Agrios 2006). Its occurrence was first noticed in Bangladesh in 2008. The incidence and severity of the disease was found to be increasing year after year.

In Bangladesh, the pathogen causes necrotic lesions on leaves, stems and fruits under natural conditions irrespective of citrus crops. Initially the lesions appear as small, slightly raised, round and light green spots on young leaves, twigs and fruits. Latter, the lesions become grayish white. Leaf surface as well as fruit skins become ruptured forming corky appearance with brown and sunken centers (Plate IA). The margins of the lesions on leaf are often surrounded by a yellowish helo.

Canker infected fruit samples were collected from Moulovibazer and Sylhet district and the pathogen was isolated following tissue planting method (Schaad and Stall 1988). For isolation, small sections of infected fruit skin were cut with a sterile scalpel and surface sterilized with 1.0% chlorox solution. About 0.5 ml sterilized distilled water was placed in a sterilized watch glass. Few pieces of surface sterilized infected tissues were placed in the water and pressed with the handle of the scalpel to crush the samples and kept for 30 minutes to release the bacteria into the water. One loopful of the bacterial suspension was streaked on yeast peptone sucrose agar (YPSA) plates. The plates were incubated at 28C for 72 hours (Schaad and Stall 1988). Bacterial colonies developed on the plate were further sub-cultured on same medium. Singe cell bacterial colonies were isolated and transferred to sterile distilled water in test tube. The water culture of the bacterium in test tubes was preserved at room temperature. The pathogenicity test of the pathogen was performed by inoculating healthy pummelo fruit. Apparently healthy fruits were surface sterilized with 1.0% chlorox. Hole (5mm diameter and 4 mm depth) was made on the surface of a fruit with a flame sterilized cork borer. One loopful of pure bacterial suspension preserved in test tube poured into the hole. Inoculated fruits were kept in a moist blotter on a tray, covered with a polythene bag to maintain high humidity and incubated at room temperature (30±2C). After seven days of incubation, characteristic symptoms of canker developed on inoculated fruit skins (Plate IB), which were almost similar to the symptoms appeared on fruits under natural conditions (Plate IA and B). Based on pathogenicity test the disease was confirmed as canker and the causal bacterium as *Xanthomonas axonopodis.* Available literature indicates that citrus canker of pummelo may be considered as the first report of the disease from the country.


Plate I. Symptoms of Canker of pummelo caused by *Xanthomonas axonopodis* [A: symptoms on naturally infected fruit and B: symptoms on artificially inoculated fruit]

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# *IN VITRO* EVALUATION OF FUNGICIDES AND BOTANICALS AGAINST *FUSARIUM OXYSPORUM* AND *MACROPHOMINA PHASEOLINA* ISOLATED FROM SOYBEAN SEEDS

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## ABSTRACT

S. A. Chaity, A. A. Khan and I. H. Mian. 2012. *In vitro* evaluation of fungicides and botanicals against *Fusarium oxysporum* and *Macrophomina phaseolina* isolated from soybean seeds. Bangladesh J. Plant Pathol. 28 (1&2): 69-72.

*Fusarium oxysporum* and *Macrophomina phaseolina* are two seed-borne pathogens, which attack many crops including soybean. An *in-vitro* test was conducted to evaluate the efficacy of six fungicides namely Bavistin 50 WP (Carbendazim), Provax-200 (Carboxin), Rovral 50 WP (Iprodione), Cupravit (Copper Oxy-chloride), Ridomil MZ 78 (Metalaxyl + Mancozeb) and Dithane M-45 (Mancozcb); and extracts of four plant species namely ginger rhizome (*Zingiber officinale*), garlic clove (*Allium sativum*), neem leaf (*Azadiracta indica*) and onion bulb (*Allium cepa*) against *F. oxysporum* and *M. phaseolina* following poison food technique using potato dextrose agar (PDA) as the basic medium. The fungicides were used @ 100, 200 and 400 ppm and the plant extracts were used @ 10, 20 and 40% concentrations. Complete (100%) inhibition in colony growth of both the pathogens was obtained with Bavistin 50 WP at all concentrations. Inhibition of colony growth was 85.74-89.26% in *M. phaseolina* and 50.55 – 67.03% in *F. oxysporum* at all concentrations of Provax-200. Rovral 50 WP gave 50.55 – 67.03% and 34.07 – 63.70% of *F. oxysporum*, and 85.74-89.26% and 82.96-88.70% inhibition of *M. phaseolina*, respectively. Provax-200 and Rovral 50 gave 50.55 – 67.03% and 34.07 – 63.70% growth inhibition of *F. oxysporum*, and 85.74-89.26% and 82.96-88.70% inhibition of *M. phaseolina*, respectively. Garlic was noted as the most effective botanical against *F. oxysporum* causing 64.64 to 89.45% growth inhibition followed by ginger (67.97 to 87.78%) at different concentrations. It was also effective against *M. phaseolina* showing 41.86, 20.56 and 14.82% inhibition in colony diameter, respectively at 40, 20 and 10% concentrations.

**Keywords**: *Fusarium oxysporum*, *Macrophomina phaseolina*, fungicide, plant extract, control.

*Macrophomina phaseolina* and *Fusarium oxysporum* are two important seed-borne pathogens of many crops causing charcoal rot and wilt, respectively. They are important pathogens of cotton, jute, maize, pulses, cucurbits, okra, sesame, etc. A study was undertaken to evaluate the efficacy of six fungicides and four plant extracts to inhibit *in-vitro* colony growth of two fungal pathogens. The selected fungicides were Provax-200 (Carboxin), Rovral 50 WP (Iprodione), Dithane M-45 (Mancozeb), Bavistin 50 WP (Carbendazim), Ridomil-MZ 78 (Metalaxyl + Mancozeb) and Cupravit (Copper Oxy-chloride); and the botanicals were extract of garlic *(Allium sativum)*, ginger (*Zingiber officinale)*, onion *(Allium cepa)* and neem *(Azadirachta indica)*. The tests were conducted following poison food technique using potato dextrose agar (PDA) as a basic medium (Dhingra and Sinclair 1985). The test fungi were isolated from soybean seeds infected with the pathogens following tissue planting methods.

Freshly prepared PDA was amended with each fungicide @ 100, 200 and 400 ppm; and water extract of each botanical @ 10, 20 and 40% (v/v) concentrations. Extracts of garlic cloves, ginger rhizomes, onion bulbs and Neem leaves were prepared by blending 100 g of each plant material in 100 ml of sterilized distilled water in a blender. The extracts were filtered through two-ply cheese cloth. Requisite quantity of individual plant extract or fungicide was added to freshly prepared warm PDA in 100 ml conical flask to have desired concentrations. The amended PDA was autoclaved at 121C under 1.05 kg/cm$^2$ pressures for 20 minutes and dispensed into 90 mm glass Petri dishes at 20 ml/dish. The medium without fungicide served as control. After solidification of the medium, the plates were inoculated with 5 mm mycelial discs cut from the margin of 7 days old cultures of *F. oxysporum* or *M. phaseolina* with a 5 mm cork borer. One mycelial disc of each test fungus was placed reversely at the center of each Petri dish. The plates were incubated in an incubator at 28 °C.

Individual experiment was conducted for each material. The experiments were conducted following completely randomized design. Data were transformed following square root transformation method and analyzed for ANOVA using MSTAT-Computer program. The means were compared following Duncan's New Multiple Range Test using the same computer program.

Radial colony diameter of *M. phaseolina* and *F. oxysporum* was 90.00 mm under control (fresh PDA without amendment) was 90 mm (diameter of plates was 90 mm). At all concentrations of Cupravit, Dithane M-45 and Ridomil MZ-78 did not show any inhibitory effect on colony growth *M. phaseolina.* Amendment of PDA with Rovral 50 WP, Provax-200 and Bavistin 50 WP caused significant inhibition in colony growth of the fungus. The colony growth was completely inhibited when PDA was amended with Bavistin 50 WP at all concentrations. Provax-200 gave 85.74, 87.58 and 89.26%, and Rovral 50 WP showed 82.96, 86.30 and 88.70% inhibition of radial growth colony at 100, 200 and 400 ppm, respectively (Table 1).

In case of *F. oxysporum*, significant inhibition of colony growth was obtained with treatments with six fungicides except Dithane M-45 at 100 ppm. The rate of inhibition varied with fungicides and their concentrations. Bavistin 50 WP gave 100% inhibition in mycelial growth of *F. oxysporum* at all concentrations. Provax-200 caused 50.55, 57.58 and 67.03%, and Rovral 50 WP gave 34.07, 53.89 and 63.70% growth inhibition at 100, 200 and 400 ppm, respectively. Three less effective fungicides, Cupravit, Ridomil MZ and Dithane M-45 showed only 8.70-19.81, 4.25-7.41-11.67% and 4.25-9.44% inhibition of radial growth of *F. oxysporum* at different concentrations (Tables 1).

All the plant extracts at all concentrations caused significant inhibition of the colony growth of the *M. phaseolina* and *F. oxysporum* compared to control. The maximum growth inhibition was achieved with garlic followed by ginger, onion and neem extracts. The botanicals gave 14.82-41.86, 11.86-24.64, 6.30-19.26% and 2.03-18.34% inhibition in colony diameter of *M. phaseolina* at 10, 20 40% concentrations, respectively (Table 2). Amendment of PDA with extracts of ginger, garlic, neem and onion at 10, 20 and 40% concentrations also significantly inhibited the growth of *F. oxysporum.* The inhibition was corroborated with concentration of each botanical. Ginger, onion and neem extracts gave 67.97-87.78, 61.47-85.19 and 45.56-82.97% inhibition of colony growth of *F. oxysporum* at 10, 20 and 40% concentrations, respectively (Tables 2).

Table 1. Effect of fungicides on *in-vitro* radial colony growth of *Macrophomina phaseolina* and *Fusarium oxysporum* on amended PDA

| Fungicides | Radial growth (mm) | | | | | |
|---|---|---|---|---|---|---|
| | *Macrophomina phaseolina* | | | *Fusarium oxysporum* | | |
| | 100 ppm | 200 ppm | 400 ppm | 100 ppm | 200 ppm | 400 ppm |
| Ridomil-MZ 78 | 90.00 a (0.00) | 90.00 a (0.00) | 90.00 a (0.00) | 83.33 b (7.41) | 82.00 b (8.85) | 79.50 b (11.67) |
| Cupravit | 90.00 a (0.00) | 90.00 a (0.00) | 90.00 a (0.00) | 82.17 b (8.70) | 78.83 c (12.41) | 72.17 c (19.81) |
| Dithane M-45 | 90.00 a (0.00) | 90.00 a (9.51) | 90.00 a (9.51) | 86.17 ab (4.25) | 83.67 b (7.03) | 81.50 b (9.44) |
| Rovral 50 WP | 15.33 b (82.96) | 12.33 b (86.30) | 10.17 b (88.70) | 59.33 c (34.07) | 41.50 d (53.89) | 32.67 d (63.70) |
| Provax-200 | 12.83 c (85.74) | 11.17 c (87.58) | 9.67 b (89.26) | 44.50 d (50.55) | 38.17 e (57.58) | 29.67 e (67.03) |
| Bavistin 50 WP | 0.00 d (100.00) | 0.00 d (100.00) | 0.00 c (100.00) | 0.00 e (100.00) | 0.00 f (100.00) | 0.00 f (100.00) |
| Control | 90.00 a (-) | 90.00 a (-) | 90.00 a (-) | 90.00 a (-) | 90.00 a (-) | 90.00 a (-) |

* Data were analyzed statistically for ANOVA after square root {$\sqrt{(X + 0.5)}$} transformation and means within the same column having a common letter(s) do not differ significantly (P= 0.05)

Table 2. Radial colony growth of *Macrophomina phaseolina* and *Fusarium oxysporum* on PDA amended with four botanicals

| Botanicals | Radial colony diameter (mm) | | | | | |
|---|---|---|---|---|---|---|
| | *Macrophomina phaseolina* | | | *Fusarium oxysporum* | | |
| | 10 (% W/V) | 20 (% W/V) | 40 (% W/V) | 10 (% W/V) | 20 (% W/V) | 40 (% W/V) |
| Ginger | 79.33 d (11.86) | 73.33 c (18.53) | 67.83 c (24.64) | 28.83 d (67.97) | 22.00 d (75.56) | 11.00 cd (87.78) |
| Garlic | 76.67 e (14.82) | 71.50 c (20.56) | 52.33 d (41.86) | 31.83 cd (64.64) | 26.50 c (70.56) | 9.50 d (89.45) |
| Neem | 88.17 b (2.03) | 79.83 b (11.30) | 73.50 b (18.34) | 49.00 b (45.56) | 28.17 c (68.70) | 15.33 b (82.97) |
| Onion | 84.33 c (6.30) | 77.17 b (14.26) | 72.67 b (19.26) | 34.67 c (61.47) | 31.00 b (65.56) | 13.33 bc (85.19) |
| Control | 90.00 a (-) | 90.00 a (-) | 90.00 a (-) | 90.00 a (-) | 90.00 a (-) | 90.00 a (-) |

* Data were analyzed statistically for ANOVA after square root {$\sqrt{(X + 0.5)}$} transformation and means within the same column having a common letter (s) do not differ significantly (P= 0.05).

* Figures within parentheses are percentage of growth inhibition based on control.

Results of the present experiments reveal that among six fungicides Bavistin 50 WP, Rovral 50 WP and Provax-200 are effective to reduce *in vitro* colony growth *M. phaseolina* and *F. oxysporum* at all concentrations. Bavistin 50 WP gave 100% growth inhibition of both fungi on amended PDA. Provax-200 caused 50.55, 57.58 and 67.03%, and Rovral 50 WP gave 34.07, 53.89 and 63.70% growth inhibition at 100, 200 and 400 ppm, respectively. Similar findings have also been reported by other investigators. Islam (2011) found complete inhibition of the fungus due to application of Bavistin at 50 WP 100 to 400 ppm concentrations. He also reported that Bavistin inhibited the radial growth of *F. oxysporum* about 95%. Ahmad *et al*. (1991) demonstrated inhibitory effect of Bavistin against *M. phaseolina*. Khan and Fakir (1995) and Haque and Gaffar (1995) demonstrated that Vitavax-200 is an effective fungicide against *M. phaseolina*. It is well established fact that Bavistin and Provax-200 are effective seed treating fungicides (Vidhyasekaran and Thiagarajan 1981, Ali and Fakir 1993).

Findings of other experiments with four botanicals reveal that all of the botanicals at 10, 20 and 40% are effective to inhibit radial colony diameter of *M. phaseolina* as well as *F. oxysporum* on amended PDA. Among the botanicals, the most effective one was extract of garlic which gave 14.82-41.86% reduction in colony diameter of *M. phaseolina* and 64.64-89.45% reduction in *F. oxysporum.* Similar findings have also been also reported by Ahmed and Sultana (1984), Dubey and Dwivedi (1991) and Islam (2011). They found that inhibition of mycelial growth and spore germination of *M. phaseolina*. Khan and Fakir (1995) and Rahman *et al*. (1999) reported that extract of garlic is effective in controlling seed-borne infection of *F. oxysporum* of jute seed.

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# A SPECIES OF *CHALARA* (CORDA) RABENHORST FROM BANGLADESH

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ABSTRACT

Shamim Shamsi and Razia Sultana. 2012. A species Of *Chalara* (Corda) Rabenhorst from Bangladesh. Bangladesh J. Plant Pathol. 28(1&2):73-75.

A species of genus *Chalara sharminensis* was recorded from dried stem of jute. Conidiophores of *C. sharminensis* were frequently associated with pycnidia of *Diplodina* sp. The fungus was tried to isolate following "Tissue planting" method on potato dextrose agar medium. The fungus did not produce any mycelia on the culture medium. This is the first report of the fungal genus from Bangladesh.

**Keywords**: *Chalara sharminensis*, Jute, Bangladesh

Jute (*Corchorus capsularis* L.) is one of the important cash crops of Bangladesh. Many fungi are associated jute plants and cause diseases of the crop. Recently, a fungus under the genus *Chalara* was found to be associated with dried stems of jute (*Corchorus capsularis* L.) as a saprophyte. Morphological characters of the fungus were recorded and compared with characteristics of 131 species and subspecies of *Chalara* so far identified throughout the world (Ellis 1971, 1976, Nag Raj *et al*. 1973, 1974, Carris 1988, McKenzie *et al.* 2002, Kowaski 2006). The characteristics of the new fungus were different from those of the previously identified species of *Chalara.* Conidial width of newly recoded fungus was 8-13 µm which is double the largest conidial width (3-5 µm) of *C. schoenoplecti* M.K.M. Wong. sp. *nov.* It indicated that the newly recorded fungus is a new species of *Chalara* and it was named as *Chalara sharminensis* sp. Nov (Plates 1, 2 and 3). Shamsi *et* Sultana. The identification was confirmed by The Commonwealth Mycological Institute, Kew, Surrey, England. The taxonomical enumeration of the fungus is given below:

**Genus: Chalara** (Corda) Rabenhorst, 1844, Krypt.-F1., 1: 38.

Colonies effuse, grey, olive brown or black, hairy or velvety. Mycelium is immersed or superficial. Stroma, setae and hyphopodia are absent. Conidiophores are macronematous, mononemetous, straight or slightly flexuous, unbranched, brown and smooth. Conidiogenous cells are monophialidic, integrated, terminal, usually determinate, occasionally percurrent, cylindrical or lageniform. Conidia are catenate, endogenous, cylindrical or oblong with truncate ends, usually colorless but sometimes brown, 0-3 septate, smooth or with the ends verruculose (Ellis 1971).

*Type: Chalara fusidioides* (Corda) Rabenhorst.

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Henry, B.W., Phytopathology, 34: 631-635, 1944.

Nag Raj, T.R. A monograph of the genus *Chalara*, in preparation.

*Distribution:* Australia, Czechoslovakia, Finland, Germany, Great Britain and U.S.A.

*Chalara sharminensis* Shamsi *et* Sultana sp. *nov*. (Plates I and II).

Colonium is effusus, latus expanus, cinerascens, puberulus, conidiophora coloniae atrobrunneus, oriens singularis seu perus aggregatae, inramusus knobby 62-171 × 10.8-15.4 (18) µm phyalidi hyalinus,18.9 -79.2 × 9.5 – 13.5 µm. Conidia endogena, catenam, hyalinus, laevis, 1-2 septatus/septalis subinde 3 septalis, cylindrdricum ambo extrima truncata 15-72 (112.6) × 8-13 µm extremaadest specimine similitudo *Chalar*a. Adest specimine similitudo *Chalara* schoenoplccti (3-5 µm) affinis, sed differt grandis conidial latitudo.

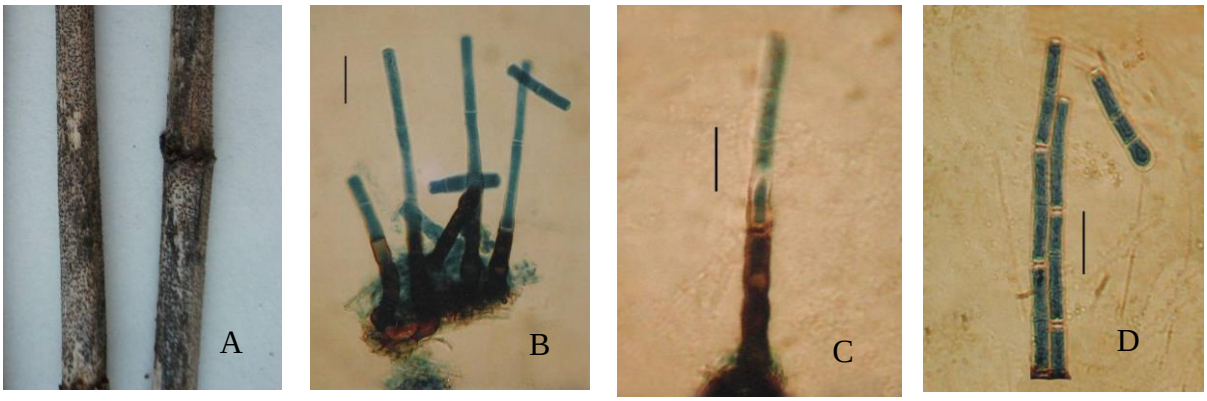


Plate 1. Light (A) and Microphotograpphs (B, C D) of *Chalara sharminensis*: [A. Association of *Chalara* colony with pycnidia of *Diplodina* sp. on jute stem. (Bar = 10 mm), B. Photomicrograph of conidia and conidiophores in fascicle. (Bar = 30 µm). C. Single conidiophore and conidia. (Bar = 25 µm). D. Conidia in chain. (Bar = 25 µm).

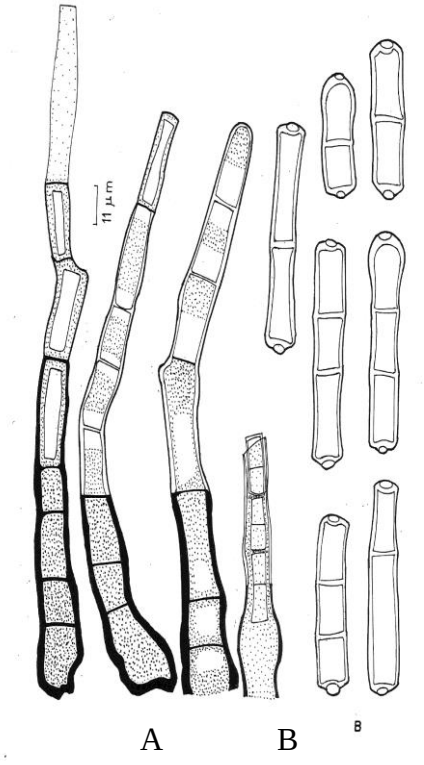


Plate 2. Camera lucida drawings of *Chalara sharminensis.* A) Conidiophores, B) Conidia

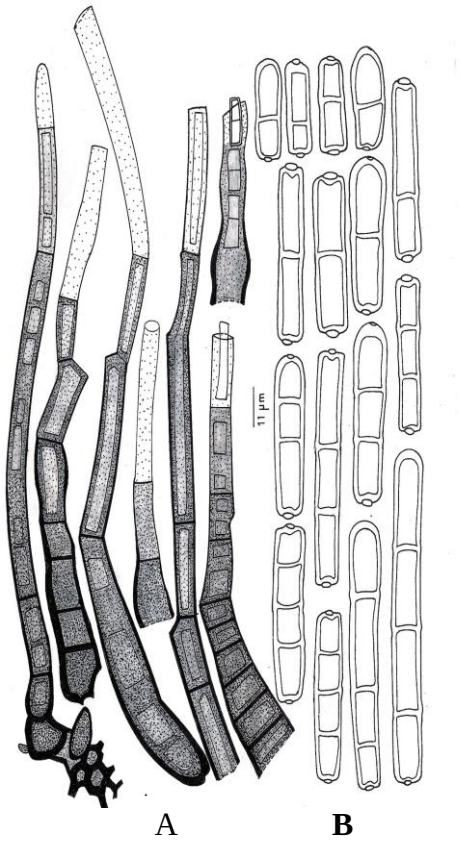


Plate 3. Camera lucida drawings of *Chalara sharminensis* (A) Conidiophore and (B) Conidia

Colony is effuse, wide spreading, grayish, hairy, Conidiophores dark brown, arising singly or in small groups, unbranched, nodose, 62-171 × 10.8-15.4 (18) µm. Phalides 18.9-79.2 × 9.5-13.5 µm. Conidia catenate, hyaline, smooth, mostly 1-2 septate (rarely 3 septate), cylindrical with truncate ends, 15-72 (112.6) × 8-13 µm. Present specimen allied to *Chalara schoenoplccti* (3-5 µm) but differs in having larger conidial width (Plates 1, 2 and 3).

*Holotypus:* On dried stem of *Corchorus capsularis* L. (Tiliaceae), Botanical Research Garden, Curzon Hall, Dhaka, Bangladesh, 2 December 2007, Shamsi 2071. *Shamim Shamsi* SS22. The specific epithet has been given in the memory of first author's late daughter Tamanna Sharmin.


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