ISSN 2412-7558 (Online)
ISSN 1012-9279 (Print)

Bangladesh Journal of Plant Pathology

Volume 29, No. 1&2, 2013

CONTENT

Bangladesh Journal of Plant Pathology

(Bangladesh J. Plant Pathol.)

Research Journal of Bangladesh Phytopathological Society

Vol. 29 **No. 1&2** **2013**

EDITORIAL BOARD

Address for correspondence:
Executive Editor
Bangladesh Journal of Plant Pathology
Department of Plant Pathology
Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706, Bangladesh

Subscription for each volume:

	Bangladesh	*Overseas*
Individual	Tk. 500.00	US $ 10
Institution	Tk. 1500.00	US$ 25

Bangladesh Phytopathological Society.

Executive Committee (2011-2013)

President:	Dr. M. A. Bari, Director (Retd.), Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh
Senior Vice President:	Professor Dr. Ashrafuzzaman Salim, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh
Vice President:	Prof. Dr. Md. Khurshed Alam Bhuiyan, Department of Plant Pathology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur -1706
Treasurer:	Dr. M. Anser Ali, Chief Scientific Officer, Plant Pathology Division, Bangladesh Rice Research Institute, Gazipur-1701, Bangladesh
General Secretary	Dr. Tapan Kumer Dey, Chief Scientific Officer, Plant Pathology Division, BARI Gazipur-1701, Bangladesh
Joint Secretary-1	Prof. Dr. Salahuddin Mahmud Chowdhury, Department of Plant Pathology, Sher-E-Bangla Agricultural University, Dhaka, Bangladesh
Joint Secretary-2	Dr. Sakhawat Hossain, Chief Scientific Officer, Oilseed Research Center, BARI, Gazipur, Bangladesh
Office Secretary	Md. Mynul Islam, Senior Scientific Officer, Plant Pathology Division, BARI Gazipur, Bangladesh
Members:	Prof. Dr. Ismail Hossain Mian, Department of Plant Pathology and Treasurer, BSMRAU, Gazipur-1706, Bangladesh
	Prof. Dr. M. A. Mannan Akanda, Department of Plant Pathology and Vice-Chancellor, Exim Bank Agricultural University, Chapainawabganj, Bangladesh
	Prof. Dr. Ismail Hossain, Department of Plant Pathology, BAU, Mymensingh, Bangladesh
	Dr. Md. Abu Taher Mia, CSO (Ret'd), Plant Pathology Division, BRRI, Gazipur
	Dr. Mohammad Hossain, CSO, Tuber Crops Research Center, BARI, Gazipur
	Dr. Md. Ibrahim Talukder, CSO, Plant Pathology Division, Bangladesh Sugarcane Research Institute, Ishurdi, Pabna
	Prof. Dr. Aminuddin Mridha, Department of Botany, Chittagong University Chittagong
	Dr. A. Muqit, SSO, Plant Pathology Division, BARI, Gazipur
	Dr. Mostafa Kamal, PSO, Plant Pathology Division, BRRI. Gazipur
Ex-Officio Member	Dr. M. A. Wadud Mian, Professor (Ret'd.), Bangladesh Agricultural University Mymensingh
	Dr. M.A. Bakr, Project Director (Ret'd.), Pulses and Oilseeds, BARI, Gazipur
Distinguished Member	Prof. Dr. Golam Ali Fakir, Ex-Vice Chancellor, Khulna University Khulna; Vice-Chancellor, Bangladesh University, Dhaka
	Dr. Hamizuddin Ahmed, Director (Ret'd), BARI, Gazipur

INFORMATION FOR CONTRIBUTORS

Membership of the Bangladesh Phytopathological Society is a prerequisite for publishing papers in the Bangladesh Journal of Plant Pathology (Bangladesh J. Plant Pathol.), or at least one of the authors must be a member of the Society. The Editorial board, however, may relax this rule in case of contribution of exceptionally merit and contributor from abroad. The journal publishes original research that signify advancement of knowledge in plant diseases, the causal agents, the factors that influence diseases, and the methods which are be used to control them. The journal is published in both print form (ISSN1012-9279) and online (ISSN 2412-7558). Materials accepted for publication must be scientifically sound and suitable for the designated readers. The manuscript should be prepared according to the guidelines provided below.

(1) Subject matters of the papers for publication should fall in the following categories:

a. Full length papers: Original research on any branch of plant pathology;

b. Review papers: Developing a new concept, hypothesis, theory, or other investigation in the field of plant pathology;

c. Phytopathological notes: Accounts of plant pathological techniques, original research or new records of plant diseases or plant pathogens.

(2) The manuscript should be typed double-space on one side of A4 size good quality paper in 12-point preferably in Times New Roman font. Margins should be 4.0 cm from the left and right, and 2.5 cm from the top and bottom of each page. Submit two copies of the manuscript to the Executive Editor, Bangladesh Journal of Plant Pathology, Plant Pathology Division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh along with hard copy, a soft copy of the manuscript in word 97-2003/XP format written in CD should be submitted.

(3) A full length paper should be arranged under the section heading: TITLE, ABSTRACT, INTRODUCTION, MATERIALS AND METHODS, RESULTS AND DISCUSSION, ACKNOWLEDGEMENT (if any) and LITERATURE CITED. Phytopathological notes should be sufficiently brief (less than 4 pages) and arranged without section headings of INTRODUCTION, MATERIALS AND METHODS, and RESULTS AND DISCUSSION.

a. Title page – Title page should be independent and contain only Title, authors' name and their address. The title should be typed in capital letters and must accurately identify and describe the contents of the manuscript. Scientific name (if any) must be in capital letter and italic. The title should be followed by authors' names. The authors' names should be followed by author's addresses. A person who did not take part in research, analysis of data and writing of the paper and who cannot assume responsibility for the technical content of the paper cannot be considered an author. Include each author's complete mailing address and institutional affiliation in the title page. Indicate the author to whom correspondence should be addressed and the author's e-mail address. Indicate whether the research is the portion of a thesis or dissertation.

b. Abstract - Start abstract from a separate page. Type the title again on this page but no authors' name and address. After the title, type a 150–200 words abstract containing the objectives, very short methodology, important findings of the research and your conclusion.

c. Keywords –The keywords and phrases following the Abstract should be alphabetically arranged and should reflect the contents of the paper. Give keywords only in one line.

(4) The author(s) are responsible to check that all references in the text appear in the section 'LITERATURE CITED' and vice versa. References should come primarily from papers published in professional journals. A small number of abstracts or text book-type references may be included; however, these should not make up the bulk of the references. Personal communication references are permitted, but, again should be kept to a minimum. In text references should be cited by giving the author's name followed by the year without any coma in between.

a. For two authors' paper, give both authors' last names.

i) Ahmed and Hamid (1995) reported the losses due to late blight of potato.

ii) The loss due to late blight of potato has been reported by Ahmed and Hamid (1995).

iii) Loss due to late blight of potato has been well documented (Ahmed and Hamid 1955).

b. Articles with more than two authors are cited by the first authors last name followed "*et al*.", and then the year:

i) Effect of environmental factors on disease development is well documented (MacHardy *et al.* 2001) instead of (MacHardy, W. E., Gadoury and Gessler. C. 2001).

ii) Disease development was recorded by MacHardy *et al.* (2001). At least 12 diseases of pulses are in Bangladesh (Ahmed *et al.* 1985) or Ahmed *et al*. (1985) recorded at least 12 diseses of pulses.

c. A string of citations should be separated by coma.

i) Effect of environmental factors on pathogens is recorded by many researchers (Rowe and Beute 1975, Sutton 1981, Pinkerton *et el*. 1998, MacHardy *et al*. 2001).

ii) Ahmed and Hamid (1955, 1989), Mian (1985), Fakir (1985) reported losses due to potato late blight.

iii) Loss due to late blight was recorded by Ahmed and Hamid (1955, 1989) and Mian (1985).

d. When a book, paper, or article has no definite author, cite it as Anon. and year, e.g., (Anon. 1996) (Anon. is the abbreviation for anonymous).

e. If you want to refer a paper found in another article, do so as (Driblick 1983 I*n:* Oobleck 1978).

(5) In the section LITERATURE CITED, references should be listed alphabetically according to the author's name. If the same author(s) are cited for more than one paper having the same order of authors' names, the papers should be listed in chronological sequence by year of publication. Anon. should be listed as 'Anonymous'.

a. Journal article with single author e.g.- Bisessar, S. 1982. Effect of heavy metals on microorganisms in soil near secondary lead smelter. Wat. Air Soil Poll. 17:305-308.

b. Journal article with two authors e.g.- Sivan, A. and Chet, I. 1993. Integrated control of *Fusarium* crown and root rot of tomato with *Trichoderma harzianum* in combination with methyl bromide or soil solarization. Crop Protec. 12:380-386

c. Journal article with multiple authors e.g.- Schneider, R. W. Williams, R. J., and Sinclair, J. B. 1976. *Cercospora* leaf spot of cowpea, models for estimating yield loss. Phytopathology 66(5): 384-388.

d. Author(s) Unknown or Not Named - If the authorship of a paper or other document is not provided, cite the author using the word "Anonymous" in the place of the author's name(s) e.g. Anonymous. 2004. Annual Internal Review. Bangladesh Rice Research Institute, Joydebpur, Gazipur. pp. 39-41.

e. Book with two authors – Dhingra, P. and Sinclair, A. 1985. Basics plant pathology methods. CRC press. Inc. Boca Raton Florida. pp.13-44 [(pp. 13-44 page means on 13 to 44), p.13, (p. 13 means on page 13), (250 pp means total page of the book)].

f. Book with multiple authors - Huth, J., Brogan, M., Dancik, B., Kommedahl, T., Nadziejka, D., Robinson, P. and W. Swanson.1994. Scientific format and style: The CBE manual for authors, editors, and publishers. 6th ed. Cambridge: Cambridge University Press. 825 pp.

g. Book: authors contributing a specific chapter - Kuret, J. and Murad. F. 1990. Adenohypophyseal hormones and related substances. *In*: Gilman, A., Rall, T., Nies, A., Taylor, P., edited. "The pharmacological basis of therapeutics". 8th ed. New York: Pergamon. pp. 1334-60.

(6) Table and graphs must be typed and drawn respectively on separate sheet. Same data must not be given in both tables and graphs. Data based on which a graph is created should be enclosed on separate sheet.

(7) All quantitative data must be in metric units. Abbreviations should be as per "Style Manual for Biological editors".

(8) Picture should be submitted on separate file in JPEG format.

(9) Manuscript not prepared following the style of the journal will not be accepted.

(10) For more information contact the Executive Editors (Email:ihmian2007@gmail.com) or consult its latest issue.

(11) Due to the high cost of publishing, page charges are required. The rates are subject to change without notice. Present page charges areare shown below:

Paper type	Black and white	Colour
		For Bangladeshi (Tk/article**)**
a. Full paper having 4 pages	2000.00	2500.00
b. Full paper having 5-8 pages	2500.00	3000.00
c. Phytopathological Note (4 pages)	1000.00	1500.00
		For foreigners ($/article)
d. Full paper having 4 pages	20.00	30.00
f. Full paper having 5-8 pages	25.00	40.00
g. Phytopathological Note (4 pages)	15.00	20.00

CHEMICAL CONTROL OF BACTERIAL SOFT ROT OF ONION CAUSED BY *BURKHOLDERIA CEPACIA*

M. M. Rahman[1], A. A. Khan[1], A. M. Akanda[1], I. H. Mian[1] and M. Z. Alam[2]

[1]Department of Plant Pathology, [2]Department of Entomology, Bangabadhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706, Bangladesh Email of first author: majibar_rhmn@yahoo.com

(This is a part of Ph.D thesis of first author)

ABSTRACT

M. M. Rahman, A. A. Khan, A. M. Akanda, I. H. Mian and M. Z. Alam**.** 2013. Chemical control of bacterial soft rot of onion caused by *Burkholderia cepacia*. Bangladesh J. Plant Pathol. 29 (1&2): 1-4.

Bactericidal properties of eight chemicals were tested *in-vitro* against onion soft rot bacteria (*Burkholderia cepacia*). Among the chemicals, acetic acid, boric acid and bleaching powder showed bactericidal activity against onion soft rot bacteria, *B. cepacia* O-15. These three chemicals were tested to treat onion bulbs against soft rot disease in storage. For treatment, fresh onion bulbs were dipped in 0.00, 0.02, 0.05 and 1.00% solutions of acetic acid, boric acid, and bleaching powder for 30 min. The treated bulbs were inoculated by spraying *B. cepacia* suspension and stored for 2-22 weeks. During the storage period, the chemicals caused 0.0-18.2% reduction in soft rot incidence and 0.00-18.0% reduction in weight loss of onion bulbs over to control. The lowest incidence of soft rot as well as weight loss was achieved with bleaching powder followed by boric acid and acetic acid. Results of both *in vitro* test and onion treatment experiment indicate that bleaching powder, boric acid and acetic acid may be used to control soft rot of onion bulbs during storage for a period of 2-22 weeks.

Key words: Bacterial soft rot, *Burkholderia cepacia*, onion, chemical control

INTRODUCTION

Bacterial soft rot (*Burkholderia cepacia*) is a common post harvest disease of onion and many other vegetables throughout the world. The disease causes severe loss of onion bulb in storage (Bdliya and Haruna 2007). Normally, chemical bactericides are not recommended for the control of soft rot disease because of high risk of health hazards (Agrios 1997). However, many scientists tested various bactericides including chemicals and microbial pesticides to control the soft rot bacteria (Chen and Lin 2000, Abd-El-Khair 2004, Wright *et al*. 2005). Researchers identified some chemicals with antimicrobial activity, which increase resistance in potato and onion against soft rot disease (Hammerschmidt and Smith 1997). Benzothiodiazole (BTH) has been idenfied as a systemic resistance inducer in many plants and effective against various plant pathogens (Gorlach *et al*. 1996, Bokshi *et al*. 2003). Increased resistance in potato tubers against *E. carotovora* subsp. *carotovora* was observed when tubers were dipped in acetyl salicylic acid (Abd-El-Sayed *et al*. 1996, Bokshi *et al*. 2003). Salt treatments also can inhibit plant pathogens or suppress their toxin production (Olivier *et al*. 1998). Salts including calcium propionate and calcium chloride reduced tissue maceration of potato tubers caused by *E. carotovora* (McGuire and Kelman 1986, Biggs *et al*. 1997, Droby *et al*. 1997).

Suppression of bacterial soft rot in potato tubers by application of an antibiotic 'kasugamycin' was investigated by Bartz (1999). Reports on chemical control of soft rot bacteria are not available in Bangladesh. Search for selection of chemicals without health hazard to human is necessary to control soft rot of onion.

Considering the above facts the present investigation was conducted to test some chemical substances for their effectiveness to control soft rot causing bacterial pathogens of onion.

MATERIALS AND METHODS

In vitro evaluation of eight chemicals against soft rot bacteria

An *in vitro* experiment was conducted to evaluate eight chemicals for their bactericidal activity against soft rot pathogen, *B. cepacia* O-15 of onion. The chemicals were acetic acid, boric acid, bleaching powder, lactic acid, calcium hydroxide, calcium chloride, potassium chloride and sodium hypochloride. Acetic acid, boric acid, lactic acid, bleaching powder and sodium hypo-chloride were tested at 0.02, 0.05 and 0.10% (w/w). Other chemicals were tested at 0.05, 0.10 and 0.20%. Yeast peptone dextrose agar (YPDA) was used as basal medium.

The YPDA was prepared following a standard method as described by Tuite (1969). After cooking the medium was amended with appropriate quantity of each chemical to have desired levels of

concentrations. Each chemical was added to YPDA, mixed thoroughly and autoclaved for 20 min at 121C under 1.1 kg/cm^2 pressures. YPDA without any chemical amendment served as control. After sterilization, the medium was poured into 90 mm glass Petri dishes at 20 ml/plate and allowed to solidify.

To prepare the inocula, *B. cepacia* O-15 was grown on YPDA at 28C for 24 hr. Bacterial cells were collected from the culture and suspended in sterilized distilled water to a concentration of ca.10^8 cfu/ml. After solidification, YPDA in the plates was inoculated with bacterial suspension and incubated at 30C in an incubator. The plates were arranged in an incubator following completely randomized design with three replications. Three additional plates having YPDA without any chemical were maintained as control. Growth of the test bacteria in the plates was observed up to 14 days of inoculation and antibacterial activity of the chemicals was determined based on initiation of colony growth.

Efficacy of the chemicals to control soft rot

Based on the results of the *in vitro* test another experiment was conducted to evaluate the efficacy of acetic acid, boric acid and bleaching powder to control soft rot disease of onion in storage. Apparently healthy bulbs of onion variety 'Taherpuri' were selected and treated with the chemicals at 0.00, 0.20, 0.05 and 1.00% concentrations. For each concentration of every chemical, 700 g of fresh onion bulbs were treated by dipping in solution of each chemical separately for 30 min and then air dried.

Fresh cultures of *B. cepacia* O-15 grown on YPDA were suspended in sterilized distilled water to prepare inocula at a concentration of ca.10^8 cfu/m. Onion bulb treated with chemicals were inoculated with the inoculum suspension of the bacteria using an atomizers and air dried again. The onion bulbs were packed in net bags and stored at room temperature for 22 weeks. For control treatment onion bulbs were treated with plain water, air dried and inoculated with the pathogen. The bulbs were checked for soft rot incidence on 2, 6, 10, 14, 18 and 22 weeks after inoculation. Data on soft rot infection and loss of weight due to soft rot in storage were recorded and expressed as percentage using the following formula described by Abd-El-Khair and Karima (2007):

$$\text{Infection \%} = \frac{\text{Number of infected bulbs}}{\text{Total number of bulbs}} \times 100$$

$$\text{Loss of weight \%} = \frac{I - W}{I} \times 100,$$

Where I= Initial weight of bulbs and
W= weight after discarding the infected tubers

Percentage of disease reduction (PDR) was calculated using the following formula (Hajhamed *et al.* 2007):

$$\text{PDR} = \frac{\text{Ack} - \text{Atr}}{\text{Ack}} \times 100,$$

Where Ack = loss in weight in control bulbs and
Atr = loss in weight of treated bulbs.

RESULTS AND DISCUSSION

***In vitro* evaluation of eight chemicals against soft rot bacteria**

Among eight chemicals tested *in-vitro* only acetic acid, boric acid and bleaching powder showed antibacterial activity against the soft rot bacteria. *In-vitro* growth of *B. cepacia* O-15 was inhibited by boric acid at all three concentrations (0.02, 0.05, 1.00%), acetic acid at two higher concentrations (0.05, 0.10%) and by bleaching powder only at the highest concentration of 1.00% (Table 1).

Efficacy of chemicals to control soft rot

At 2, 6, 10, 14, 18 and 22 weeks of storage, the soft rot incidence was 14.8, 42.9, 75.00, 82.10, 100.00 and 100.00% and loss in weight of onion bulb was 17.10, 43.5, 883.9, 100 and 100%, respectively. At different weeks of storage, application of three chemicals caused 0.0-18.2% reduction in soft rot incidence (Fig. 1) and 0.00-18.0% reduction in weight loss of onion bulbs (Fig. 2) over control. The lowest incidence of soft rot as well as weight was achieved with bleaching powder followed by boric acid and acetic acid (Fig. 1 and 2).

Table 1. Antibacterial activity of three chemicals at 0.0 to 1.0% concentrations against soft rot bacteria of onion (*Burkholderia cepacia*)

Chemicals tested	Concentration (%)			
	0.0	0.02	0.05	1.0
Acetic acid	-	-	+	+
Boric acid	-	+	+	+
Bleaching powder	-	-	-	+

+ = positive; - = negative

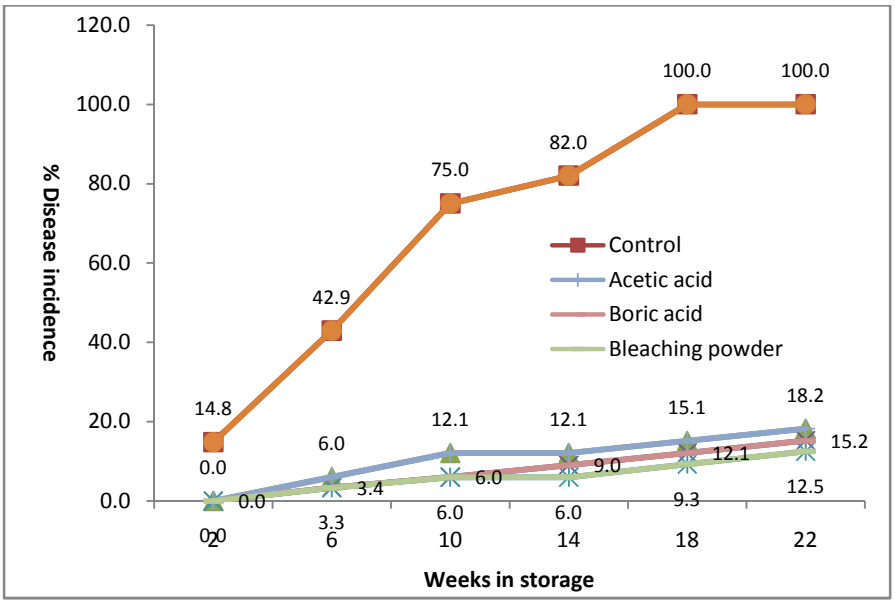


Figure1. Effect of three chemicals on soft rot incidence of onion in storage for 2-22 weeks at 4 weeks interval

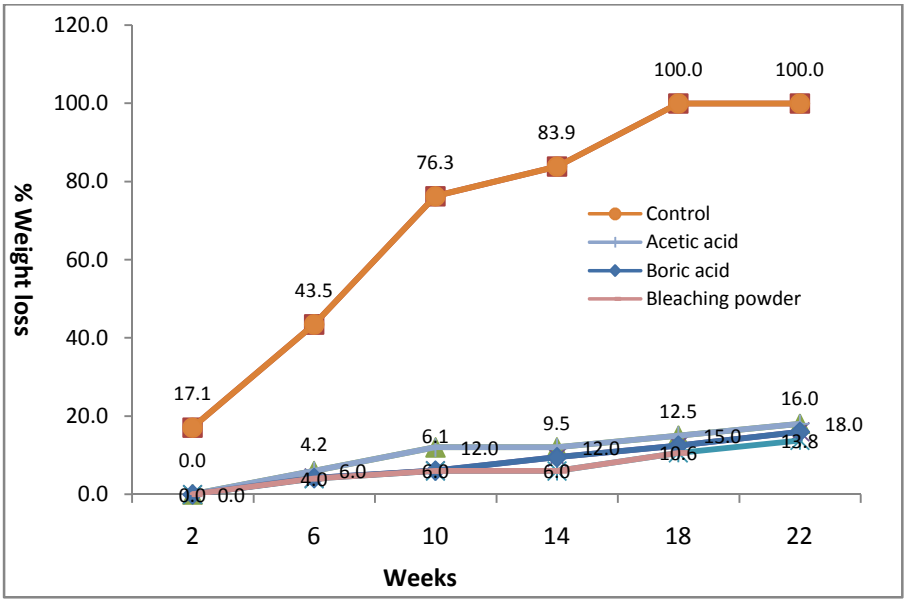


Figure 2**.** Effect of three chemicals on weight loss of onion bulbs in storage for 2-22 day at 4 weeks of intervals

Results of the *in-vitro* test reveal that the most effective chemical to inhibit *in-vitro* growth of soft rot causing bacteria (*B. cepacia* O-15) of onion was bleaching powder followed by boric acid and acetic acid. Other investigators also found that organic acids can inhibit growth of *B. cepacia* causing soft rot of potato (Bokshi *et al.* 2003). Based on findings of the present *in vitro* test acetic acid, boric acid and bleaching powder were selected for treatment of onion to control soft rot disease during storage. Findings of the experiment reveal that prestorage treatment of onion bulbs with bleaching powder, boric acid and acetic acid is effective to reduce soft rot incidence and weight loss of onion bulbs. Many researchers also reported similar results. Hajhamed *et al.*(2007) found that potassium sulfate, ammonium phosphate and calcium chloride as salt compounds significantly decreased severity of bacterial soft rot

disease of potato. Saleh and Huang (1997) reported that benzoic acid and sodium benzoate at 1, 5 and 10 mM inhibited soft rot bacterial growth and were effective in controlling the disease in both tomato fruits and potato tubers. Salts including calcium propionate and calcium chloride reduced tissue maceration of potato tubers due to attack of *E. carotovora* (Biggs *et al*. 1997, Droby *et al*. 1997, Olivier *et al*. 1998). Findings of the two experiments indicate that acetic acid, boric acid and bleaching powder may be recommended to control soft rot of onion caused by *B. cepacia*.

LITERATURE CITED

Abd El-Sayed, Wafaa M., Abd El-Ghaffar, N. Y. and Shehata, A. M. 1996. Application of salicylic acid and aspirin for induction of resistance to tomato plants against bacterial wilt and its effect on endogenous hormones. Ann. Agric. Sci., Ain Shams Univ., Cairo, 41:1007-1020.

Abd-El-Khair, H. 2004. Efficacy of starner in controlling the bacterial soft rot in onion. Ann. Agril. Sci., Cairo. 49(2): 721-731.

Abd-El-Khair, H. and Karima, H.E.H. 2007. Application of some bactericides and bioagents for controlling the soft rot disease in potato. Res. J. Agric. and Bio. Sci., 3(5): 463-473.

Agrios, G. N. 1997. Control of plant diseases. *In Plant Pathology*, 4th edn. California: Academic Press. pp. 200-216.

Bartz, J. A. 1999. Suppression of bacterial soft rot in potato tubers by application of kasugamycin. American J. Potato Res. 76(3): 127-136.

Bdliya, B. S. and Haruna, H. U. 2007. Efficacy of solar heat in the control of bacterial soft of potato tubers caused by *Erwinia carotovora* subsp. *carotovora*. J. Plant Protec. Res., Nigeria Vol. 47 No. 1.

Biggs, A. R., El-Kholi, M. M., El-Neshawy, S. and Nickerson, R. 1997. Effect of calcium salts on growth, polygalacturonase activity, and infection of peach Fruit by *Monilinia fructicola*. Plant Dis. 81: 399-403.

Bokshi, A. I, Morris, S. C. and Deverall, B. J. 2003. Effects of benzothiadiazole and acetylsalicylic acid on b-1,3-glucanase activity and disease resistance in potato. J. Plant Pathol. 52: 22–27.

Chen, C. W. and Lin, C. Y. 2000. Control of *Erwinia* soft rot disease of Calla lily. Pl. Pathol. Bull. 2000. 9(3): 107-114.

Droby, S., Wisniewski, M. E., Cohen, L., Weiss, B., Touitou, D., Eilam, Y. and Chalutz, E. 1997. Influence of $CaCl_2$ on *Penicillium digitatum* grapefruit peel tissue and biocontrol activity of *Pichia guilliermondii*. Plant Dis.87: 310-315.

Gorlach, J., Sandra, V., Gertrud, K., Georges, H., Uli, B., Karl-Heinz, K., Oostendrop, M., Staub, T., Ward, E., Kessmann, H. and Rayals, J. 1996. Benzothiadiazole, a novel class of inducers of systemic acquired resistance in wheat. Plant cell8: 629-643.

Hajhamed, A. A., Sayed, W. M. A. E., Yazied, A. A. E. and Ghaffar, N. Y. A. E. 2007. Suppression of bacterial soft rot disease of potato.Egypt J. Phytopathol.35(2): 69-80.

Hammerschmidt, R. and Smith, J. B. 1997. Acquired resistance to disease in plants. Hort. Rev. 18: 247-289.

McGuire, R. G. and Kelman, A. 1986. Calcium in potato cell wall in relation to tissue maceration by *Erwinia carotovora.* Phytopathology76: 401-406.

Olivier, C., Halseth, D. E., Mizubuti, E. S. G. and Loria, R. 1998. Postharvest application of organic and inorganic salts for suppression of silver scurf on potato tubers. Plant Dis. 82: 213-217.

Saleh, O. I. and Huang, J. S. 1997. Bacterial soft rot disease of tomato fruits in Florida, USA: Identification, response of some American and Egyptian cultivars of solanaceous plants and chemical control. Assiut J. Agril. Sci. 28(2): 11-26.

Tuite, J. 1969. Plant pathoglogical methods. Fungi and Bacteria. Bur.Pub. Co. Minneapolis, Minn. USA. 293 pp.

Wright, P. J., Triggs, C. M., and Burge, G. K. 2005. Control of bacterial soft rot in calla (*Zantedeschia* spp.) by pathogen exclusion, elimination and removal. New-Zealand J. Crop Hort. Sci. 33(2):117-123.

FIRST RECORD ON *BOTRYTIS* BLIGHT (*BOTRYTIS GLADIOLORUM*) OF GLADIOLUS FROM BANGLADESH

S. S. Siddique[1], A. U. Ahmed[2], M. S. Akter[1], M. M. Islam[1] and I. H. Mian[3]

[1]Scientific Officer, Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), RARS, Jessore; [2]Principal Scientific Officer, Plant Pathology Division, BARI, [3]Professor, Department of Plant Pathology, Bangabandhu Sheik Mujibur Rahman Agricultural University, Gazipur, Bangladesh

ABSTRACT

S. S. Siddique, A. U. Ahmed, M. S. Akter, M. M. Islam and I. H. Mian. 2013. First report on *Botrytis* Blight (*Botrytis gladiolorum*) of gladiolus from Bangladesh. Bangladesh J. Plant Pathol. 29 (1&2):5-10.

Botrytis gray mold disease like symptoms appeared on gladiolus during 2012 and 2013 crop season grown in Jessore regions of Bangladesh. The disease caused spots on leaves, flower buds and inflorescence. In severe infection, the disease caused both flower and leaf blight and corm rot. *Botrytis gladiolorum* was consistently isolated from infected gladiolus plants. For confirmation of the disease, Koch's postulate was performed through artificial ino-culation of healthy leaves of gladiolus grown in pots in a glass house. Conidial suspension of *B. gladiolorum* isolated from naturally infected plants used as inocula for inoculation. Characteristic symptoms of *Botrytis* blight developed on inoculated gladiolus plants were identical as recorded from the field. Based on inoculation test it was confirmed that the disease was *Botrytis* blight of gladiolus and the causal fungus was *B. gladiolorum*. This is the first record on the occurrence of *Botrytis* blight and its causal pathogen, *B. gladiolorum* in Bangladesh.

Key words: *Botrytis* gray, *Botrytis gladiolorum*, incidence

INTRODUCTION

Gladiolus (*Gladiolus hortulanus*), also known as queen of the bulbous plants, is cultivated in Bangladesh for its beautiful flower spikes having a long life as cut flower. Its magnificent inflorescence with variety of colors and number of pretty florets makes it attractive to the growers and cut flower users (Chanda *et al.* 2000, Bose *et al*. 2003, Pant 2005). The cut flower is one of the most important commercial crops in Jessore region of Bangladesh. The requirements of cut flower in the country are supplied by the growers of these regions.

Gladiolus plant is attacked by a number of diseases throughout the world. Of them *Botrytis* blight caused by *B. gladiolorum* is very destructive one. The disease is manifested by spots on leaf, flower bud, inflorescence and stem, and corm rot. Drayton (1928) reported *Botrytis* disease of gladiolus from Canada in 1928. The disease has also been reported from Holland (Drayton 1929), England (Moore 1939), New York (Dodge and Laskaris 1941), Australia (Wade 1945), India (Sohi (1992, Singh *et al.* 2005), Pakistan (Mirza and Shakir 1991) and Iran (Mirzaei *et al*. 2008). Mirza and Shakir (1991) reported *B. gladiolorum* from corm and leaves of gladiolus in Pakistan. Sohi (1992) worked on diseases of ornamental plants and reported *B. gladiolorum* from corms and leaves of gladiolus in India. Blight caused by *B. gladiolorum* is noted as the major threat for gladiolus production in India (Singh *et al.* 2005).

The disease has not yet been reported from Bangladesh. In recent years, disease problems appeared in Jessore regions of Bangladesh as one of the major limiting factors for growing gladiolus. In 2013-2014 crop season, a new disease appeared in farmer's fields of the regions. The disease was manifested by characteristic symptoms of *Botrytis* blight as spots on leaf, flower bud, flower and stem and rotting of corm. The disease severity was very high and caused leaf and inflorescence blight. Almost all plants in a field were found to be infected by the disease. Moreover, the market price of flower sticks was reduced. The symptoms appeared in the field was recorded and compared with the symptoms reported by other workers (Mirza and Shakir 1991, Sohi 1992, Singh *et al.* 2005 and Mirzaei *et al*. 2008). The comparison reveals that the disease may be *Botrytis* blight. To identify the disease an investigation was conducted during the flower season of 2013 in Jessore region of Bangladesh.

MATERIALS AND METHODS

Diseased samples of leaf, flower buds and stem of gladiolus were collected from farmers' fields of Jessore regions. The fungus associated with the specimens was isolated following tissue planting methods on potato dextrose agar (PDA) (Mian 1995). Collected leaf specimens were cut into small pieces, sterilized with 1.0% chlorox (NaHCl) solution for 1 min, rinsed in sterile distilled water for three times and placed in Petri dishes containing PDA. The isolated fungus was purified following hyphal tip method (Mian 1995). To identify the fungus, morphological characters such as

conidiophore length, conidial and sclerotial dimensions were recorded, and the associated fungus was identified based on the morphology (Mirzaei *et al*. 2008).

Pathogenicity of the isolated fungus was performed under control conditions by inoculating healthy gladiolus with spore suspension of *B. gladiolorum* isolates. Gladiolus plants were grown in earthen pots (20 cm height and 20 cm rim diameter). The isolates were multiplied on PDA in Petri dishes. Ten days after incubation, conidia were harvested from the cultures by flooding the plates with sterilized distilled water and scraping with sterilized glass slides. The conidial suspension was filtered through muslin cloth to remove mycelium fragments. The suspension was adjusted to $6x10^4$ conidia ml^{-1} using sterilized distilled water. At flowering stage, apparently healthy gladiolus leaves were inoculated with the conidial suspension. For inoculation, the inoculum suspension was sprayed over the plants. Plants under control were sprayed with plain water. Both inoculated and control plants were covered with polythene sheet to keep the plants humid for 48 hours. The pots with plants were placed in a glass house having ambient temperature of 20-22C until development of symptoms. Characteristic symptoms of the disease appeared within 12 days of inoculation. The inoculated fungus was re-isolated from the inoculated plant parts showing characteristic symptoms following the procedures as mentioned earlier. Pieces of leaf specimens were also plated on moist blotting paper in Petri dishes and incubated at 21C. The fungus grew on the leaf samples were isolated, purified and morphological characteristics of the fungus were recorded.

RESULTS AND DISCUSSION

Symptoms of *Botrytis* blight of gladiolus observed on leaf, stem, flower and corm in the farmer's fields and in inoculated plants of gladiolus are described below:

Symptoms on leaf

Initially, reddish brown tiny spots appeared on the leaves which became round to oval and sometimes irregular in shape (Plate 1 A). The spots enlarged gradually and turned into pale brown in color with reddish brown margin and dark yellow center (Plate 1 B). In case of severe infection, several spots coalesced together and formed large lesions and a blighted symptom appeared (Plate 1 C). At later stage of infection, moldy structure of mycelium, conidiophores and spores appeared on the blighted leaves (Plate 1 D& E). Severe lesions appeared on leaf sheath which girdled the sheath around the stem (Plate 1 F).

Symptoms on stem

Infection of stem started from leaf sheath (Plate 1 F). From the sheath, lesions moved downwards and reached the stem causing stem girdling. The lesion encircles the stem and soft rot symptoms appeared. Sometime stem girdling occurred at the point of infection (Plate 2 A). Grayish fungal growth also observed at the point of infection of stem (Plate II B).

Symptoms on flower

Minute water-soaked lesions developed on flower buds and flowers of infected plants at 72 hr after inoculation. Lesions increased in size and coalesced to form patches within 7 to 10 days of infection. Shoots became blighted and died in 12-14 days. All the infected organs were covered with gray mold within 16-18 days. Re-isolation of the causal fungus from the inoculated plants consistently yielded the inoculated fungus. On petals and sepals translucent water soaked spots appeared with light brown margin and pale colored centre (Plate III A). As the spots enlarged, dead tissue turned into brown and the flowers became rotten (Plate III B). In severe cases entire flower can be rotted and gray mass of spore appeared on rotted portion (Plate III C). Plants under control plants did not show any symptom of the disease.

Symptoms on corms

Initially, small reddish brown lesions appeared on corms of gladiolus (Plate IV A). Gradually, several lesions coalesced together and turned into large black lesion (Plate IV B). Sometime large black mummified areas appeared on the neck region of the corm. In the neck region, large brown spots were observed (Plate IV C).

Morphological characteristics of the causal fungus

After 48 hr of incubation, whitish mycelial growth appeared on the infected leaf pieces of gladiolus used as inocula and plated on moist blotting paper in Petri dish (Plate V A). Similarly, after 5 days of incubation colonies of *B. gladiolorum* grew from pieces of gladiolus leaf were placed on PDA in Petri dishes (Plate V B). Conidiophores bearing conidia (Plate II C) appeared on the colonies produced on leaf pieces as well as PDA (Plate V A and C). Later on, the colony turned into brown and produced black sclerotia after 14-16 days of incubation (Plate V D and E). Conidiophores were dark brown and twisted (Plate V A, B, D and E). Conidia were ellipsoid and ovoid or oval in shape, pale brown in color and 14.2-20.7 mm x 9.3-12.9 mm in dimension (Plate V F).

Plate 1. Photographs showing leaf symptoms of *Botrytis* blight (*B. gladiolorum*) of gladiolus [A. Tiny spot, B. enlarge spot, C. blighted leaf, D & E. blighted leaf, F. lesion on sheath].

Plate II. Photographs showing stem symptoms of *Botrytis* blight (*B. gladiolorum*) of gladiolus [A. stem girdling with lesions, B. severe lesions on stem having mycelium, sporangiophores and conidia].

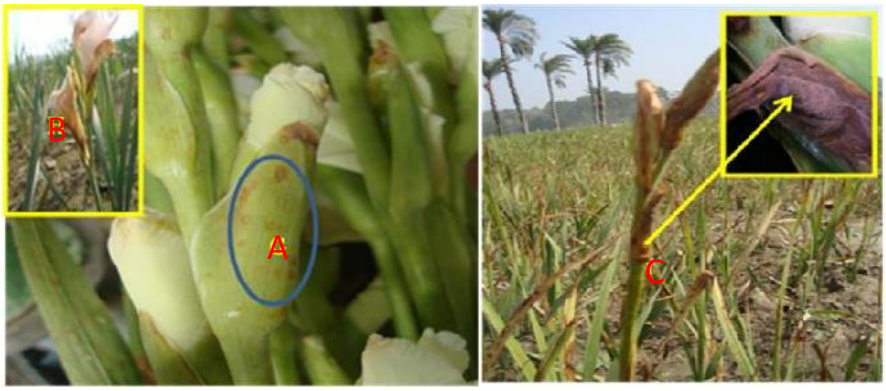


Plate III. Photographs showing flower symptoms of *Botrytis* blight (*B. gladiolorum*) of gladiolus [A. spots on flower bud, B. blighted flower having mycelium, sporangiophores and conidia].

The symptoms of *Botrytis* blight of gladiolus recorded from inoculated as well as naturally infected plants in the present investigation are similar to the symptoms reported by other researchers (Mirzaei *et al*. 2008, Sung Kee Hong *et al*. 2003, Pscheidt and Ocamb 2013). The morphological characteristics of colonies, mycelia, conidiophores, conidia and sclerotia of *B. gladiolorum* recorded in the present investigation are almost similar to the descriptions of Ellis, (1971), Wang *et al.* (1996), Kishi 1998, Sung Kee Hong *et al*. (2003) and Mirzaei *et al*. (2008).

It has been recorded that *B. gladiolorum* infects different flowers under Iridaceae family like gladiolus, freesia, ixia, crocus, and iris in North America, Europe, Africa, New Zealand, China, and Japan (Boerema and Hamers 1989, Gould 1954, Kishi 1998, Mckenzie 1990, Plate and Schneider 1972, Wang *et al.* 1996, Sung Kee Hong *et al*. 2003, Mirzaei *et al*. 2008).

Findings of the present investigation clearly reveal that *Botrytis* blight caused by *B. gladiolorum* regularly attacks the gladiolus plants in Jessore regions of Bangladesh. However, the disease has not yet been reported from the country. So, the present report may be considered as the new record of *Botrytis* blight of gladiolus and its causal fungus, *B. gladiolorum* in Bangladesh**.**

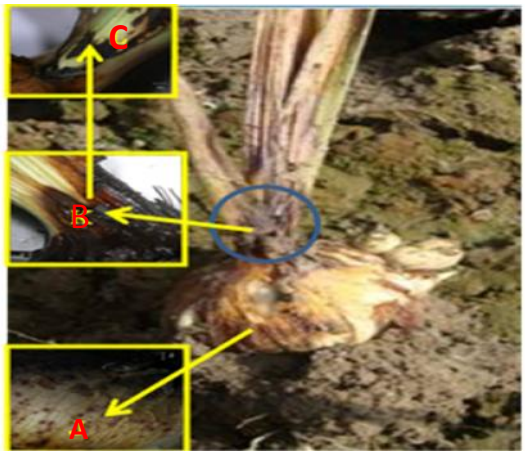


Plate IV. Photographs showing corm symptoms of *Botrytis* blight (*B. gladiolorum*) of gladiolus [A. spots on flower bud, B. blighted flower having mycelium, conidiophores and conidia, C. large brown spot on neck region]**.**

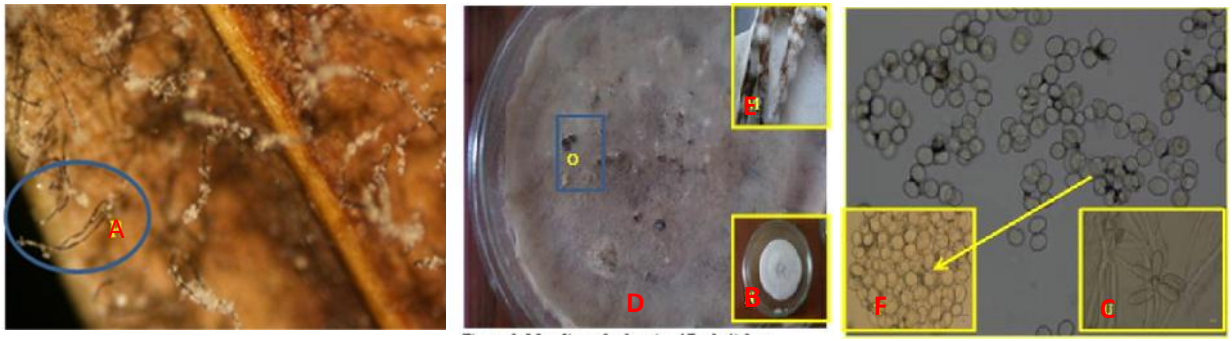


Plate V. Photographs showing corm symptoms of *Botrytis* blight (*B. gladiolorum*) of gladiolus [A. mycelium grew on leaves placed on moist blotter, B. mycelium grew on leaves placed on PDA, D and E. sclerotia formation, C & F. conidiophores bearing conidia, F. Conidia]**.**

LITERATURE CITED

Boerema, G. H. and Hamers, M. E. C. 1989. Checklist for scientific names of common parasitic fungi. Neth. J. Plant Pathol. 95 (Suppl.3):4-5.

Bose, T. K., Yadav, L. P., Pat P., Parthasarathy, V. A. and Das, P. 2003. Commercial Flowers, Naya Udyog, Kolkata, India. Vol. 2.

Chanda, S., Barma, G. and Roychowdhury, N. 2000. Influence of different levels of nitrogen, phosphorus and potassium on growth and flowering of gladiolus. Hort. J. 13 (1): 76-86.

Dodge, B. O. and Laskaris, T. 1941. Botrytis corm rot of Gladiolus. J.N.Y. bot. Gdn. 42, 92-5. (Tran. Brit. Mycol. Soc. 1949; 32. 291-304).

Drayton, F .L. 1929. Bulb growing in Holland and its relation to disease control. Sci. Agri. IX. 494-509. (Tran. Brit. Mycol. Soc. 32: 291-304, 1949).

Drayton, F. L. 1928. Reported of the Dominion botanìst for 1927. Rep. Dept. Agric. (Botany) Can., p27. Tran. Brit. Mycol. Soc. 1949: 32. 29l-304.

Ellis, M. B. 1971. Dematiaceous Hyphomycetes. Kew Surrey, England, 608pp.

Gould, C. J. 1954. *Botrytis* diseases of gladiolus. Plant Dis. Rep. Suppl. 224:2-33.

Kishi, K. 1998. Plant diseases in Japan. Zenkaku Noson Kyoiku Co., Ltd., Tokyo, Japan. 1276 p.

McKenzie, E. H. C. 1990. New plant disease records in New Zealands: miscellaneous fungal pathogens II. New Zealands J. Crop. Hort. Sci. 18:65-73.

Mian, I. H. 1995. Methods in Plant Pathology. Published by IPSA-JICA Project. IPSA, Salna Gazipur

Mirza, J. H. and Shakir, A. S. 1991. First report of fungal pathogens on Gladiolus from Pakistan. Pak. J. Phytopathol., 3: 74-76.

Mirzaei, S., Mohammadi Goltapeh, E., Shams-Bakhsh, M. and Safaie, S. 2008. Identification of *Botrytis* spp. on Plants Grown in Iran. J. Phytopathol. 156(1):21-28. DOI: 10.1111/j.1439-0434.2007.01317.x

Moore. W. C. 1939. Discases of bulbs. Bull. Minist. Agric. Fish. N0. 117. 112-116. (Brit. Mycol. Soc. Trans., 1949. 291-304.)

Pant, S. S. 2005. Effect of different doses of nitrogen and phosphorus on the corm and cormel development of gladiolus (*Gladiolus* sp.) cv. american beauty. J. Inst. Agric. Anim. Sci. 26: 153-157.

Plate, H. P. and Schneider, R. 1972. Ungewöhnliches Auftreten von *Botrytis gladiolorum* an Freesien. Gartenwelt 7:146-147.

Pscheidt, J. W. and Ocamb, C. M. (Senior Eds.). 2013. Gladiolus-Botrytis leaf and flower spot and corm rot. Pacific Northwest Plant disease Management Handbook, Oregon State University. pp 1-3.

Singh P. J., Sidhu, G. S. and Kumar Ramesh. 2005. Effect of Pre- and Post-inoculative Sprays of Fungicides on Blight of Gladiolus caused by Botrytis Gladiolorum. J. Orna. Hort. 8:2:139

Sohi, H. S. 1992. Diseases of ornamental plants in India. Indian Council of Agri. Research. New Delhi, p. 195

Sung Kee Hong, Wan Gyu Kim, Weon Dae Cho and Hong Gi Kim. 2003. Occurance of Gray Mold in Freesia and Gladiolus Caused by *Botrytis gladiolorum* in Korea. *Plant Pathol. J.* 19(2): 102-105.

Van Poeteren. N. 1938. Verol. Plziekt. Dienst Wageningen. 89. 92 pp. (Tran. Brit. Mycol. Soc. 1949; 32. 291-304).

Wade, G. C. 1945. Botrytis corm rot of gladiolus, its cause and control. Proc. Roy. Soc. Vict. LVII (N.S), 81-118.

Wang, X. Y., Zhang, L. X. and Zhang, Z. Y. 1996. A new species of *Botrytis* and 5 known *Botrytis* species in China. Acta Phyto- pathol. Sinica 26:79-85.

PESTALOTIOPSIS GUEPINII (DESM.) STAY. – A NEW PATHOGEN OF BLACK SPOT DISEASE OF ROSE IN BANGLADESH

Shamim Shamsi[1] and Anita Ghosh[2]

[1]Professor and [2] Post *Graduate student*
Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh
E-mail address: Prof.shamsi@gmail.com

ABSTRACT

Shamim Shamsi and Anita Ghosh. 2013. *Pestalotiopsis guepinii* (desm.) Stay. – a new pathogen of black spot disease of rose in Bangladesh. Bangladesh J. Plant Pathol. 29 (1&2): 11-14.

An investigation was conducted during November 2009 to October 2010 to determine causal fungi of black spot disease of rose (*Rosa centifolia* L.). Black spot infected leaf samples were collected from different locations of Dhaka city. The fungi associated with the samples were isolated and identified. The principal fungal pathogen associated with the diseased specimens was *Diplocarpon rosae* Wolf (imperfect stage *Marssonina rosae*). Other fungal pathogens associated with the disease were *Pestalotiopsis guepinii* and its two culture types (*Pestalotiopsis guepinii*-1, *P. guepinii*-2). The fungi belong to the class Coelomyecetes under the Division Deuteromycota. The fungi were frequently isolated from black spot infected leaf samples of the rose. After inoculation of detached leaves and seedlings of rose, *P. guepinii* and its two culture types developed characteristics symptoms of black spot. The pathogen was reisolated from black spot infected inoculated leaves to fulfill Koch's postulate. The findings of the investigation indicate that *P. guepinii* is another fungal pathogen of black spot of rose in addition to *D. rosae*. This is a new record about causal agents of black spot disease of the cut flower.

Keywords: Black spot, rose, *Pestalotiopsis guepinii*

INTRODUCTION

Rose (*Rosa centifolia* L.) is grown throughout the world for their beautiful flower and fragrance. The ornamental plant is also grown in Bangladesh. *Diplocarpon rosae* Wolf and its imperfect stage *Marssonina rosae* (Lib.) Died is well documented pathogen of black spot of rose. Black spots are circular with a perforated edge and reach a diameter of 14 mm. Severely affected plants however, do not show the circular spots as they coalesced together and form large lesion (Debner 1988). From India, Mukerji and Bhasin (1986) reported leaf spot of rose caused by *Pestalotiopsis versicolor*. Islam *et al*. (2010) reported seven diseases of rose from Bangladesh. The diseases, in order of their prevalence, are *Botrytis* blight (*Botrytis cinerea*), *Cercospora* leaf spot (*Cercospora puderi*), rose mosaic (Rose Mosaic Virus), black spot (*Diplocarpon rosae),* die-back (*Botryodiplodia theobromae*), *Alternaria* leaf spot (*Alternaria alternata*) and stem canker (*Crytosporella umbrina* (Speg.) Stey. In Bangladesh, reports on association of fungal pathogens with black spot symptoms are not available. The present investigation was undertaken to identify the fungal pathogens associated with black spot disease of rose plant other than *D. rosae*.

MATERIALS AND METHODS

Healthy and diseased leaves of rose were collected from different locations of Dhaka city during November 2009 to October 2010. Fungi were isolated from black spot infected leaf samples following "Tissue Planting method" on PDA medium and pure culture of the isolated fungi were prepared following single spore method (Tuite 1969). Morphological characteristics of the fungi were recorded under a compound microscope and identified using standard key books (Barnett and Hunter 2000, Booth 1971, Ellis 1971, 1976, Sutton 1980, Ellis and Ellis 1997). All diseased specimens and associated fungi were preserved in the Herbarium of the Department of Mycology and Plant Pathology, University of Dhaka.

Pathogenicity test of the isolated fungi was performed following modified detached leaf assay technique (Azad and Shamsi 2011). Fresh, healthy and mature leaves of rose were collected, washed with distilled water, surface sterilized with 1.0% Clorox for five minutes and rinsed in sterilized water. Ventral and dorsal sides of the leaflets with and without pricking with needles were inoculated with 2 mm diameter mycelial block of the isolated fungi previously grown on PDA medium for seven days. Another set of leaves with and without pricking and without inoculation were maintained, which served as controls. Three replicated leaflets were used for each treatment. The inoculated leaflets were placed in Petri dishes containing water soaked filter paper and cotton ball to maintain sufficient humidity to initiate infection. The plates were incubated at 25-28C. The inoculated and non inoculated leaflets were checked for symptom development starting from 3 days of inoculation and continued up to 7-10 days.

Seedling inoculation method was also followed to confirm pathogenicity of the isolated_fungi. Healthy seedling of rose plant was transplanted in pots (30 cm Diam.) containing sterilized soil at three seedlings per pot and allow to grow for three month in a nethouse providing necessary water and nutrients. Healthy leaves of seedlings were washed with sterilized water and surface sterilized with 1.0% Chlorox and rinsed with sterilized distilled water.

Surface sterilized leaves were pricked with sterilized needle. Pricked and unpricked leaves were inoculated with the test fungi by rubbing sporulating PDA culture of the test fungus. Leaves under control received only fresh PDA medium without fungal inoculum. Plants were covered with polythene bags to maintain proper humidity level and to avoid contamination. Three seedlings were inoculated for each treatment. The inoculated plants were placed in a clean bench. The plants were examined daily and continued up to 10 days to record the development of symptoms. Symptoms developed on artificial inoculated leaves were recorded and compared with the symptoms of those observed on naturally inoculated leaves. The fungus was resonated from the inoculated leaves of rose to fulfill Koch's postulates.

RESULTS AND DISCUSSION

Marssonina rosae was isolated from black spot infected leaves of rose plants. In addition to *M. rosae*, another fungal pathogen, *Pestalotiopsis guepinii* and its two culture types (*Pestalotiopsis guepinii*-1, *P. guepinii*-2) were frequently isolated from leaves having black spot symptoms (Plate I A, B and C). Their cultural characteristics are summarized in Table 1. Other fungi associated with black spot infected leaves of rose were *Cladosporium cladosporioides* (Fresen.) de Vries, *F. oxysporum* Berk. & Curt. and a species of *Penicillium*.

Table 1. Comparative study of *Pestalotiopsis guepinii* and its culture types isolated from black spot infected rose leaves

Isolate No.	Name of isolate	Characteristics of the fungus on PDA medium		
		Seven days old colony character	Acervulus	Dimension of Conidia (µm)
SS 2034	*P. guepinii*	Colony compact & diameter 17 mm	Formed within 7-10 days, large, many, compactly arranged	Length 18.4-23.6 Width 5.6-7. 2
SS 2041	*P. guepinii*$_1$	Colony compact & diameter 18 mm	Formed within 7 days minute, numerous, scattered	Length 20.0-28.0 Width 4-6.8
SS 2041	*P. guepinii*$_2$	Colony fluffy & diameter 18 mm	Formed after 25 days, colony fluffy	Length 20.4-26.8 Width 6.4-8.4

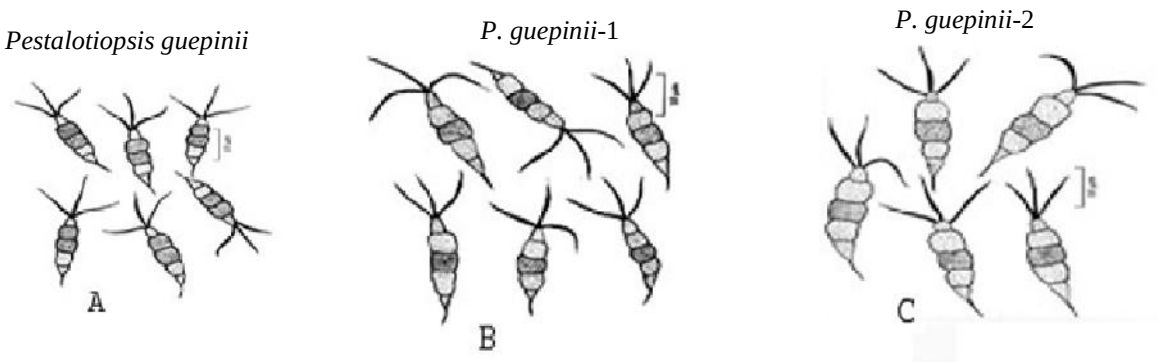


Plate I. Camera lucida drawings of conidia of *Pestalotiopsis guepinii* (A) and its two cultural types (B&C).

Taxonomic enumeration of *Pestalotiopsis guepinii*

Colonies on PDA having pH 6.0 and incubated temperature 25±1C are white, cottony. Hyphae hyaline, septate and profusely branched. Acervuli are black, shinning, conspicuous, conidiomata 200 µm long. Conidiophores short hyaline, 10-15 µm long and 1-2 µm wide, mostly aseptate, with 1-2 proliferation. Conidia blackish brown, mostly three septate with 2-5 (mostly 3) hyaline appendages at the apex and a short hyaline appendages at the base, apical appendages 16.5 - 26.1 µm long and a basal 4.2 - 7.8 µm long (Plates I and II). Based on these morphological characteristics, the fungus was identified as *Pestalotiopsis guepinii* (Desm.) Stay. (Sutton 1980).

Characteristic symptoms of black spot developed on rose leaves which were pricked dorsally and inoculated with *Pestalotiopsis guepinii* as dark brown lesion. The fungus was reisolated from inoculated rose leaves showing characteristic symptoms to fulfill the Koch's postulates. Two culture types of *P. guepinii* (1&2) also produced characteristic symptoms of black spot on inoculated rose leaves. *Pestalotiopsis guepinii* produced 8-10 mm dark lesion around the point of inoculation. Subsequently *P. guepinii*-1 produced 10-11 mm and *P. guepinii-2* produced 8-12 mm irregular dark lesion around the point of inoculation. Control leaves did not show any symptoms of disease (Plate III).

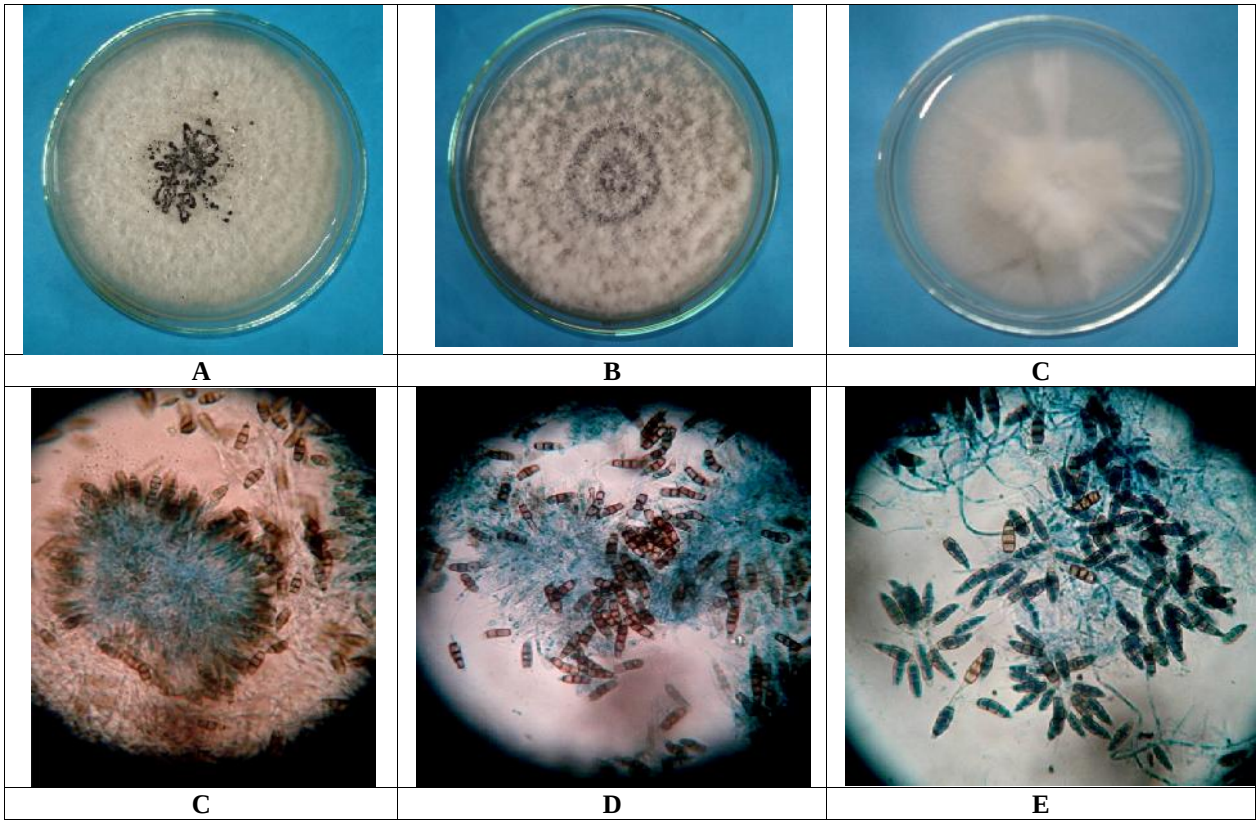


Plate II. Ten days old PDA culture of *Pestalotiopsis guepinii*, *P. guepinii*-1 and *P. guepinii*-2 (A-B) and acervulus with conodiophos and conidia of three pathogens (D-F).

Results of the present study reveal that *P. guepinii* and its two culture types can develop symptoms of black spot only when test leaves are pricked with dorsally before inoculation. It indicates that injury is essential for penetration of the fungus. Black spot caused by *D. rosae* and its imperfect stage has been reported earlier from Bangladesh (Islam *et al.* 2010) as well as abroad (Willium 1949, Debner 1988, Mukerji and Bhasin 1986). Reports on the association of *P. guepinii* with black spot infected rose leaves under natural conditions and its pathogenicity to cause the disease on inoculated rose leaves are not available in Bangladesh or abroad. The findings indicate that *P. guepinii* is another pathogen of black spot of rose and this is a new information on causal agent of the disease.

Plate III. Symptoms of black spot disease developed on detached leaves of rose inoculated with *P. guepinii*, *P. guepinii*-1 and *P. guepinii*-2 [A: Uninoculated leaf (control), B-D: Leaves inoculated with three isolates of *P. guepinii*, E: Control plant, F—K: Plants inoculated with three isolates of *P. guepinii*].

LITERATURE CITED

Azad, R. and Shamsi, S. 2011. Identification and pathogenic potentiality of fungi associated with *Huttuyania cordata* Thunb. Dhaka Univ. J. Biol. Sci. 20:(2):131-138.

Barnett, H. L. and Hunter, B. B. 2000. Illustrated Genera of Imperfect Fung. (4th edn.). Bur. Pub. Co. Minn. 218 pp.

Debner, T., Drewes-Alvarez, R. and Rockstroh, K. 1998. Identification of five physiological races of black spot, *Diplocarpon rosae* Wolf on roses. Pl. Breed. 117(3): 267-270.

Ellis, M. B. 1971. Dematiaceous Hyphomycetes. The CMI, England. 608 pp.

Ellis, M. B. 1976. More Dematiaceous Hyphomycetes. The CMI, England, pp. 507.

Ellis, M. B. and Ellis, J. P. 1997. Micro fungi on Land plants. An Identification Handbook. 868 pp.

Islam, M., Alam, M. A., Rahman, Q. A., Moinuddin, F. M. and Kabir. M. H. 2010.The diseases of rose at Bangladesh agricultural University campus, Mymensingh. IJSAT. pp. 15-20.

Mukerji, K. and Bhasin, G. 1986. Plant diseases of India a source book . Tata McGrew-Hill pub. Com., New Delhi. pp. 467.

Sutton, B.C. 1980. The Coelomycetes. Fungi Imperfecti with Pycnidia, Acervuli and Stromata. CMI, England. 696 pp.

Tuite, J. 1969. Plant pathoglogical methods. Fungi and Bacteria. Bur. Pub. Co. Minneapolis, Minn. USA. 293 pp.

IN-VITRO EVALUATION OF ANTIFUNGAL ACTIVITY OF PLANT EXTRACTS AGAINST *RHIZOCTONIA ORYZAE-SATIVAE* CAUSING AGGREGATED SHEATH SPOT OF RICE

S. B. Jahan[1], M. A. Ali[2], S. Alam[1], Z. R. Moni[3] and M. A. Alam[3]

[1]Department of Botany, Rajshahi University, Rajshahi; [2]Plant Pathology Division, [3]Plant Breeding division, Bangladesh Rice Research Institute, Joydebpur, Gazipur-1701, Bangladesh.
Email of first author: shapnattc@yahoo.com

(The paper is a part of M.Ph thesis of the first author)

ABSTRACT

S. B. Jahan, M. A. Ali, S. Alam, Z. R. Moni and M. A. Alam. 2013. *In-vitro* evaluation of antifungal activity of plant extracts against *Rhizoctonia oryzae-sativae* causing aggregated sheath spot of rice. Bangladesh J. Plant Pathol. 29 (1&2):15-19.

I*n-vitro* experiments were conducted to test the antifungal activity of garlic clove (*Allium sativam*), ginger rhizome (*Zingiber officinales*), and leaves of henna (*Lawsonia inermis*), water pepper (*Poligonum hydropiper*), ivy gourd (*Coccinia cordifolia*) and neem (*Azadirachta indica*) against mycelia growth of *Rhizoctonia oryzae-sativae*, the causal fungus of aggregated sheath spot of rice. Aqueous extracts of all the botanicals were prepared, mixed with liquid potato dextrose agar (PDA) medium at 0, 5, 10, 15, 20 and 25% concentrations and poured into 90 mm Petri dishes at 20 ml per dish. After solidification of PDA, 90 mm glass Petri dishes were inoculated with mycelia blocks of *Rhizoctonia oryzae-sativae* cut from 5 days old PDA culture of the pathogen at one block per plate. It was found that the plant extract reduced radial colony diameter of the pathogen appreciably at different concentrations of the botanicals compared to control (0%). Among the tested plant extracts, garlic and henna was the most effective material against *R. oryzae-sativae* showing 50% reduction in colony diameter at 3.25% concentration, which indicated lowest LD (lethal dose) 50 value followed by henna extract at 3.75%. Lowest LD 90 value also showed by garlic extracts at 17.25% followed by henna extract at 19%. Garlic and henna considerably decreased sclerotia germination at all the concentrations tested in the present study.

Key words: *Rhizoctonia oryzae-sativae*, aggregate sheath spot, *in-vitro* screening, plant extracts

INTRODUCTION

Aggregated sheath spot caused by *Rhizoctonia oryzae-sativae* is usually considered as a minor disease of rice but it can be a very aggressive disease of the crop under favourable conditions (Lanoiselet *et al*. 2007). It occurs in many of the rice growing countries of the world (Gunnel *et al.* 1984, 1992, Cedeno *et al.* 1998, Lanoiselet *et al.* 2001). In Bangladesh, aggregate sheath spot was reported for the first time in 1988 by Shahjahan *et al.* (1988). The disease caused yield losses of 20% in Australia, 4 to 9% in Uruguay (Lanoiselet *et al.* 2005) and in Bangladesh the disease may reduce rice yield by 14.74%.

Chemical, physical and cultural methods are recommended to control aggregated sheath spot of rice. Available reports reveal that extracts of many plant species possess antifungal and antibacterial properties (Hasan *et al*. 2005, Ogbo and Oyibo 2008, Dubey *et al*. 2009). Several higher plants and their constituents may be used successfully in plant disease control (Singh *et al*. 1993). Adityachaudhury (1991) mentioned that use of plant extracts and phytoproducts is gaining attention due to their bio-degradability, low toxicity and mini-mum residual toxicity in the ecosystem. Antifungal activities were found in *Eucalyptus, Syzygium aromticum, Azadirachta indica, Rosmarinus officinalis* against *Rhizoctonia solani, R. oryzae, R. oryzae-sativae* and *Sclrotium hydrophilum* (Aye *et al*. 2011). Cinnamon oil was found efficient plant product that inhibited *in vitro* colony diameter of *R. oryzae-sativae* as well as suppression of aggregate sheath spot disease of rice under greenhouse condition. The present piece of research was undertaken to evaluate efficacy of six plant extracts to inihiting invitro vegetative growth and sclerotia germination of *R. oryzae-sativae*.

MATERIALS AND METHODS

Six locally available plant species namely garlic (*Allium sativam*), ginger (*Zingiber officinales*), henna (*Lawsonia inermis*), water pepper (*Poligonum hydropiper*), ivy gourd (*Coccinia cordifolia*) and neem (*Azadirachta indica*) were collected. Water extracts of garlic clove, ginger rhizome, and leaves of henna, water pepper, ivy gourd and neem were prepared. For preparation of the plant extracts, 100 g of each material was washed in sterile distilled water, 100 ml sterile water was added (1:1 w/v), crushed in a mortar and

pestle and passed throught 2-ply cheese cloth. Each plant extract was filtered through filter paper (Whatman no.1), which was considered as standard plant extract of each plant species and tested for antifungal activity.

Poison food technique was followed to test the plant extracts using potato dextrose agar (PDA) as the basic medium (Dhingra and Sinclair 1985, Ali and Archer 2003). The aqueous extracts were thoroughly mixed with warm PDA (40C) at 5, 10, 15, 20 and 25% concentration and poured into sterile Petri plates (90 mm). PDA without any extract served as the control. An isolate (No. MY-1) of *R. oryzae-sativae* was obtained from Plant Pathology Division, Bangladesh Rice Research Institute (BRRI), Gazipur and multiplied on fresh PDA. Mycelial disc were cut from the actively growing section of 3-day old culture of the pathogen. The disc was placed at the center of each Petri plate containing amended or unamended PDA at one disc per plate. The inoculated plates were incubated at room temperature (25-28C). The plates were arranged on the desks in the Laboratory of Plant Pathology Division, BRRI following completely randomized design. Four replicated plates were used for each treatment. The redial colony diameter was measured in all treatments when the mycelium reached the rim of the Petri plate under control. Percent growth inhibition was calculated based on diameter of colony under control plates.

Inhibition of sclerotia germination was determined by the method of Chaizuckam and Davis (2010). PDA medium was amended with the plant extracts at 0, 5, 10, 15, 20 and 25% following the procedures as mentioned earlier. Ten sclerotia of the pathogen (isolate MY-1) were collected from 14 days old PDA culture, soaked in sterile water for 10 min. Water soaked sclerotia were transferred to amended PDA plates. After tree day of incubation at room temperature number of germinated sclerotia was counted. The experiment was repeated once.

Data on radial mycelial growth were subjected to statistical analysis using CropStat (version 7.2) computer software. Paired-t test was performed to assess the effect of plant extract on sclerotial germination comparing with control.

RESULTS AND DISCUSSION

In-vitro mycelial growth of *R. oryzae-sativae* in the control plates reached the rim within 4 days of incubation. Every plant extract significantly reduced *in-vitro* radial colony diameter of the fungus at all concentrations compared to control. The degree of reduction corroborated with their concentrations (Table 1).

Percentage of mycelial growth inhibition at different concentrations of five plant extracts over control is shown in Figure 1. In general, the highest reduction was achieved with garlic extract followed by henna, neem and ginger extracts. The reduction in colony growth under the extracts ranged 67.67-100.00%, 33.39-78.33 and 27.22-70.56%, respect-tively. The lowest reduction of 12.22-32.78% was recorded from treatments with water pepper followed by ivy gourd which showed 15.56-61.67% reduction in colony growth. Irrespective of plant extract, growth inhibition percentage increased gradually with the increase of concentration. The relationship between growth inhibition and concentration was linear and positive. The correlation coefficient for each extract was significant (Fig. 1 and Table 2).

All of the plant extracts caused inhibition of sclerotia germination of *R. orya sativae*. The most effective one was garlic followed by henna. Significant reduction in sclerotia germination was achieved with all concentrations of two plant extracts compared to control. The least effective plant extract was water pepper followed by Ivy gourd (Table 3).

Table 1. Effect of different concentrations of six plant extracts on *invi-tro* mycelial growth of *R. oryzae sativae*

Plant extract	Radial diameter (mm) at different concentration of plant extract (%)					
	0	5	10	15	20	25
Garlic	45.00	14.50	12.25	8.25	0.00	0.00
Ginger	45.00	38.00	27.75	21.25	18.50	13.25
Henna	45.00	15.50	13.00	9.250	3.75	2.50
Water pepper	45.00	39.50	36.50	34.75	33.00	30.25
Ivy gourd	45.00	32.75	31.00	27.75	20.50	17.25
Neem	45.00	29.75	23.00	17.75	13.25	9.75

LSD (P=0.05) to compare treatments means (except control) is 1.47 and to compare treatment means with control mean1.31

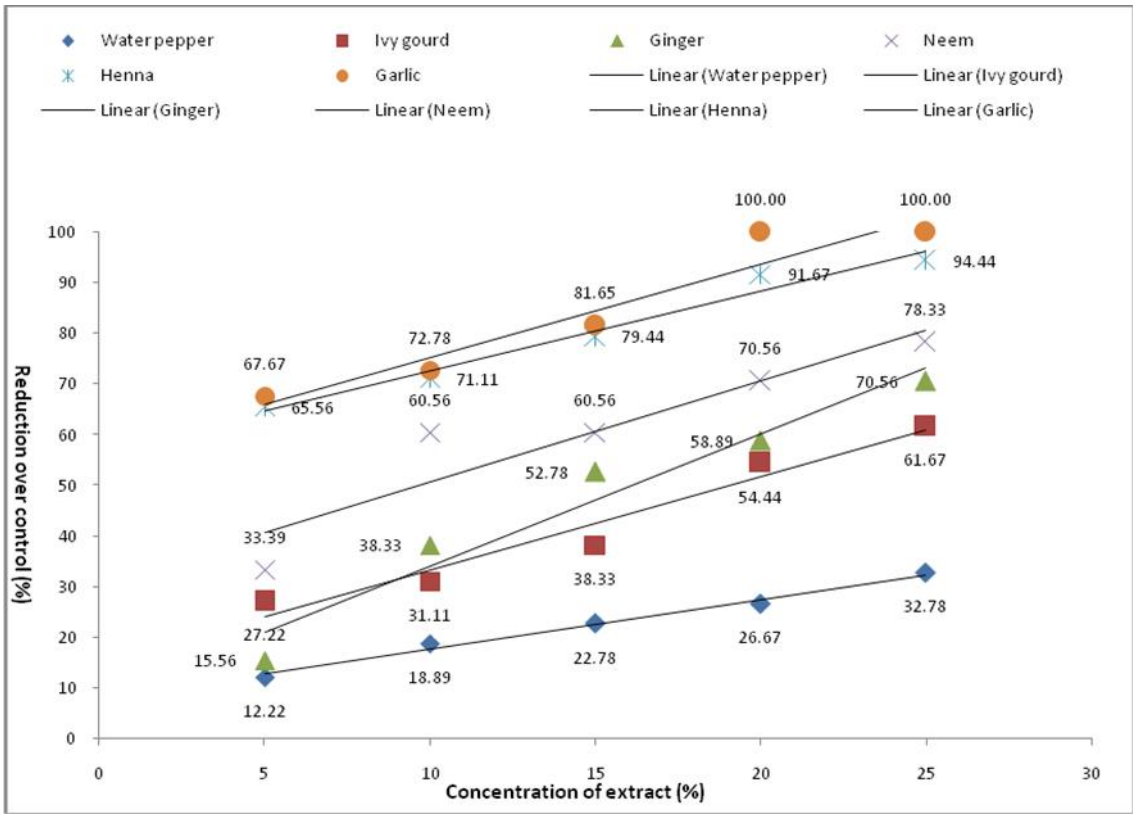


Fig. 1. Relationship of percentage of reduction over control in *in-vitro* radial colony diameter of *R. oryzae-sativae* with concentration of five plant extracts.

Table 2. Regression equation, coefficient of determination and coefficient of correlation of relationship between growth reduction and concentration of different plant extract

Botanicals	Regression Equation	Coefficient of determination (R^2)	Coefficient of correlation (r)
Water pepper	y = 0.978x + 7.99	0.963	0.989*
Ivy gourd	y = 1.844x + 14.88	0.977	0.955*
Ginger	y = 2.611x + 8.06	0.980	0.950*
Neem	y = 1.997x + 30.71	0.929	0.864*
Henna	y = 1.566x + 56.94	0.990	0.971*
Garlic	y = 1.837x + 56.85	0.963	0.928*

Results of the present investigation reveal that water extracts of garlic, zinger, henna, ivy gourd and neem posses antifungal activity showing cause of inhibition of *in-vitro* colony growth of *R. oryzae-sativae*. The most effective botanical is garlic followed by henna. They can reduce mycelial growth of *R. oryzae-sativae* at least by more than 60% at their lowest concentration. Garlic at 20% concentration and above concentration is effective for complete inhibition of in-vitro growth. Henna extract is able to reduce mycelia growth of the pathogen by 94.44% at 25% concentration. *In-vitro* antifungal activity of botanicals tested in the present study have also been reported by many other investigators (Chaijuckam and Devis 2010, Aye *et al*. 2011, Rahman *et al*. 2012, Chaity *et al*. 2012).

Table 3. Antifungal activity of different plant extracts with different concentrations on sclerotia germination of *R. oryzae-sativae*

Plants Extract	Concentration of plant extracts (%)				
	5	10	15	20	25
Garlic	8.0***	7.25***	7.0***	0	0
IVY Gourd	9.5	9.0	9.0	8.75	8.25**
Ginger	8.25**	8.25**	8.0***	7.5***	7.25***
Henna	9.5	9.5	9.5	9.0	9.0
Water pepper	9.5	9.5	9.25	8.75	8.5*
Neem	9.25	9.0	8.75	8.5*	8.0***
Control (without plant extract)	9.5				

Significant difference between plant extracts with different concentration and the controls at 0.1%, 1% and 5% level is indicated by ***, ** and * respectively

The factors responsible for antifungal activity of the botanical tested have not been studied in present investigation. However, other investigators reported that sulfur rich protein Ajoene derived from garlic had antifungal activities against *Aspergillus niger*, *Candida albicans* (Yoshida *et al.*1987). Other workers also showed the presences of antifungal properties in *A. sativam* (Misra and Dixit 1976, Agarwal 1978). Garlic has already been reported to have antifungal activity against *R. oryzae-sativae* (Chaijuckam *et al.* 2010). The sensitivity of fungi and even isolates of the same species to plant extracts may vary. Such as, garlic extract at 5% completely inhibited vegetative growth of California isolates of *R. oryzae-sativae* (Chaijuckam *et al.* 2010) but in case of Bangladeshi isolate of *R. oryzae-sativae*, it required 20% concentration.

In the present experiment *R. oryzae-sativae* was added in the list of sensitivity to the henna extract. The henna leaf extract caused 65.56% - 94.44% inhibition at 5 - 25% concentrations. Leaf extract of henna completely controlled the growth of *Drechslera oryzae, Sclerotium oryzae, S. rolfsii and Rhizoctonia solani* at 20% (w/v) concentration. The presence of antifungal compound (2- hydroxyl- 1, 4 napthoquinine) in the leaf extract of henna had been identified which might be responsible for microbial growth inhibition (Tripathi *et al.* 1978).

Neem leaf extract showed 33.89 - 78.33% inhibition at 5 - 25% concentration. The plant extract inhibited the mycelial growth of *R. solani, R. oryzae sativae R. oryzae*, and *Sclerotium hydrophilum* by 87.5, 80.0, 92.5 and 49.2% respectively (Aye and Matsumoto 2011). Similarly, findings of the present investigation showed satisfactory reduction in colony growth on *R. oryzae-sativae* by using neem leaf extract. In present test, ginger showed moderate inhibition against *R. oryzae-sativae*. Pakrashi (2003) demonstrated that Ginferenone A, a diarylheptenone constituent of ginger, showed strong antifungal action against *Pyricularia oryzae* and moderate anticoccidium effect *in vitro*.

Lower inhibition percentage were noted in this investigation with *P. hidropiper* and *Coccinia cordifolia*. Hasan *et al.* (2009) observed that *P. hidropiper* root extract on chloroform had strong antifungal activities against *A. niger*, *A. fumigatus*, *A. flavus, C. albicans*, *Rhizopus oryzae* and *Tricophyton rubrum.* Garlic and henna extracts significantly decreased sclerotia germination at all concentrations while, neem extract at 20% and 25% reduced sclerotia germination significantly compared to the control. The findings of the present investigation reveal that garlic and henna contain effective properties against *R. oryzae-sativae* (Table 3).

ACKNOWLEDGEMENT

This work was supported by Plant Pathology Division, Bangladesh Rice Research Institute, Gazipur. First author appreciates the Ph.D. grant by University Grant Commission, Bangladesh.

LITERATURE CITED

Adityachaudhury, N. 1991. Phytochemicals: their potency as fungicides and insecticides and their prospects of manipulating natural production. *In*: Sen and Dutta (eds.) Biotechnology in crop protection. Kalynana, India.

Agarwal, P. 1978. Effect of root and bulb extracts of *Allium* spp. on fungal growth. Trans. Brit. Mycol. Soc., 70 (3): 439-441.

Ali, M. A. and Archer, A. 2003. Evolution of some new fungicides against sheath blight disease of rice caused by *Rhizoctonia solani*. Bangladesh J. Pl. Pathol. 19 (1&2):13-20.

Aye, S. S. and Matsumoto, M. 2011. Effect of some plant extracts on *Rhizoctonia* spp. and *Sclerotium hydrophillum*. J. Medi. Pl. Res.5: 3751-3757.

Cedeno, L., Nass, H., Carrero, C., Cardona, R., Rodriguez, H., and Aleman, L. 1998. *Rhizoctonia oryzae-sativae*, agent of the aggregated stain of rice in Venezuela. Interciencia 23: 248-251.

Chaijuckam, P. and Davis, R.M. 2010. Efficacy of natural plant products on the control of aggregate sheath spot of rice. Pl. Dis..94(8): 986-992.

Chaity, S. A., Khan, A. A. and Mian, I. H. 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): 59-62.

Dhingra, O. D. and Sinclair, J. B. 1985. Basic plant method. CRP Press, Inc.Boca Raton, Florida, 132 pp.

Dubey, R. C., Kumar, H. and Pandey, R. R. 2009. Fungitoxic effect of Neem xtracts on growth and sclerotial survival of *Macrophomina phaseolina in vitro*. J. Am. Sci., 5: 17-24.

Gunnel, P. S. and Webster, R. K. 1984. Aggregate sheath spot of rice in California, Pl. dis. 68, 529-531.

Hasan, M. M., Chowdhry, S. P., Alam, S., Hossain, B. and Alam, M. S. 2005. Antifungal effect of plant extracts on seed-borne fungi of wheat seed regarding seed germination. Pak. J. Biol. Sci., 8: 1284-1289.

Hasan, M. F., Das, R., Khan, A., Hossain, M. S. and Rahman, M. 2009. The Determination of antibacterial and antifungal activities of *Polygonum hidropiper* root extract. Adv. Biol. Res. 3(1-2): 53-56.

Lanoiselet, V. M., Ash, G. J., Cother, E. J., Priest, M. J. and Watson, A. 2001. First report of *Waitea circinata* causing sheath spot and *Rhizoctonia oryzae-sativae* causing aggregate sheath spot on rice in south-eastern Australia. Plant Pathol. 30:369-370.

Lanoiselet, V.M., Cother E. J., Ash, G. J., and Harper, J. D. I. 2005. Yield loss in rice caused by *Rhizoctonia oryzae-sativae* in Australia. Australian Pl. Pathol. 34: 175-179.

Mistra, S. B. and Dixit, S. N. 1976. Fungicidal spectrum of the leaf extract of *Allium sativum*. Indian Phytopath. 29(4): 208-231.

Ogbo, E. M. and Oyibo, A. E., (2008). Effects of three plant extracts (*Ocimum gratissimum, Acalypha wilkesiana ans Acalypha macrostachya)* on post harvest pathogen of Persea americana. J. Med. plants Res., 2: 311-314.

Pakrashi, S. C., Pakrashi, A. 2003. Ginger: A Versatile Healing Herb. P. 42.

Rahman, M. Z., Mafuzul Haque, A. H. M., Zaman, M. A., Amin, M. F. and Das, A. K. 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.

Singh, H. N. P., Prasad, M. and Shinha, K. K. 1993. Efficacy of leaf extracts of some medicinal plants against disease development in banana. Lett. Microbial., 17: 269-271.

Tripathi, R. D., Srivastava, H. S., and Dixit, S. N. 1978. A fungistosic principle from the leaves of Lawsonia inermis Linn. Experientia, 24:51-52.

Yoshida, S., Kasuga, S., Hayashi, N., Ushiroguchi, T., Matsmura, H. and Nakagawa, S. 1987. Antifungal Activity of Ajoene Derived from Garlic. Appl. Environ. Biol. 53(3): 615-617.

IDENTIFICATION OF PATHOTYPES OF *XANTHOMONAS ORYZAE* PV. *ORYZAE* CAUSING BACTERIAL BLIGHT OF RICE IN BANGLADESH

S. M. K. H. Chowdhury[2], I. H. Mian[1] and M.A.I. Khan[3]

[1]Professor and [2]Graduate Student, Department of Plant Pathology
Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706, Bangladesh, and
[3]Senior Scientific Officer, Bangladesh Rice Research Institute, Gazipur-1701, Bangladesh

(This is a part of MS thesis of first author)

ABSTRACT

S. M. K. H. Chowdhury, I. H. Mian and M.A.I. Khan. 2013. Identification of pathotypes of *Xanthomonas oryzae* pv. *oryzae* causing bacterial blight of rice in Bangladesh. Bangladesh J. Plant Pathol. 29 (1&2):21-27.

An investigation was conducted to identify pathotypes or races of *Xanthomonas oryzae* pv. *oryzae* causing bacterial blight of rice depending on the reaction patterns to a set of nine near-isogenic lines (NILs) or differentials. Ten isolates of *X. oryzae* pv. *oryzae* collected from different locations of Bangladesh were tested on the NILs namely IRBB2, IRBB4, IRBB5, IRBB7, IRBB10, IRBB11, IRBB13, IRBB14 and IRBB21 harboring the resistance genes *Xa2*, *Xa4*, *xa5*, *Xa7*, *Xa10*, *Xa11*, *xa13*, *Xa14* and *Xa21*, respectively. The resistance frequency was maximal in IRBB21 followed by IRBB4, IRBB5 and IRBB7. Among the differentials (NILs), the resistance frequency was the lowest in IRBB13 followed by IRBB10, IRBB2, IRBB14 and IRBB11. Based on susceptibility of the differentials and virulence of the isolates in terms of lesion length and infection frequency the isolates were grouped into 8 different pathotypes or races. Of them three isolates (ISO3, ISO4 and ISO6) showed identical virulence on the differentials and they were grouped into pathotype 1. Other seven isolates were grouped into 7 different pathotypes (Pathotype 2 to 8). The most avirulent isolate was ISO-1 followed by ISO-5 and ISO-10. Only one differential, IRBB21 showed resistant reaction to all isolates whereas IRBB13 was susceptible to all isolates except ISO1.

Key word: *Xanthomonas oryzae* pv. *oryzae*, pathotype, rice differential.

INTRODUCTION

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* is one of the major diseases of rice (*Oryza sativa* L.) in Bangladesh. The disease appears every year with different degree of severity (Jalaluddin *et al*. 2005). It is noted as the most destructive and wide spread bacterial disease in the tropics and sub-tropics (Mew 1989, Ezuka 2000). The average yield of rice in Bangladesh is lower compared to the other rice growing countries of the world. There are many constraints responsible for low yielding, among them disease are considerable as the most important one. Bacterial blight disease has been recognized as one of the most damaging disease of rice in Bangladesh. But, comprehensive report on the effects of BB on rice yield in Bangladesh is not available. Reports from other rice growing countries reveal that yield loss due to BB varied from 2% to 74% depending on location, season, crop growth stage and genotype (Kumar *et al.* 2013).

Present recommendations for the management of bacterial blight of rice are use of host resistance, modifications in cultural practices, use of biological control agents, botanical extracts, natural products, and conventional and non-conventional chemicals. Rice genotypes showing resistance to bacterial blightin different countries are also recorded Ou 1985, Anon. (Ou 1985, Anon. 1988). Use of resistant varieties is the most effective and economic management practice against bacterial blight of rice. When different strains of a bacterial pathogen are present, it is recommended to grow resistant varieties possessing field resistant genes to overcome development of new races in the pathogen populations (Khan *et al.* 2009). Khan *et al.* (2009) identified virulence differentiation of 41 isolates of *X. oryzae* pv. *oryzae* collected from different areas of Bangladesh, based on strain-cultivar interaction using 9 near isogenic lines of rice with different resistance genes. Leaves were inoculated following leaf clipping method. The virulence of the strains was differentiated distinctly. Weak interaction patterns were found between bacterial isolates and rice cultivars, but there were specific interactions. They suggested that *X. oryzae* pv. *oryzae* could be divided into 6 pathotypes.

Environment, rice cultivation with new varieties, BB incidence/severity might cause a shift in pathogen-host interaction. Therefore, regular investigations especially before development of durable resistant rice varieties to BB, is necessary to identify the existing pathotypes or races of the causal pathogen. Considering the above point, the present investigation was undertaken to identify pathotypes or races of *X. oryzae* pv. *oryzae* (*Xoo*) isolates collected from selected areas of Bangladesh.

MATERIALS AND METHODS

Leaf samples of rice infected with *Xanthomonas oryzae* pv *oryzae* (BB) were collected from farmers' fields of Gazipur, Comilla, Chittagong, Rangpur and Dinajpur districts in Bangladesh during 2010-2011. Ten isolates of *X. oryzae* pv. *oryzae* were established from the collected samples using peptone sucrose agar (PSA) medium (Jalaluddin *et al.* 2005). Single colony of pure culture of each isolate was prepared and maintained in PSA medium slants. Bacteria were mixed in sterilized clay suspension and stored under room condition for long term use (Hossain 2001).

The isolates were designated using an abbreviation 'ISO' for isolate and a serial number. Pathogenicity of the isolates was tested on a set of nine near-isogenic lines (NILs) namely IRBB2, IRBB4, IRBB5, IRBB7, IRBB10, IRBB11, IRBB13, IRBB14 and IRBB21 harboring the resistance genes *Xa2*, *Xa4*, *xa5*, *Xa7*, *Xa10*, *Xa11*, *xa13*, *Xa14* and *Xa21*, respectively (Huang *et al.* 1997). This set of NILs was developed by IRRI, Philippines using background of IR24.

Seeds of the NILs were collected from Bangladesh Rice Research Institute (BRRI). The BRRI obtained them from International Rice Research Institute (IRRI), Manila, Philippines. The experiment was conducted in the Experimental Farm of BRRI, Gazipur during T. Aman season, 2011. Seedlings of each differential were raised in earthen pots containing sandy loam soils mixed with well decomposed cow dung. Thirty days old seedling was transplanted in the field at one seedling per hill maintaining row to row and hill to hill distances of 20 cm. Ten sets of nine NILs were planted for ten isolates maintaining 40 cm set to set distance.

The isolates of *X. oryzae* pv. *oryzae* were grown on PSA slants separately for 72 hours at 30°C. Inoculum of each isolate was prepared by mixing the cultured bacteria in 10 ml sterile distilled water in a test tube. Before inoculation, the concentration of the bacterial suspension was adjusted to 10^8 CFU/ml using sterile distilled water. At the maximum tillering stage, 8-10 leaves/hill were inoculated by clipping with sterile scissors dipped in the bacterial suspensions. Three hills of each of the NILs were inoculated with individual isolate (Khan *et al*. 2009).

For data collection, a total of 20 leaves infected with BB were collected after 21 days of inoculation. Data on lesion length on each leaf was measured. Disease reactions of the NILs to the isolates were categorized based on lesion length, where less than 3 cm was considered as resistant (R) and more than 3 cm was rated as susceptible (S) according to Li *et al.* (2008). The isolates of *X. oryzae* pv. *oryzae* produced more than 3 cm long lesion on inoculated leaves were considered as virulent and the NILs were considered as susceptible (S), and those produced less than 3 cm long lesion were noted as avirulent and the NILs as resistant (R).

RESULTS AND DISCUSSION

Reactions of nine near-isogenic lines (NILs)

Mean lesion length of bacterial blight developed on nine near-isogenic lines (NILs) owing to inoculation with ten isolates of *X. oryzae* pv. *oryzae* varied greatly. Each of ten isolates produced a range of lesion size, showing the largest size lesion on a single NIL and the shortest on another NIL. These two extreme values were significantly different. The intermediate values some were significantly different from the highest value and some were different from the lowest value (Table 1).

Significantly the highest mean lesion length was recorded from NIL IRBB10. The second highest mean lesion length was found on IRBB11 followed by IRBB4 and IRBB2. The lesion length of three rice NILs was statistically similar but significantly higher compared to other NILs except IRBB10. The average lesion length on leaves of IRBB5, IRBB13 and IRBB7 was also statistically similar and significantly higher compared to only IRBB14 and IRBB21. The lowest lesion length was found in IRBB21 followed by IRBB14. The response of two NILs to the pathogen was significantly different (Fig. 1).

The mean lesion length on nine-rice NILs caused by isolates ISO4, ISO6 and ISO3 of *X. oryzae* pv. *oryzae* was statistically similar and significantly higher compared to other seven isolates. The mean lesion length developed by ISO2, ISO8 and ISO9 was also statistically similar and significantly higher compared to ISO1, ISO5 and ISO10. The differences in average lesion length of later three isolates were not significant (Fig. 2).

Virulence diversity among ten isolates

The isolates of *X. oryzae* pv. *oryzae* were polymorphic for virulence to nine NILs of rice possessing the resistance genes *Xa2*, *Xa4*, *xa5*, *Xa7*, *Xa10*, *Xa11*, *xa13*, *Xa14* and *Xa21*. Ten isolates collected from naturally BB infected rice plant grown in the districts of Rangpur, Dinajpur, Gazipur, Jhalokati, Comilla and Chittagong were classified into 8 pathogenic groups, which were tentatively designated as pathotype 1 to 8 on the basis of their pathogenicity on nine NILs. The reaction patterns of the 8 pathogenic groups were respectively SSSSSSSSR, SRRRSRSRR, RRRRSRSRR, RSSSSSSSR, SRRRSSSRR, RRRRSRSSR, RRRRRRSRR and RRRRRRRRR. None of the isolates showed virulence effect on all the NILs (Table 2).

Table 1. Lesion length of bacterial blight on nine near isogenic lines (NILs) and a variety of rice caused by ten isolates of *Xanthomonas oryzae* pv. *oryzae* after inoculation.

NILs	Mean lesion length (cm) developed by ten isolates										
	ISO1	ISO2	ISO3	ISO4	ISO5	ISO6	ISO7	ISO8	ISO9	ISO10	Mean
IRBB2	0.71	13.86	19.81	23.73	0.51	7.37	2.57	8.50	0.83	1.60	7.95
IRBB4	0.41	0.54	17.81	25.43	0.60	22.10	10.70	0.83	0.55	0.60	7.96
IRBB5	0.36	2.40	15.03	13.63	0.72	19.33	10.74	0.39	0.94	0.62	6.42
IRBB7	0.42	0.88	11.60	11.42	0.77	19.28	8.09	1.14	1.13	0.85	5.56
IRBB10	1.18	10.24	17.37	26.23	4.02	20.17	7.65	11.57	12.95	1.20	11.26
IRBB11	0.67	1.77	17.55	17.57	0.73	17.46	21.21	4.44	2.79	1.53	8.57
IRBB13	1.60	5.94	8.92	9.06	4.62	9.01	3.70	8.98	3.65	3.47	5.90
IRBB14	1.43	0.99	9.61	3.13	0.82	13.37	5.50	1.79	4.47	1.76	4.29
IRBB21	0.55	2.71	1.72	1.09	1.62	2.66	1.15	1.07	0.72	2.42	1.57
Mean	0.81	4.37	13.27	14.59	1.60	14.53	7.92	4.30	3.11	1.56	-

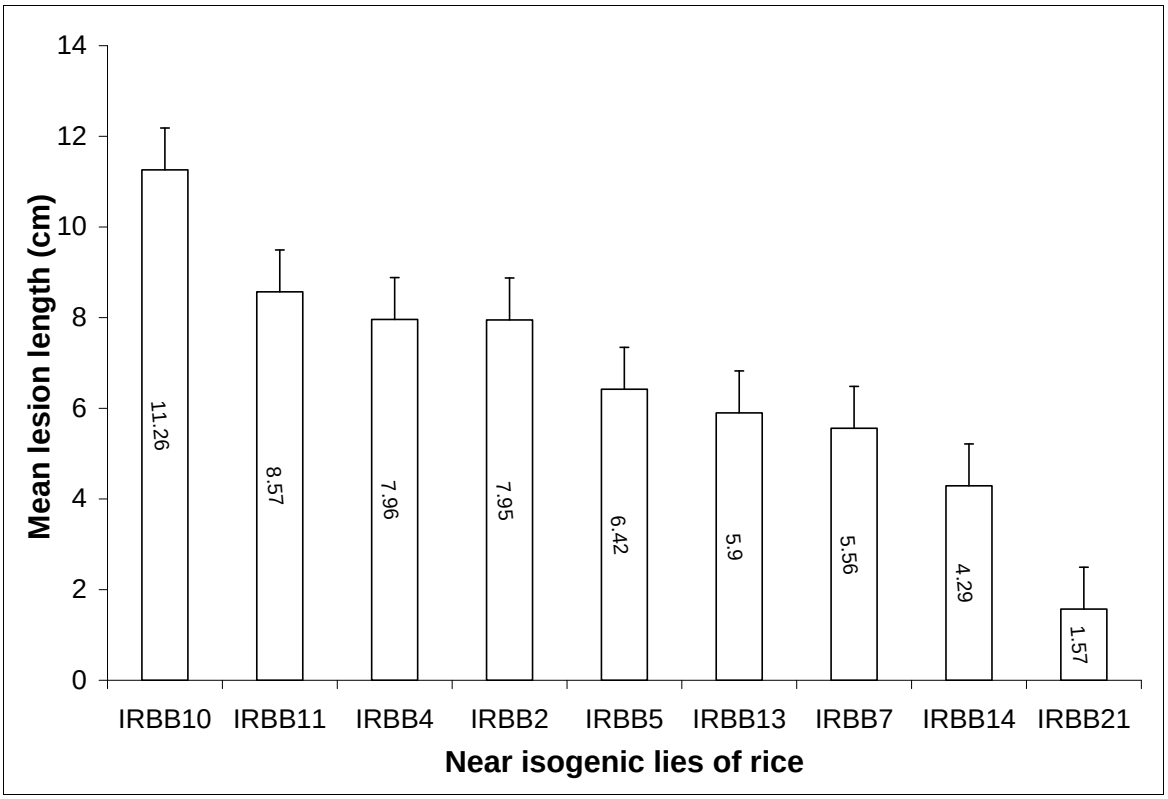


Figure 1. Mean lesion length of bacterial blight on nine near-isogenic lines of rice due to inoculation with isolates (*Xanthomonas oryzae* pv. *oryzae*)

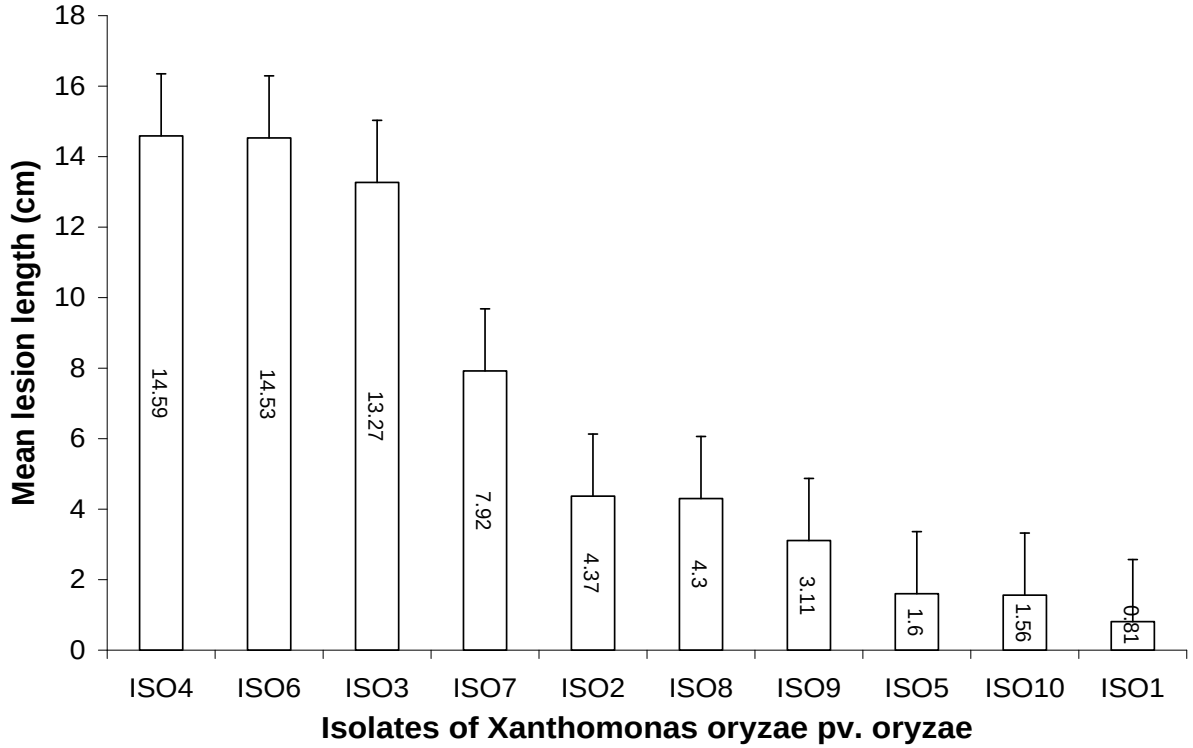


Figure 2. Mean lesion length of bacterial blight caused by ten isolates of *Xanthomonas oryzae* pv. *oryzae* on nine near-isogenic lines of rice

Table 2. Pathogenic diversity of the 10 isolates of *Xanthomonas oryzae* pv. *oryzae* based on the reaction of nine near-isogenic lines (NILs)

Patho type	Representative isolates	Near-isogenic lines (NILs) and known resistance genes[a]								
		IRBB2 (*Xa2*)	IRBB4 (*Xa4*)	IRBB5 (*xa5*)	IRBB7 (*Xa7*)	IRBB10 (*Xa10*)	IRBB11(*Xa11*)	IRBB13 (*xa13*)	IRBB14 (*Xa14*)	IRBB21 (*Xa21*)
1	ISO3, ISO4, ISO6	S	S	S	S	S	S	S	S	R
2	ISO2	S	R	R	R	S	R	S	R	R
3	ISO5	R	R	R	R	S	R	S	R	R
4	ISO7	R	S	S	S	S	S	S	S	R
5	ISO8	S	R	R	R	S	S	S	R	R
6	ISO9	R	R	R	R	S	R	S	S	R
7	ISO10	R	R	R	R	R	R	S	R	R
8	ISO1	R	R	R	R	R	R	R	R	R

[a] Near-isogenic lines (NILs) harboring a single gene for resistance were used to characterize pathotypes among 10 isolates of *X. oryzae* pv. *oryzae*. S = susceptible and R = resistant. Note: A mean lesion length of < 3 cm was considered as resistance and >3 as susceptible (*Li *et al.* 2008).

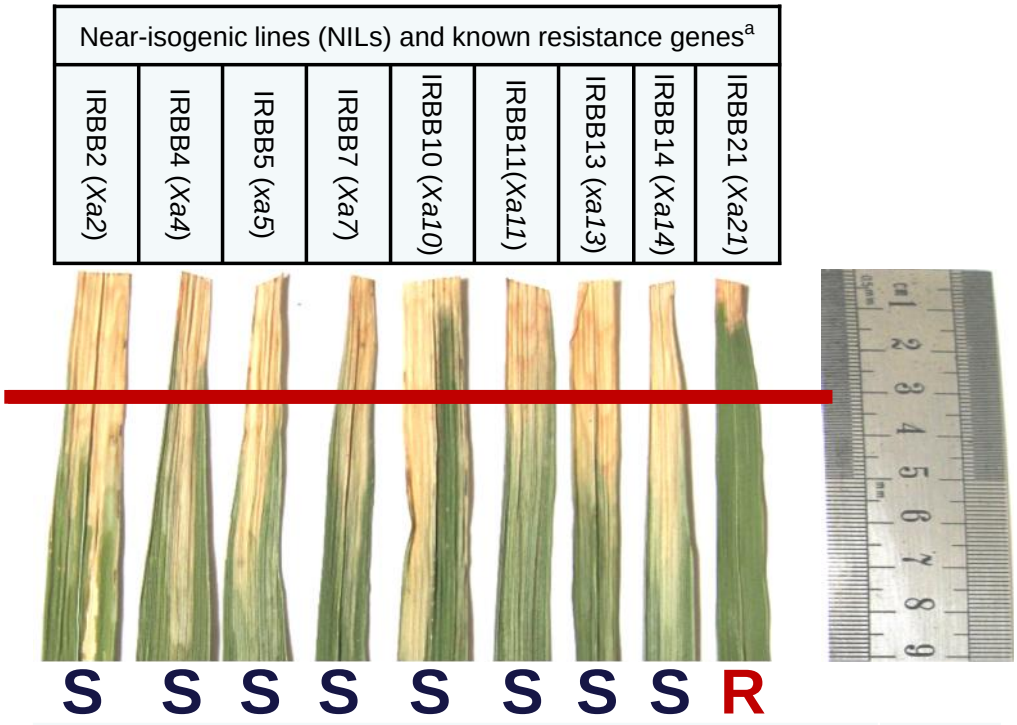
All tested isolates were avirulent to only one differential (IRBB21). Isolates ISO3, ISO4 and ISO6 were virulent to all NILs except IRBB21 which were grouped into pathotype 1. The isolate, ISO1 was avirulent to all the NILs which was placed in another pathotype designated as pathotype 8. Isolate ISO7 was avirulent to IRBB2 and IRBB21 containing resistance genes *Xa2* and *Xa21* respectively. Other isolates (ISO2, ISO5, ISO7, ISO8, ISO9 and ISO10) showed variable reaction patterns on the NILs and they were classified into different pathotypes and designated as pathotype 2 to 7, respectively (Table 2 and Fig. 3).

Differential characteristics of near-isogenic lines

Ten isolates were tested to assess the differential characteristics of nine NILs. Genotype IRBB21 showed resistant reaction to all isolates showing lesion length of 0.55-2.71 cm with mean 1.57 cm. IRBB13 (*xa13*) showed resistant reaction to only ISO1 having lesion length of 1.60 cm and IRBB10 possessing resistant gene *Xa10* was resistant to ISO1 and ISO10 showing lesion length of 1.18 cm and 1.20 cm, respectively. Three genotypes, IRBB4, IRBB5 and IRBB7 containing the resistant genes *Xa4*, *xa5*, *Xa7* respectively were resistant to 6 isolates (ISO1, ISO2, ISO5, ISO8, ISO9 and ISO10). In other NILs, IRBB2, IRBB11 and IRBB14 showing resistant reaction against 5 different isolates and lesion length ranged 0.51-23.73, 0.67-21.21 and 0.82-13.37 cm with means of 7.95, 8.57 and 4.29 cm, respectively (Tables 1&2 and Fig. 1).

Resistance frequency of isolates on rice near-isogenic lines (NILs)

The resistance frequency of the isolates on the rice near-isogenic lines having specific gene for resistance is shown in Table 3 and Fig. 2. The BB resistance gene showed 10 to 100% resistant reaction to the isolates of *X. oryzae* pv. *oryzae* tested in the present study. The highest resistance frequency was found in IRBB21 (*Xa21*) followed by IRBB4 (*Xa4*), IRBB5 (*xa5*) and IRBB7 (*Xa7*). The lowest resistance frequency was found in IRBB13 (*xa13*) followed by IRBB10 (*Xa10*), IRBB2 (*Xa2*), IRBB14 (*Xa14*) and IRBB11 (*Xa11*).



[a] Near-isogenic lines (NILs) containing a single gene for resistance

S = susceptible and R = resistant. A mean lesion length of < 3 cm was considered as resistance and >3 as susceptible.

Figure 3. Pictorial views of reaction pattern of nine near-isogenic lines (NILs) to BB isolates of Pathotype 1.

Table 3. Resistance genes of rice near-isogenic lines (NILs) and their resistance frequency to 10 Bangladeshi isolates of *X. oryzae* pv. *oryzae*

NILs	Resistance gene	Resistance frequency (%)[a]
IRBB2	*Xa2*	50
IRBB4	*Xa4*	60
IRBB5	*xa5*	60
IRBB7	*Xa7*	60
IRBB10	*Xa10*	20
IRBB11	*Xa11*	50
IRBB13	*xa13*	10
IRBB14	*Xa14*	50
IRBB21	*Xa21*	100

[a]Resistance frequency was calculated as the ratio of isolates including resistance reaction vs. total isolates tested on each NILs.

Pathotypes or races of bacterial plant pathogens are generally grouped on the basis of virulence phenotypes on a set of differential varieties of rice (Jeung *et al*. 2006, Liu *et al*. 2007, Khan *et al*. 2009). In the present investigation, nine NILs of rice with distinct genes for bacterial blight resistance were tested in determining virulence of ten isolates of *X. oryzae* pv. *oryzae* collected from different locations of Bangladesh. The symptoms developed by those isolates were clear to distinguish virulent and avirulent isolates (Fig. 3). Based on susceptibility of the differentials and virulence of the isolates, in terms of lesion length and infection frequency, the isolates were grouped into 8 different pathotypes. Similar results were reported by other investigators who worked in other countries with other isolates of the pathogen (Noda *et al.* 2001, Khan *et al.* 2009). Khan *et al.* (2009) tested 41 Bangladeshi isolates of *X. oryzae* pv. *oryzae* using the same set of differentials and grouped them into 6 pathotypes. Pathotypes 1 contained 33 isolates, 2 contained 3 isolates and pathotype 3 contained 2 isolates. Each of pathotypes 4, 5 and 6 contained only one isolate. The BB resistance gene showed 9.09 to 63.64% resistant reaction to the isolates used by them. In the present study, 8 pathotypes were identified. Among them ISO3, ISO4 and ISO6 were most virulent. Among the differentials IRBB13 (*xa13*) and IRBB10 (*Xa10*) were susceptible to almost all the tested isolates. In China, *xa5* and *xa13* genes were found resistant to almost all the isolates tested by Noda *et al.* (2001). It indicates that Bangladeshi isolates tested in the present investigation are different from those in China (Li *et al.* 2008, Liu *et al.* 2007). The NIL IRBB21 (*Xa21*) was found resistant to all pathotypes tested in the present study. IRBB5 and IRBB7 showed complete resistance to all isolates, and IRBB2 and IRBB11 were susceptible to all isolates in Vietnam, indicating that Bangladeshi isolates are different from those in Vietnam (Lai Van *et al.* 1999).

Based findings of te investigation it may be concluded that wide variations exist in the populations of *X. oryzae* pv. *oryzae* that cause bacterial blight of rice in Bangladesh. There are at least 8 different pathotypes in the populations of the BB pathogens occur in the country. The isolate ISO4, along with ISO3 and ISO6, was the most virulent whereas ISO1showed the most avirulent pathotype. All isolates were avirulent to only one differential, IRBB21 (*Xa21*) tested in the present study.

ACKNOWLEDGEMENTS

The authors express their gratefulness to Research Management Committee (RMC) of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur-1706, Bangladesh for providing fund to undertake the research work. The authors also express their indebtedness to Plant Pathology Division, Bangladesh Rice Research Institute (BRRI), Bangladesh for providing research materials and laboratory facilities to undertake the study.

Literature Cited

Anonymous. 1988. Bacterial √blight of rice.In: proceedings of the International Workshop on Bacterial Blight of Rice, 14-18 Mar 1988. Los Baños, Laguna : IRRI. 235 pp.

Ezuka, A. A. 2000. A Historical Review of Bacterial Blight of Rice. Bull. Natl. Ins. Agro. Res. 15:211.

Farooq, H. M. and Ahmad, N. 2007. Bacterial leaf blight — a serious rice disease. DAWN, November 05, 2007, Pakistan.

Hossain, M. A. 2001. Rice cultivar- *Xanthomonas oryzae pv. oryzae* interactions for development of bacterial blight disease in Bangladesh. Ph. D. Thesis. Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh. 151 pp.

Huang, N., Angeles, ER., Domingo, J., Magpantay, G., Singh, S., Zhang, G., Kumaravadivel, N., Bennett, J. and Khush. GS. 1997. Pyramiding of bacterial leaf blight resistance genes in rice: marker-assisted selection using RFLP and PCR. *Theor Appl Genet* **95**: 313-320.

Jalaluddin, M., Yamamoto, T., Nakai, H. and Tsuyumu, S. 2005. Pathogenic variability and DNA fingerprinting of *Xanthomonas oryzae pv. oryzae* from Bangladesh. SABRAO J. Breed. Gene. 37(1): 1-10.

Jeung, J. C., Heu, S.K., Shin, M.S., Vera Cruz, C. M. and Jena, K. K. 2006. Dynamics of *Xanthomonas oryzae pv. oryzae* populations in Korea and their relationship to know bacterial blight resistance gene. Phytopathology 96: 867-875.

Khan, M.A.I., Mansur, M. A., Ali, M.A and Mia, M.A.T. 2009. Pathogenic diversity of *Xanthomonas oryzae pv. oryzae* in Bangladesh. Bangladesh J. Plant Pathol. 25(1&2): 1-6.

Kumar, A., Guha, A., Bimolata, W., Reddy, A.R., Laha, G.S., Sundaram, R.M., Pandey, M.K., Ghazi, I.A. 2013. Leaf gas exchange physiology in rice genotypes infected with bacterial blight: An attempt to link photosynthesis with disease severity and rice yield. AJCS 7(1): 32-39.

Li, G., Song, C. F., Pang, X. M., Yang, Y. and Wang, J. S. 2008. Analysis of pathotypic and genotypic diversity of *Xanthomonas oryzae pv. oryzae* in China. J. Phytopathology 157: 208-218.

Lai Van E., T. Noda, and Pham Van Du. 1999. Resistance assessment of rice cultivars to *Xanthomonas oryzae* pv. *oryzae* and pathogenicity testing of bacterial leaf blight isolates in Vietnam. Japan Intl. Res. Cen. Agric. Sci. Tsukuba, Ibaraki, Japan pp. 120-130.

Liu, H., Yang, W., Hu, B. and Liu, F. 2007. Virulence analysis and race classification of *Xanthomonas oryzae pv. oryzae* in China. J. Phytopath. 155: 129-135.

Mew, T.W. 1989. An overview of the world bacterial blight situation. In: T. Ogawa and G.S. Kush (eds). Bacterial blight of rice . International Rice Research Institute, Manila, Philippines. pp 7-12.

Noda, T., Li, C., Li, J., Ochiai, H., Ise, K. and Kaku, H. 2001. Pathogenic diversity of *Xanthomonas oryzae pv. oryzae* strains from Yunnan province, China. JARQ 35(2): 97-103.

Ou, S. H. 1985. Rice diseases. 2nd ed. Great Britain (UK). CMI. 380 pp.

EFFECT OF TUBER-BORNE INOCULUM OF *RHIZOCTONIA SOLANI* ON THE DEVELOPMENT OF STEM CANKER AND BLACK SCURF OF POTATO

M. M. Rahman[1], M. A. Ali[2], M. U. Ahmad[2] and T. K. Dey[3]

[1]Principal Scientific officer, TCRSC, BARI, Bogra, [2]Professor, Department of Plant Pathology, BAU, Mymensingh. [3]Director, PRC, BARI, Ishurdi, Pabna Banladesh
Email of first author: matiarbari@yahoo.com

(This is a part of Ph.D. dissertation of the first author)

ABSTRACT

M. M. Rahman, M. A. Ali, M. U. Ahmad and T. K. Dey. 2013. Effect of tuber-borne inoculum of *Rhizoctonia solani* on the development of stem canker and black scurf of potato. Bangladesh J. Plant Pathol.29 (1&2):29-32.

An experiment was conducted to determine the effect of level of *Rhizoctonia solani* infected seed tubers on germination, plant growth and development of stem canker and black scurf diseases of potato. The levels of infected seed tubers tested in the experiment were 0, 5, 10, 20, 50, and 100%. Germination, stem number per hill, plant height, disease incidence and percent disease incidence (PDI) under control (0% infected seed tuber) were 98.33%, 5.1, 60.58 cm, 18.33% and 7.29, respectively. At 5-100% infected seed tubers, the three growth parameters decreased to 86.33-94.08%, 4.43-4.70/hill, 54.33-59.55 cm, whereas DSI and PDI increased to 27.08-51.24% and 9.16-21.25, respectively. Decrease in growth parameters and increase in DSI as well as PDI were corroborated with the levels of infected seed tubers. Planting 5-100% infected seed tubers increased production of diseased tubers by number as well as by weight compared 100% healthy seed tubers (control). At 100% healthy seed, number of russet, deformed and sclerotia bearing tubers were 8.25, 5.50 and 4.00/plot. Use of potato seeds mixed with 5-100% *R. solani* infected tubers reduced the three parameters within the range of 12.75-20.00, 9.75-17.00 and 90.50/plot, respectively. The results revealed that increase in inoculum level caused increased in infection on progeny tubers. Number of russet, deformed and sclerotia bearing tubers were higher when 100% infected tubers were sown. The other levels of tuber-borne *R. solani* incoculum showed low of infection.

Key words: *Rhizoctonia solani*, stem canker, black scurf, potato, seed tuber

INTRODUCTION

Potato is the major vegetable crop in Bangladesh. It also plays an important role for food security in the country (Hashem 1990). The area under potato cultivation is increasing year after year and farmers are adopting it as a cash crop. During 2008-2009, about 6.89 million tons of potato was produced from 0.46 million hectares of land with an average yield of 14.86 t/ha (Uddin *et al*. 2010). The national average yield of potato is much lower as compared to other potato growing countries like the Netherlands, where the average potato yield is 41.3 t/ha (Chadha 1995, Swaminathan 2000).

The major constraint of potato production in Bangladesh is prevalence of epidemic diseases and shortage of quality seed tuber. A total of 39 diseases (both biotic and abiotic) of potato have been recorded in the country (Ali and Khan 1990). The major soil and tuber-borne diseases of potato are black scurf and stem canker, bacterial wilt and common scab. Among them stem canker and black scurf caused by *Rhizoctonia solani* (Kuhn) is the most common and widespread disease throughout Bangladesh (Ali and Dey 1994).

Rhizoctonia solani infects the underground stem and produces necrosis called stem canker, whereas tuber infection produces symptoms on skin in the form of black sclerotia called black scurf. The pathogen is also involved in the early dying syndrome of potato plants (Kotcon *et al.* 1985). Tuber-borne inocula act as the main source for introducing the disease into the new areas (Wicks *et al*. 1996). The highest level of inoculum causes the highest infection resulting in maximum reduction in plant growth and tuber yield (Rahman *et al*. 1996a). So, it is essential to know the effect of tuber-borne inoculum on disease development. The present study was undertaken to determine the effect of inoculum levels of seed tuber infected with *R. solani* on the development of stem canker and black scurf of potato.

MATERIALS AND METHODS

Healthy as well as *R. solani* infected seed tubers of a susceptible variety, Diamant were collected from the Breeder Seed Production Centre (BSPC) Debiganj under Panchagarh district. Both healthy and infected potato seed tubers were mixed before planting at 0, 5, 10, 20, 50 and 100% infected seed tubers. The experiment was conducted at the Tuber Crops Research Sub-Centre (TCRSC) of Bangladesh Agricultural Research Institute (BARI), at Chalopara, Bogra district during 2007-2008 potato growing season. The experiment was laid out in a randomized complete block design with four replications. The unit plot size was 3 m × 3 m. Block

to block and plot to plot distances were 100 cm and 50 cm, respectively. Row to row and tuber to tuber distances were 60 cm and 25 cm, respectively.

Recommended doses of fertilizers and manure were applied as suggested by Tuber Crops Research Centre, BARI, Gazipur. Cowdung was incorporated in the soil during land preparation at the rate of 10 t/ha. Urea, Triple super phosphate (TSP), Muriate of potash (MOP), Gypsum, Zinc sulphate and Boric acid were applied at the rate of 360, 220, 250, 120, 14 and 6 kg per hectare, respectively. Half of urea and the entire amounts of TSP, MP, Gypsum, Zinc sulphate and Boron were applied at the time of final land preparation. Tubers were planted on 30 November, 2007. Rest half of urea was applied at 30 days after planting. Weeding was done at 25 and 50 days after planting. Earthing up was done at 30 days after planting. Irrigation was applied at 25 and 45 days after planting. Dursban 50WP (Chloropyrifos) @ 0.5% and Admire (Imidacloprid) @ 0.1% were applied to control cutworm and aphid, respectively. Secure (Pyriminamine) @ 0.1% was sprayed at 10 days intervals as preventive measure against late blight of potato. The crop was harvested on 27th February, 2008.

Data on germination, number of stems per hill, plant height, disease incidence, percent disease index (PDI), tuber infection and yield were recorded. Data on disease incidence were recorded at 70 days after planting. To collect data on disease incidence, 20 plants were uprooted carefully from each plot, washed with tap water and checked for infection. Number of infected and healthy plants was counted in each plot and incidence percentage was calculated based on total number of plants checked.

Disease severity was indexed on a 0-6 scale (Dey 2010), where 0=No symptom on stolon, 1=Minute brown lesion on stolon or root, 2=Moderately brown lesion on stolon and curling tendency on central leaf, 3=Stolon symptom discolored accompanied by brown discoloration on roots, 4=Brown to black discoloration on underground parts, tissue discoloration and tissue squeezed/curling of growing leaves, 5=Profuse emergence of auxiliary leaves and leaf size reduced markedly with pale green
margin, and 6=Production of aerial tubers with green colour. The plants were checked individually and categorized into different group according to the indexing scale. The number of stem canker infected plants under each grade was recorded and percent disease index (PDI) was calculated using the following formula:

$$PDI = \frac{\text{Class frequency}}{\text{Number of plants assessed X highest score}} \times 100$$

Incidence of black scurf was recorded at harvest. The black scurf infected tubers were separated into three groups such as russet, deformed and sclerotia (Chand and Logan 1982). The number of tubers under each group was counted and the respective weight was recorded. Number and weight of healthy tubers of each plot was also recorded.

Data on different parameters were analyzed using computer program MSTAT-C. Differences among the treatment means were compared following Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Germination, stem number per hill, plant height, disease incidence (DSI) and percent disease incidence (PDI) under control (0% infected seed tubers) were 98.33%, 5.1, 60.58 cm, 18.33% and 7.29, respectively. At 5-100% infected seed tubers, the three growth parameters decreased to 86.33-94.08%, 4.43-4.70/hill, 54.33-59.55 cm, whereas the two disease related parameters (DSI and PDI) increased to 27.08-51.24% and 9.16-21.25, respectively. Decrease in growth parameters and increase in DSI as well as PDI were corroborated with the levels of infected seed tubers. However, decrease in germination and increase in DSI as well as PDI were significant compared to control but decrease in stem number per hill and plant height were statistically similar at 5-100% infected seed tubers (Table 1).

Table 1. Effect of tuber-borne inoculum levels of *Rhizoctonia solani* on germination, growth parameters and disease incidence of potato

Tuber-borne inocula (%)	Germination (%)	Stem/ hill	Plant height (cm)	Disease incidence (%)	Disease severity (PDI)
0	98.33 a	5.10a	60.58 a	18.33 e	7.29 e
5	94.08 b	4.43 a	59.55 a	27.08 d	9.16 d
10	90.49 c	4.65 a	58.25 a	33.33 c	12.08 c
20	86.33 d	4.83 a	57.88 a	38.33 bc	13.75 c
50	84.99 d	4.70 a	56.08 a	43.33 b	16.87 b
100	80.95 e	4.48 a	54.33 a	51.24 a	21.25 a

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

Planting 5-100% infected seed tubers significantly increased production of diseased tubers by number as well as by weight compared 100% healthy seed tubers (control). At 100% healthy seed, number of russet, deformed and sclerotia bearing tubers were 8.25, 5.50 and 4.00/plot. Use of potato seeds mixed with 5-100% *R. solani* infected tubers reduced the three parameters within the range of 12.75-20.00, 9.75-17.00 and 90.50/plot, respectively. Weight of russet, deformed and sclerotia bearing tubers were 600.0, 515.0 and 200.0 g/plot under control. All parameters decreased the three parameters to 600.0-1370.0, 515.0-1200.0 and 200.0-67.00 g/plot. The increase in number and weight of deformed and sclerotia bearing tubers were significant compared to control (100% healthy seed). The highest number and weight of diseased tubers were harvested from plots planted with 100% infected seeds followed by 50 and 20% infected seed tubers. The differences in production of diseased tubers by number as well as weight at three higher levels of infected seed tubers were significant with the exception of weight of russet tuber at 100 and 50% infected seed tubers. The minimum increase in diseased tubers having russet and deformed symptoms and bearing sclerotia were harvested from plots planted with 5% infected seed tubers followed by 10% (Table 2).

The highest yield of 21.50 t/ha was harvested from plots planted with 100% healthy seeds which was statistically similar to the yield of the plots planted with 5, 10 and 20% infected seed tubers. The lowest yield of 17.50 t/ha was harvested from plots planted with 50% infected seed tubers which was statistically similar to the yield at 100% infected seed tubers deformed and sclerotia bearing tubers. The yield at two higher levels of infected seed tubers was significantly lower compared to control (Table 2). It was found that the tuber yield decreased gradually with the increase in levels of infected seed tubers. Their relationship was negative and linear (Fig. 1).

In the present investigation, the highest germination, plant growth and lowest disease incidence and percent disease incidence were recorded from control (100% healthy seed tubers) followed by infected seed tuber levels of 5, and 10%. On the other hand, the lowest germination, plant growth and highest disease incidence as well as severity were recorded at 100% infected seed tubers (Tables 1 and 2).

The findings of the present investigation were found to be similar to the findings of many other researchers (Chand and Logan 1982, Read *et al.* 1989, Rahman *et al*. 1996a, 1996b, Naz *et al*. 2008). They found that severity of stem canker and stolon infection by *R. solani* increased with increasing levels of *R. solani* inoculum. Inoculum levels showed significant negative impact on germination of seed tuber and plant height. The treatment having higher level of tuber inocula significantly differed from other treatments in the degree of tuber infection. Read *et al.* (1989) reported that high inoculum levels caused severe infection with delay in shoot growth and decrease in stem height and foliage weight. Based on findings of the present study it may be concluded that higher level of seed tuber-borne inoculum of *R. solani* leads to increase in stem canker and black scurf infection in the subsequent crop resulting in decrease of tuber yield and quality of potato.

Table 2. Influence of levels of infected *Rhizoctonia solani* seed tuber on germination, plant growth, yield and disease incidence of potato

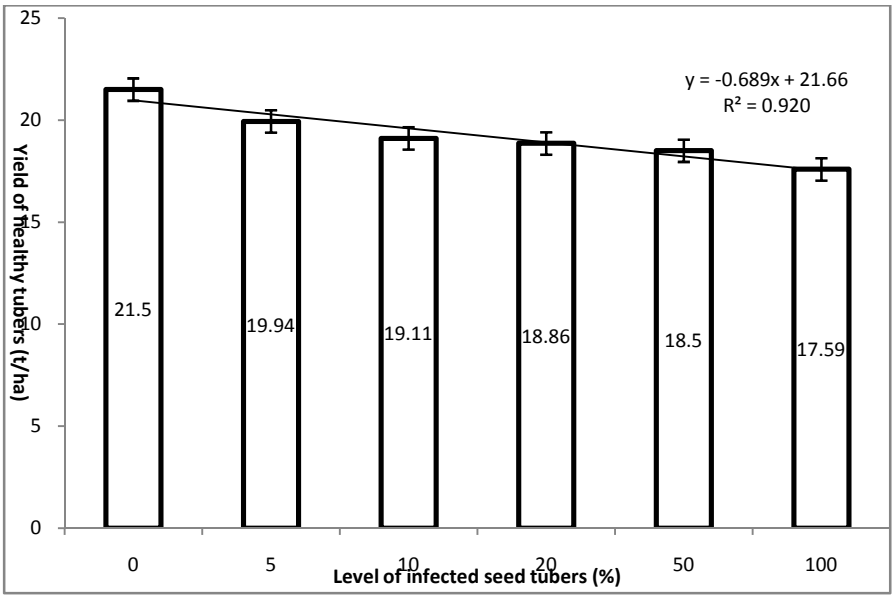
Tuber-borne inocula (%)	Number of infected tubers/plot			Weight of infected tubers/plot (g)			Yield (t/ha)
	Russet	Deformed	Sclerotia	Russet	Deformed	Sclerotia	
0	8.25 d	5.50 d	4.00 f	600.0 c	515.0 e	200.0 f	21.50 a
5	12.75 c	9.75 c	18.00 e	900.0 b	617.0 d	1090.0 e	19.94 ab
10	13.75 c	10.75 c	24.75 d	970.0 b	820.0 c	1170.0 d	19.11 ab
20	14.45 c	11.00 c	44.50 c	998.0 b	850.0 c	3110.0 c	18.86 ab
50	17.25 b	15.00 b	60.50 b	1250.0 a	920.0 b	4320.0 b	18.50 b
100	20.00 a	17.00 a	90.50 a	1370.0 a	1200.0 a	6700.0 a	17.59 b

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

LITERATURE CITED

Ali, M. S. and Dey, T. K. 1994. Pathological research on tuber crops in Bangladesh. *In:* Proc. Workshop on Tubers Crops on Transfer of Technology of CDP crops under Research Extension Linkage Programme. pp.159-165.

Ali, M.S. and Khan, A.L. 1990. Pathological constraints of seed potato production in Bangladesh. *In*: Proc. of the international seminar, Seed production in Bangladesh (Eds. Rashid, M.M.; M.A. Siddique and M.M. Hussain). BADC, Dhaka.



Figurer 1. Relationship between levels of seed tubers infected with *Rhizoctonia solani* and yield of healthy tubers.

Chadha, K.L. 1995. Integrated management of bacterial wilt (Eds, Hardly, B and E.R. French). CIP, Lima, Peru. 19 (1-2): 13-20.

Chand, T. and Logan, C. 1982. Reaction of ten potato cultivars to stem canker and black scurf of potato caused by *Rhizoctonia solani*. Annals of Applied Biology. 100: 102-103.

Hashem, A. 1990. An introduction to the potato seed industary of Bangladesh. International seminar. Seed potato in Bangladesh, BADC, Dhaka, Bangladesh. 1-15.

Dey, T. K. 2010. Tuber crops diseases-their identification, method of recording, rating scale and grading system. Oilseed Res. Center, Bangladesh Agril. Res. Inst. Gazipur. pp. 28-31.

Kotcon, J. B., Rouse, O.I. and Mitchell, J. E. 1985. Interactions of *Verticillium dahliae*, *Colletotrichum coccodes*, *Rhizoctonia solani* and *Pratylenchus penetrans* in the early dying syndrome of Russet Burbank potatoes. Phytopathol. 75: 68-74.

Naz, Farah, Rauf, C. A., Abbasi, N. A., Haque, I. U. and Ahmad, I. 2008. Influence of inoculum levels of *Rhizoctonia solani* and susceptibility on new potato germplasms. Pakistan. J. Bot. 40 (5): 2199-2209.

Rahman, M.L., Hossain, M.M., Ashrafuzzaman, M. and Islam, T. 1996a. Effect of inoculum levels of *Rhizoctonia solani* on the incidence of black scurf disease of potato. Bangladesh J. Plant Pathol. 12 (1-2): 21-22.

Rahman, M.L., Hossain, M.M., Islam, T. and Ashrafuzzaman, M. 1996b. Effect of inoculum levels of *Sclerotium rolfsii* on the development of wilt in potato. Bangladesh J. Plant Pathol. 12 (1-2): 27-28.

Read, P. J., Hide, G. A., Firmaer, J.P. and Hall, S.M. 1989. Growth and yield of potatoes as affected by severity of stem canker (*Rhizoctonia solani*). Potato Res. 32: 9-15.

Swaminathan, M.S. 2000. Potato for global security. *In*: Potato Global Research and Development. Indian Potato Assoc, CPRI, Shimla, H.P, India. 302 p.

Uddin, M. A., Yasmin, S., Rahman, M. L., Hossain, S. M. B. and Choudhury, R.U. 2010. Challenges of potato cultivation in Bangladesh and developing digital databases of potato. Bangladesh J. Agril. Res. 35 (3): 453-463.

Wicks, T, Morgan, J. B. and Hall, B. 1996. Influence of soil fumigation and seed tuber treatment on the control of *Rhizoctonia* solani on potatoes. Australian J. Exp. Agric. 36: 339-345.

INTEGRATED APPLICATION OF INSECTICIDE AND FUNGICIDE TO CONTROL OkYVCMV AND *PSEUDOCERCOSPORA* LEAF SPOT OF OKRA SEED CROPS

M. G. Kibria[1] and I. H. Mian[2]

[1]Senior Scientific Officer, Plant Pathology Section, HRC, BARI, [2]Professor, Department of Plant Pathology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh

(The paper is a part of Ph. D. thesis of the first author)

ABSTRACT

M. G. Kibria and I. H. Mian. 2013. Integrated application of insecticide and fungicide to control OkYVCMV and *Pseudocercospora* leaf spot of okra seed crops. Bangladesh J. Plant Pathol. 29 (1&2):33-38.

A field experiment was conducted to test the efficacy of integrated application of pesticides to control Okra yellow vein clearing mosaic virus (OkYVCMV) and *Pseudocercospora* leaf spot caused by *Pseudocercospora abelmoschi* (PLS) of okra seed crop, and to increase yield and quality of okra seeds. All pesticidal treatments gave considerable reduction over control in incidence of OkYVCMV, severity of PLS, and population of whitefly (*Bemisia tabaci*). The reduction over control was 55.95, 43.33, 35.35 and 21.02% in OkYVCMV incidence and 89.18, 72.45, 62.36 and 31.52% in PLS due to treatments T_1= pre-sowing seed treatment with Gaucho 72 WS (Imidacloprid) at 5 g/kg seed + soil treatment with Furadan 5G (Carbofuran) at 30 kg/ha)+ 4 foliar sprays with Admire (Imidacloprid) at 1 ml/L+ 4 foliar sprays with Emivit 50 WP at 3 g/liter, T_2=seed treatment with Gaucho 72 WS+ 4 foliar sprays with Admire + 4 foliar sprays with Emivit 50 WP, T_3=seed treatment with Gaucho 72 WS+ 4 foliar sprays with Emivit 50 WP, and T_4=seed treatment with Gaucho 72 WS, respectively. Reduction in population of whitefly due to the pesticidal treatments were 69.06, 58.39 and 17.78% at 30 days after sowing, and 73.90, 65.76 and 40.29% at 60 days after sowing. The treatments improved plant growth, pod and seed yield and seed quality considerably. The seed yield was increased by 133.22, 80.54, 64.91 and 23.05% over control due to the treatments. The best treatment was T_1 to control both diseases and to improve yield and quality of seeds.

Key words: Okra, OkYVCMV, *Pseudocercospora* leaf spot.

INTRODUCTION

Okra, also known as lady's finger (*Abelmoschus esculentus* L.) is one of the popular vegetable crops in Bangladesh. It is cultivated throughout the year except 3-4 cool months. It suffers from a number of diseases caused by fungi, bacteria, nematode and viruses (Talukdar 1974, Fakinr 2000, Rangaswami and Mahadevan 2006). Among them, Okra Yellow Vein Clearing Mosaic Virus (OkYVCMV) and *Psudocercospora* leaf spot (P. *abelmoschi*) are two major diseases. OkYVCMV infects at all growing stages of okra causing severe reduction in plant growth and yield. The disease is transmitted by the vector whitefly (*Bemisia tabaci*). Plants infected at early stages remain stunted. The fruits of the infected plant exhibit pale yellow color, deformed, small and tough in texture. Seeds of infected plants are smaller and shriveled. If the plants are infected within 20 days after germination (DAG), the yield loss may be up to 94%. Infection of plants at 50 and 60 DAG may cause 84 and 49% yield losses, respectively (Sastry and Singh 1974).

Attempt was made by many researchers to control the disease through insect vector control and found that application of insecticide minimized whitefly population and the incidence of OkYVCMV (Pun *et al.* 2005a, 2005b, Gowdar *et al*. 2007). Significant reduction in OkYVCMV incidence and whitefly populations was achieved with foliar spray of Acetamiprid, Dimethoate, Imidacloprid, Meta-systox, Monocrotophos, Nuvacron, Thiamethoxam, Triazo Ahmed *et al*. 2001, Misra 2005, Gowdar *et al*. 2007). Pesticidal seed treatment with insecticide (Imidacloprid) (Praveen *et al*. 2007) and soil application of Carbofuran at the time of sowing are effective measures to control OkYVCMV through its vector suppression (Murthy and Reddy 1992, Praveen *et al*. 2007).

Pseudocercospora leaf spot (PLS) of okra caused by *Pseudocercospora abelmoschi* is prevalent in late summer and early winter. The disease may cause severe problem in seed crop causing smaller fruits and shriveled seeds. Spraying okra field with 500 g Zineb or Maneb suspended in 250 liters of water at 15 days interval after the first appearance of the symptoms proved effective for controlling *Pseudocercospora* leaf blight disease. The best control of *P. abelmoschi* was obtained from foliar spray of Copper oxychloride, followed by Carbendazim and Mancozeb. Carbendazim gave the highest yield increase, followed by Copper Oxychloride and Mancozeb (Srivastava *et al.* 1992, Rahman *et al*. 2000).

Both OkYVCMV and *Pseudocercospora* leaf spot are common in Bangladesh (Talukdar 1974). These are considered as serious diseases of okra,

especially when it is grown in late summer. Comprehensive research has not yet been accomplished in the country to find out effective control methods against the diseases. Available literature reveal that effective control of OkYVCMV and *Pseudocercospora* leaf spot may be achieved through vector control using insecticides and foliar spray with fungicides, respectively. Integrated application of the pesticides may give better control compared to application of either insecticides or fungicides. Present piece of research was conducted to test the efficacy of integrated application of insecticide and fungicide to control OkYVCMV and *Psuedocercospora* leaf spot of okra.

MATERIALS AND METHODS

An experiment was conducted to test the efficacy of three insecticides and a fungicide applied in an integrated approach to control Okra Yellow Vein Clearing Mosaic Virus (OkYVCMV) and *Psuedocercospora* leaf spot (*Pseudocercospora abelmoschi*) of okra. Three insecticides namely Gaucho 72 WS (Imidacloprid), Furadan 5G (Carbofuran) and Admire (Imidacloprid) were applied to control OkYVCMV through vector control, and the fungicide Emivit 50 WP was used to control *Psuedocercospora* leaf spot. Individual components of treatments in the present experiment were: C_1 = Pre-sowing insecticidal seed treatment with Gaucho 72 WS (Imidacloprid) @ 5 g/kg seed, C_2 = Pre-sowing soil treatment with Furadan 5G (Carbofuran) @ 30 kg/ha), C_3 = Four foliar sprays with Admire (Imidacloprid) @ 1 ml/L, and C_4 = Four foliar sprays with Emivit 50 WP @ 3 g/liter. Spraying of Admire was started from 15 DAS and continued for 4 times at 15 days interval. Furadan 5G was applied in the furrows at sowing. Seeds were treated with the insecticide just before sowing. Spray of fungicide was started when symptoms of PLS appeared and continued for 4 times with 7 days interval. The integrated treatments, T_1= C_1+ C_2+ C_3+ C_4, T_2= was C_1+C_3+ C_4, T_3= C_1+ C_4 and T_4= C_1. An additional treatment T_5 was maintained which received neither insecticide nor fungicide and served as control.

The experiment was conducted in the experimental farm of Bangabandhu Sheikh Mujibur Rahman Agrilcultural Univeristy (BSMRAU), Gazipur during 2009. The experiment was laid out following 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 maintaining isolation distance as well as 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 (Anon. 2005). Manure was applied as cowdung at the rate of 4 t/ha. 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 dressing and incorporated with soil at 3rd and 5th week after sowing. A previously selected susceptible okra genotype was used in the experiment. Seeds of the genotype were soaked in tap water for 12 hours, air dried and treated with Gaucho 72 WS. Treated seeds were sown in the field maintaining plant to plant and row to row distances of 60 and 50 cm, respectively. After 6 to 7 days of germination, the seedlings were thinned to have one plant per hill. 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). Irrigation was applied after each top dressing of urea and whenever necessary. Every time, irrigation was followed by mulching and weeding. Other necessary intercultural operations were done throughout the cropping season for proper growth and fruit production (Razzaque *et al.* 2000).

Data on incidence of OkYVCMV was recorded on 60 days after sowing (DAS). Number of diseased and healthy plants in each unit plot was counted. Incidence of OkYVCMV was expressed in percentage based on total number of plants checked. The disease incidence (DI) was computed following a formula as shown below:

$$\% \text{ DI} = \frac{\text{Number of infected plants}}{\text{Total number of plants checked}} \times 100$$

Data on whitefly population was also recorded. To count population of adult whitefly, five plants were selected randomly from each unit plot and 5 leaves were selected from each plant at different nodal positions (one at the top, two in the middle and two at the bottom). The counts were taken in the morning when whiteflies are less active as suggested by Basu (1995). Number of whitely per leaf was counted at 30 and 60 DAS. Precaution was taken not to disturb the insect at the time of counting. Severity of *Pseudocercospora* leaf spot (PLS) was indexed on a 0-8 scale as suggested by Rahman and Nahar (1990) at 60 DAS. Percent Disease Index (PDI) for the leaf spot was computed according to the following formula as described by Mian (1995):

$$\text{PDI} = \frac{\text{Sum of all disease ratings}}{\text{Total number of leaves checked X maximum disease grade}} \times 100$$

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 were 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.

Fully ripened pods were collected from each of the unit plot and seeds were separated from the pods, sun dried and cleaned. Seed yield per plant were computed. Recorded data were subjected to statistical analysis following standard procedure (Gomez and Gomez 1984) using MSTAT-C statistical software. Whenever necessary, data were transformed using ArcSin transformation method before performing analysis of variance.

RESULTS AND DISCUSSION

Incidence of OkYVCMV

Incidence of OkYVCMV on okra seed crop varied from 28.31 to 64.27% under different treatments including control (T_1 to T_5). The highest incidence of the disease was recorded from control plot. All pesticidal treatments (T_1 to T_4) caused significant reduction in disease incidence over control. The reduction over control was 55.95, 43.33, 35.35 and 21.02% respectively under treatments T_1, T_2, T_3 and T_4 (Table 1).

Severity of *Pseudocercospora* leaf spot (PLS)

The percent disease index (PDI) of PLS on okra in plots under different treatments including control ranged 5.64-52.12. The highest disease severity was found under control. Like OkYVCMV, PDI of PLS was also significantly reduced due to application pesticidal treatments (T_1 to T_4) compared to control. The reductions over control were 89.18, 72.45, 62.36 and 31.52% under T_1, T_2, T_3 and T_4 treated plots, respectively (Table 1).

Population of whitefly

Average populations of whitefly on okra seed crop ranged from 0.91-2.98 per leaf at 30 DAS and 1.25-4.79 per leaf per plant at 60 DAS recorded on five treatment approaches (T_1 to T_4) including control (T_5). Treatments T_1, T_2 and T_3 significantly reduced the whitefly populations over control (T_5). The maximum reduction was achieved by T_1 treated plot followed by T_2 and T_3 showing 69.06, 58.39 and 17.78% reduction over control at 30 DAS, and 73.90, 65.76 and 40.29% at 60 DAS, respectively (Table 2).

Plant height and Fruit yield per plant

Plant height of the seed crop was 105.89, 97.90, 93.80, 90.01 and 84.12 cm found in the plots treated with T_1 to T_5, respectively. All pesticidal treatment increased plant height significantly over control. The maximum increase was obtained in T_1 treated plot followed by T_2, T_3 and T_4 treated ones (Table 3).

Number of fruits per plant under the T_2, T_3, T_4 and T_5 was 16.11, 13.83, 13.15, 11.91 and 10.44, respectively. Significant increase in fruit yield was obtained with all the pesticidal treatments (T_1 to T_4). Differences in number of fruit per plant were significant (Table 3).

Seed number per fruit and per plant, seed yield, seed size and Seed quality

All qualitative parameters of seeds in terms of germination, seedling growth and seed vigor were improved tremendously due to application of all pesticidal treatments. The lowest number of seeds per fruit was found in treated control plots (T_5). The fruit number increased significantly over control due to application of the treatments T_1 to T_4. The highest fruit yield was obtained from T_1 treated plot followed by T_2 and T_4 treated plots showing 59.96, 56.39, 55.16 and 49.59 seeds per fruit. The lowest seed number of 476.63 seeds per plant was harvested from control plot. It was increased to 965.90, 779.81, 725.17 and 590.82 due to pesticidal treatments T_1, T_2, T_3 and T_4, respectively. The increase was significant compared to control (Table 3).

Seed yield was 56.44, 43.69, 39.91, 31.23 and 24.20 g/plant in plots treated with different treatments (T_1, T_2, T_3 and T_4). Significantly the highest seed yield was achieved with all pesticidal treatments over control. The yield increase in T_1, T_2, T_3 and T_4 treated plots was 133.22, 80.54, 64.91 and 23.05%, respectively. Seed size in terms of 1000-seed weight increased significantly over control (T_5) due to four pesticidal treatments (T_1,- T_4). The maximum increase in 1000-seed weight was achieved with T_1 followed by T_2, T_3 and T_4 showing 1000-seed weight of 58.43, 56.03, 55.03 and 52.85 g, respectively. (Table 4).

Shoot and root length and Seed vigour index

The root and shoot length ranged from 6.04-13.05 cm and 5.32-9.59 cm, respectively obtained in different treated plots. Both the parameters were significantly higher in all four pesticidal treated plot compared to untreated control plot. The highest shoot and root length were found in the plot treated with T_1 followed by T_2, T_3, T_4 and T_5 treated plots (Table 5).

Table 1. Influence of integrated pesticidal treatment on incidence of OkYVCMV and severity of *Pseudocercospora* leaf spot (PLS) infecting okra seed crop at days after sowing

Treatment	OkYVCMV		*Pseudocercospora* leaf spot	
	Incidence (%)	% Decrease over control	PDI	% Decrease over control
T_1 = seed treatment + fungicide spray + insecticide spray + soil treatment	28.31 d* (32.15)**	55.95	5.64 d (13.74)	89.18
T_2 = seed treatment + fungicide spray + insecticide spray	36.42 c (37.12)	43.33	14.36 c (22.27)	72.45
T_3 = seed treatment + fungicide spray	41.55 c (40.14)	35.35	19.61 c (26.28)	62.36
T_4 = seed treatment	50.76 b (45.44)	21.02	35.69 b (36.68)	31.52
T_5 = Control	64.27 a (53.29)	-	52.12 a (46.22)	-

*Means within the same column having a common letter(s) are not significantly different (P=0.05).
**Figures within parentheses are ArcSin(X+1) transformed values.

Table 2. Influence of integrated pesticidal treatment on population of whitefly of okra seed crop

Treatment	Population of whitefly at 30 DAS		Population of whitefly at 60 DAS	
	Number per leaf per plant	% Reduction	Number per leaf per plant	% Reduction
T_1 = seed treatment + fungicide spray + insecticide spray + soil treatment	0.91 c	69.46	1.25 c	73.90
T_2 = seed treatment + fungicide spray + insecticide spray	1.24 c	58.39	1.64 c	65.76
T_3 = seed treatment + fungicide spray	2.45 b	17.78	2.86 b	40.29
T_4 = seed treatment	2.74 ab	8.05	3.82 ab	20.25
T_5 = Control	2.98 a	-	4.79 a	-

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

Table 3. Influence of integrated pesticidal treatment on plant height, number of fruit per plant, and number of seed per fruit and per plant of okra seed crop

Treatment	Plant height (cm)	Fruit per plant	Seed per fruit	Seed number per plant
T_1 = seed treatment + fungicide spray + insecticide spray + soil treatment	105.89 a	16.11 a	59.96 a	965.90 a
T_2 = seed treatment + fungicide spray + insecticide spray	97.90 b	13.83 b	56.39 ab	779.81 b
T_3 = seed treatment + fungicide spray	93.80 c	13.15 b	55.16 b	725.17 b
T_4 = seed treatment	90.10 c	11.91 c	49.59 c	590.82 c
T_5 = Control	84.12 d	10.44 d	45.65 d	476.63 d

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

The vigour of seedling raised from seeds harvested from the plots of different treatment viz., T_1, T_2, T_3, T_4 was 1838.09, 1513.17, 1239.34, 1075.88 and 732.61, respectively. The parameters under all pesticidal treatments were significantly higher compared to control (T_5). The highest seedling vigour was found under the treatment T_1 followed by T_2, T_3, and T_4 (Table 5).

BCR

Fruit yield per hectare varied ranged 6.38-10.79 tons under five treatments. The BCR recorded under five treatments including control ranged 1.39-1.84. The highest fruit yield as well as BCR was obtained with T_1 followed by T_2, T_3 and T_4. Effect of the treatments on all parameters were not significantly different (Table 6).

Results of the present study reveal that pre-sowing insecticidal seed treatment with Gaucho 72 WS (Imidacloprid) @ 5 g/kg seed, insecticidal soil treatment with Furadan 5G (Carbofuran) @ 30 kg/ha) at sowing, 4 foliar insecticidal spray with Admire (Imidacloprid) @ 1 ml/L and 4 foliar fungicidal spray with Emivit 50 WP @ 3 g/liter applied to okra seed crop in integrated approaches gave significant decrease in incidence of OkYVCMV, PLS, and population of whitefly (*Bemisia tabaci*) over control. The treatments gave significant improvement in plant growth, pod and seed yield and seed quality. Chakraborty and Mukhopadhyay (1977), Khan and Mukhopadhyay (1985), Anju *et al.* (1993), Singh *et al*. (1998), Rana *et al*. (2006) and Praveen *et al*. (2007) tested insecticidal measures to control OkYVCMV and found effective results. Rahman *et al.* (2000) reported successful control of PLS with fungicidal spray and obtained higher seed yield of okra. But none of the investigator tested the control measures in integrated approaches.

Table 4. Influence of integrated pesticidal treatment on okra seed crop influencing seed yield and seed size

Treatments	Seed yield (g/plot)	Seed yield increase over control (%)	1000-Seed weight (g)
T_1 = seed treatment + fungicide spray + insecticide spray + soil treatment	56.44 a	133.22	58.43 a
T_2 = seed treatment + fungicide spray + insecticide spray	43.69 b	80.54	56.03 b
T_3 = seed treatment + fungicide spray	31.23 c	23.05	52.85 c
T_4 = seed treatment	24.20 d	-	50.78 d

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

Table 5. Influence of integrated pesticidal treatment on okra seed crop quality parameters of seed

Treatment	% Germination	Shoot length (cm)	Root length (cm)	Seed vigour index
T_1 = seed treatment + fungicide spray + insecticide spray + soil treatment	81.20 a (64.30)	13.05 a	9.59 a	1838.09 a
T_2 = seed treatment + fungicide spray + insecticide spray	77.57 b (61.73)	11.47 a	8.04 b	1513.17 b
T_3 = seed treatment + fungicide spray	73.12 c (58.77)	9.74 b	7.21 c	1239.34 c
T_4 = seed treatment	69.51 d (56.49)	8.75 b	6.73 cd	1075.88 d
T_5 = Control	64.49 e (53.42)	6.04 c	5.32 d	732.61 e

Figures within parentheses are ArcSin(X+1) transformed values.
Means within the same column having a common letter(s) are not significantly different (P=0.05).

Table 6. Influence of integrated application of three insecticides and a fungicide against OkYVCMV and *Pseudocercospora* leaf spot of okra on benefit cost ration

Treatment	Fruit Yield t/ha	Gross return/ha	Total cost/ha	Net return/ha	BCR
T_1	10.79	161882	88106	73776	1.84
T_2	9.44	141546	82785	58761	1.71
T_3	8.74	131130	77571	53559	1.69
T_4	7.82	117242	73029	44213	1.61
T_5	6.38	95728	68775	26953	1.39

LITERATURE CITED

Ahmed, N. E., Kanan, H. O., Sugimoto, Y., Ma. Y. Q., and Inanaga, S. 2001. Effect of imidaclopid on incidence of *Tomato yellow leaf curl virus.* Plant Dis. 85: 84- 87.

Anju, H., Gupta, M. D. and Handa, A. 1993. Management of bhindi yellow vein mosaic virus disease. Indian Phytopathol. 46(2): 123-130.

Anonymous. 1996. International Rules for Seed Testing. International Seed Testing Association (ISTA). Seed Sci. Tech. 24 (Suppl.: 29-72.

Anonymous. 2005. Fertilizer Recommendation Guide-1997.Bangladesh Agricultural Research Council, Dhaka -1215. Bangladesh. 109 pp.

Baki, A. A. and Anderson, J. D. 1972. Physiological and Biological determination of seeds. *In*: Seed Biol. - 11, Aca. Prees, New York. Pp.283-315.

Basu, A. N. 1995. *Bemicia tabaci* (Ginnadius): Crop pest and principal whitefly vector of plant viruss. Oxford and IBH Pub. Co. Pvt. Ltd., New Delhi, India. 183 pp.

Chakraborty, R. and Mukhopadhyay, S. 1977. Effect of some pesticides on the YVMV disease of bhindi, *Pesticides* II: 19-22.

Fakir, G. A. 2000. An annolated lst of seed-borne diseases in Bangladesh. Seed Pathology Laboratory. Bangladesh Agricultural University, Mymensing, Bangladesh.

Gomez, K. A. and Gomez, A. A. 1984. Statistical procedures for Agricultural Research. John niley and Sons Pub., New York. pp. 91-97.

Gowdar, S.B., Babu, H.N.R. and Reddy, N.A. 2007. Efficacy of insecticides on okra yellow vein mosaic virus and whitefly vector, *Bemisia tabaci* (Guenn.). Ann. Pl. Prot. Sci. 15(1): 116-119.

Khan, M. A. and Mukhopadhyay, S. 1985. Effect of different pesticides combination on the incidence of yellow vein mosaic virus disease of okra (*Abelmoschus esculentus*) and its whitefly vector, *Bemisia tabci* Genn. Indian J. Virol. I: 147-151.

Mian, I. H. 1995. Methods in Plant Pathology. IPSA-JICA project publication, Institude of Post Graduate Studies in Agriculture, Gazipur, Bangaladesh. 24:136 pp.

Misra, H. P. 2005. Efficacy of some newer insecticides against the whitefly, *Bemisia tabaci* infesting okra. Orissa J. Hort. 33(2): 76-78.

Murthy, K.V. V. S and Reddy, D. R. R. 1992. Chemical control of yellow mosaic disease of bhendi. Indian J. Plant Prot. 20 (2):198-201.

Praveen, K. S., Sajjan, A. S., Patil, R. K., Dharmatti, P. R. and Kurdikeri, M. B. 2007. Influence of seed treatment and foliar spray with insecticides and neem production growth and seed yield in okra. Karnataka J. Agril. Sci., 20 (2): 388-390.

Pun, K. B., Sabitha, D. and Balasubramanian, G. 2005a. Prediction of whitefly population and Okra yellow vein mosaic virus disease incidence in okra. Indian J. Virol. 16(1/2): 19-23.

Pun, K. B., Sabitha, D. and Jeyarajan, R. 2005b. Management of Okra yellow vein mosaic virus disease and its whitefly vector. Indian J. Virol. 16(1/2): 32-35.

Rahman, M. L. and Nahar, N. S. 1990. Effect of plant density and cultivars on the incidence of cercospora leaf spot of cowpea. Bangladesh J Plant Pathol. 6(1&2): 13-15.

Rahman, M. A., Ali. M., Mian, I. H., Begum, M. M. and Uddin, M.K. 2000. Pesticidal control of *Pseudocercospora* leaf spot and shoot and fruit borer of okra seed crop. Bangladesh J. Plant pathol. 16: 1-2, 31-34.

Rana, S. C., Singh, P. B., Pandita,V. K. and Sinha, S. N. 2006. Evaluation of insecticides as seed treatment for control of early sucking pests in seed crop of okra. Ann. Pl. Prot. Sci. 14(2): 364-367.

Rangaswami, G. and Mahadevan, A. 2006. Diseases crop plants in India. New Delhi: Prentice-Hall of India. pp. 316-318.

Razzaque, M. A., Sattar, M. A., Amin, M. S., Quayum, M. A. and Alam, M.S. 2000. Krishi Projukti Hatbai (in Bangla). Bangladesh Agricultural Research Institute, Gazipur, Bangladesh. p.464.

Sastry, K.S.K. and Singh, S.J. 1974. Effect of yellow mosaic virus infection on growth and yield of okra crop. Indian Phytopath. 27: 294-297.

Singh, B.R. and Singh., M. 1989. Control of yellow-vein mosaic of okra by checking its vector whitefly through adjusting dates of sowing, insecticidal application and crop barrier. Indian J. Virol. 5 (1-2): 61-66.

Singh, B.R. and Singh., M. 1989. Control of yellow-vein mosaic of okra by checking its vector whitefly through adjusting dates of sowing, insecticidal application and crop barrier. Indian J. Virol. 5 (1-2): 61-66.

Singh, D., Verma, N. and Naqvi, Q. A. 1998. Control of yellow vein mosaic of okra by seed selection and insecticidal application. Indian J.Virol. 14(2): 121-123.

Srivastava, L. S., Gupta, D. K. and Verma, R. N. 1992. Chemical control of Cercospora blight disease of okra in Sikkim. Indian J. Mycol. Pl. Pathol., 22(1): 73-74.

Talukdar, M. J. 1974. Plant diseases in Bangladesh. Bangladesh J. Agril. Res. 1(1):61-81.

CHARACTERIZATION OF *OROBANCHE* SPECIES OCCURRING IN BANGLADESH

Manisha Saha[1] and I. H. Mian[2]

[1]Graduate Student and [2]Professor, Department of Plant Pathology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh
Email of corresponding author: ihmian2007@gmail.com

(The paper is a part of M.S thesis of the first author)

ABSTRACT

Manisha Shaha and I. H. Mian. 2013. Characterization of *Orobanche* species occurring in Bangladesh. Bangladesh J. Plant Pathol. 29 (1&2): 39-44.

A survey was conducted in the district of Jessore, Kushtia, Pabna and Rajshahi to identify and characterize *Orobanche* species occurring in those locations of Bangladesh. Two distinct species of *Orobanche* were found to occur in the country. The first one was found to attack mustard, cabbage and cauliflower, and the second one attacked sunflower, tomato and tobacco. *Orobanche* sp. collected from mustard showed higher shoot length, canopy diameter, sepal number, petal length, ovary length, ovary diameter, and style length. Flowers were with 2 bractlets, calyx divided at the base posteriorly, and entire anteriorly, stamen number was 4 (all equal), with wooly anthers. On the other hand, *Orobanche* sp. collected from tomato had higher stem girth, leaf length, leaf width, sepal length, stigma diameter and stamen length. Flowers were without bractlets, calyx divided at the base anteriorly as well as posteriorly, stamen number was 4 (2 longer than other 2), with glabrous anthers. The morphological characters clearly showed that the two *Orobanche* observed on two hosts species were distinctly different species. The morphological characters were compared with a standard key and *Orobanche* collected from mustard was identified as *O. aegyptiaca* L. and the parasite attacking tomato was identified as *O. cernua* L.

Key words: *Orobanche* species, host range, survey.

INTRODUCTION

Orobanche, commonly known as broomrape, is a genus of parasitic flowering plants. It is a holoparasite affecting several hundred crop species including rapeseed-mustard, tobacco, potato, brinjal, cabbage, cauliflower, turnip and many other Solanaceous and Cruciferous plants (Musselman 1980). They lack chlorophyll and their leaves are vestigial. Above-ground stems are produced only for the purpose of flowering and setting seeds. After ripening of the fruits, seeds of *Orobanche* shed in soil, they undergo a conditioning period of several weeks. After conditioning, seeds germinate in response to host stimulant and radicle (germtube) emerges. Germination is followed by haustorial initiation, attachment of the host roots, and lastly penetration of the vascular tissues (Musselman 1980, Kreutz 1995).

Species of *Orobanche* are aggressive and damaging parasitic weeds which have a tremendous impact on agriculture causing considerable yield losses, in many countries, especially in the drier and warmer areas of Europe, Africa and Asia (Dhanapal *et al*. 2009). The losses may vary from 5 to 100% (Stewart and Press 1990). The average crop loss, across all broomrape species, is approximately 34% (Linke *et al*. 1989, Diaz *et al.* 2006).

A comprehensive review published in India (Dhanapal *et al*. 2009) reveal that practices to control broomrape include physical methods (weeding, soil tillage, flooding, irrigation, pola-rization, flaming), chemical methods (soil fumigation, herbicide application, use of germination stimulants), biological methods (use of resistant or tolerant varieties), cropping systems with trap and catch crops, intercropping, and biological control (with insects or fungi).

In Bangladesh, *Orobanche* attack different crops. However, list of the susceptible crops are not available in the country. At at least two species of the pest namely *O. aegyptiaca* and *O. cernua* occur in Bangladesh (Begum and Huq 1983). Comprehensive report on crop loss due to the pest is not available in the country. A recent hand book on agricultural technologies published by Bangladesh Agricultural Research Institute reveals that *Orobanche* is a common pest of mustard. In Bangladesh, recommendations to control the pest include removal of *Orobanche* plants before flowering, avoidance of growing mustard in known infested fields, destruction of the weed with 2,4-D and use of appropriate amount of TSP fertilizer (Islam *et al*. 2004). Alam *et al*. (2008) identified some resistant germplasm of mustard in a screening test conducted during 2007-2008.

The present paper reported the results of an investigation conducted to characterize the species of *Orobanche* attacking crop plants in Bangladesh.

MATERIALS AND METHODS

The investigation was conducted in the district of Jessore, Kushtia, Pabna and Rajshahi. *Orobanche* infested fields of mustard, sunflower, tomato, cabbage, cauliflower and tobacco were visited and host range of the parasite was recorded. Apparently healthy and matured *Orobanche* plants were collected from each of the host crops. Canopy diameter covered by *Orobanche* plants and growth habit of shoot were recorded.

To collect data on plant characters, the selected *Orobanche* plants were uprooted by digging with a spade along with root systems of respective hosts. They were washed with tap water carefully to minimize root damage of host plants. Whole plants and their individual parts were checked with naked eyes or under a hand lens or using a compound microscope according to necessity. Data on characteristics of shoot, leaf, flower and fruit were recorded.

For recording leaf characters, 20 fully open leaves were selected randomly from the plants. Lamina length from base attached with the stem to the tip, lamina width at the widest point and lamina shape were recorded.

A total of 20 fully open flowers were checked and data on flowers types, flowers arrangement (solitary or clustered), color of petals and anther, number and length of sepal, petal and stamen, length of style and ovary, diameter of stigma and ovary, and shape and diameter of pollen were recorded.

RESULTS AND DISCUSSION

Orobanche and their hosts identified

During the investigation, mustard, sunflower, tomato, cabbage, cauliflower and tobacco were found to be infected with *Orobanche*. The morphological appearance of the pests infecting sunflower, tomato and tobacco was distinctly different from the parasites that attacked mustard, cabbage and cauliflower (Plate-IA&B). Morphological characters of stem, leaf and flowers recorded during the investigation were compared with a standard key and *Orobanche* from mustard family was identified as *Orobanche aegyptiaca* L. and the parasite infecting other crops was identified as *Orobanche cernua* L (Lie Dang Shu 1998).

In mustard fields, *O. aegyptiaca* infected up to 100% host plants. In severe cases, infected host plants died before maturation (Plate-IC). In infected tomato fields, *Orobanche* shoot emerged in cluster showing up to 42% host plants infection. Infected tomato plants gradually died and weathered before maturation of fruits (Plate-ID).

Both *O. aegyptiaca* and *O. cernua* were found to grow closed to the stem base or a little far from it depending on length of root systems of their hosts. The shoot of *O. cernua* attacking tomato is robust as compared to *O. aegyptiaca* that attacks mustard. The appressorium of the *Orobanche* developed at the base of stems was thicker on tomato than those infecting mustard (Plate-IE&F). The number of parasite shoots per square meter was higher in mustard fields than tomato fields. It might be due to the fact that the parasite attacking mustard has higher tendency to branch than those of tomato.

Patrick and Miller (1994) also found similar results. They reported that in some species (such as *O. aegyptiaca*, *O. ramosa*) branching of the shoot is normal, whereas in others (*O. cernua, O. minor, O crenata*) branching is rare.

Reports from various sources reveal that seeds of *Orobanche* germinate in response to the host stimulant and the radicle, also known as germtube, emerges. If the radicle reaches the host root, its tip thickens and attaches itself to root surface. The thickening is known as appressorium. Finally, haustorium initiates from the appressorium, which engulfs the host roots and penetrates the vascular tissues of the host and makes parasitic relationship with the host (Plate-IIA&B). With this organ, the parasite withdraws water, minerals and organic compound from the hosts (Visser and Dorr 1986, Stewart and Press 1990, Nun and Mayer 1993).

Morphology of *Orobanche aegyptiaca*

Plant: The canopy diameters of *O. aegyptiaca* in mustard fields ranged 1.40-3.80 cm with a mean of 2.44 cm and standard deviation 0.65 cm. Stems were erect, roundish, thinner, purplish white and glandular pubescent. The shoots were branched above the middle point but some were unbranched. They were smooth having crowded leaves and flowers. The plant height ranged 17.00-31.00 cm with a mean of 23.88 cm and standard deviation 3.71 cm. The range of stem girth at the widest point was 1.00-2.00 cm with a mean of 1.69 cm and standard deviation of 0.35 cm (Table 1).

Leaf: The leaves were alternate, acute, and lanceolate. The base of leaves was wide and tip was pointed. Stem under and around a leaf was light greenish yellow. Leaf length ranged 0.60-1.10 cm with a mean of 0.77 cm and standard deviation 0.15 cm. The maximum leaf width ranged 0.30-0.60 cm with a mean of 0.41 and standard deviation 0.09 cm (Plates I&II and Table 1).

Flower: Flowering initiated in January and ended in March. Inflorescence was spicate, bract ovate-lanceolate, with 2 bractlets, flowers sub-sessile, bisexual, alternate, axial and attached with the stem at an acute angle (Plate I&II). Calyx was campanu-

Plate-1. Photographs showing *Orobanche aegyptiaca* infecting mustard (left) and *O. cernua* infecting tomato (right).

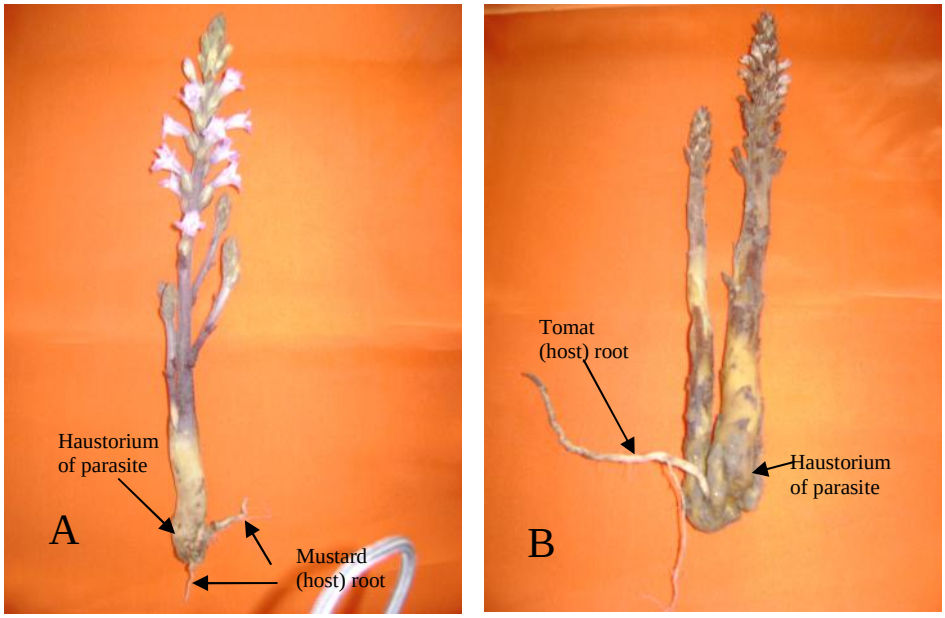


Plate-II. Haustoria of *Orobanche* engulfing host roots of mustard (A) and tomato (B).

nulate with 4 sepals, divided at the base posteriorly, entire anteriorly, light greenish yellow colour, sepal length 0.80-1.20 cm with a mean of 1.01 cm and standard deviation 0.16 cm. Corolla was funnel shaped with five lobed petals, usually rounded, tip of petal free and blue purple and purplish white along the tube, petal length 1.50-2.00 cm with a mean of 1.73 cm and standard deviation 0.20 cm. Stigma diameter ranged 0.10-0.20 cm with a mean of 0.12 cm and standard deviation 0.04 cm. Ovary was bicarpelary with terminal single style, ovary length ranged 0.40-0.80 cm with a mean of 0.65 cm and standard deviation 0.14 cm, ovary diameter at widest point ranged 0.25-0.40 cm with a mean of 0.35 cm and standard deviation 0.05 cm. Number of stamens was 4 (all equal), with wooly anthers, length ranged 0.60-0.80 cm with a mean of 0.70 cm and standard deviation 0.08 cm. Style length ranged 0.05-0.60 cm with a mean of 0.48 cm and standard deviation 0.04 cm. Pollen diameter ranged 6.24-10.92 µm with a mean of 8.05 µm. Pollen shape was round with spines on the surface (Table 1).

Table 1. Measurements of different plant parts of *Orobanche* attacking mustard

Parameter	Range	Mean	Standard deviation
Shoot height (cm)	17.00-31.00	23.88	3.71
Maximum stem girth (cm)	1.00-2.00	1.69	0.35
Maximum canopy diameter (cm)	1.40-3.80	2.44	0.65
Leaf length (cm)	0.60-1.10	0.77	0.15
Maximum leaf width (cm)	0.30-0.60	0.41	0.09
Number of sepals	4.00-4.00	4.00	0.00
Sepal length (cm)	0.80-1.20	1.01	0.16
Petal length (cm)	1.50-2.00	1.73	0.20
Stigma diameter (cm)	0.10-0.20	0.12	0.04
Ovary length (cm)	0.40-0.80	0.65	0.14
Ovary diameter (cm)	0.25-0.40	0.35	0.05
Stamen length (cm)	0.60-0.80	0.70	0.08
Style length (cm)	0.50-0.60	0.48	0.04

Pollen diameter (µm)	6.24-10.92	8.05	1.09

Morphology of *Orobanche cernua*

Plant: The stems were erect, robust, rigid, thicker, light brown at immature stage, dark brown at mature stage, glabrous near stem base or minutely pubescent from middle upwards, and unbranched with crowded leaves and flowers. The plant height ranged 14.00-32.00 cm with a mean of 23.13 cm and standard deviation 5.27 cm. Maximum stem girth ranged 1.70-4.00 cm with a mean of 3.26 cm and standard deviation 0.85 cm, and the canopy diameters ranged 1.40-2.00 cm with a mean of 1.68 cm and standard deviation 0.25 cm (Plate-III and Table 2).

Leaf: The leaves were scale like, alternate, acute, ovate-lanceolate along with bracts, bractlets and calyx. The base of leaves was swollen due to development of flower bud, and pointed tip. Leaf length ranged 1.00-1.50 cm with a mean of 1.27 cm and standard deviation 0.20 cm. The maximum leaf width ranged 0.40-0.70 cm with a mean of 0.58 and standard deviation 0.10 cm (Plate III and Table 2).

Flower: Flowering initiated in January and ended in March. Inflorescence was spicate, bract ovate-lanceolate, without bractlets, acuminate, flower sub-sessile, bisexual, alternate, axial and attached with the stem at an acute angle. Calyx was campanulate with 2 distinct sepals, divided at the base both anteriorly and posteriorly, greenish brown, length 0.7-1.0 cm with a mean of 1.88 cm and standard deviation 0.11 cm. Corolla was funnel shaped with five lobed petals, usually rounded, tip of petal free and white with blue tint at the tip, purplish white along the tube, length 1.30-2.00 cm with a mean of 1.68 cm and standard deviation 0.25 cm. Stigma diameter ranged 0.10-0.20 cm with a mean of 0.16 cm and standard deviation 0.05 cm. Ovary was bicarpelary with terminal single style, length ranged 0.30-0.70 cm with a mean of 0.57 cm and standard deviation 0.13 cm, diameter 0.20-0.30 cm with a mean of 0.27 cm and standard deviation 0.03 cm. Number of stamens was 4 (2 longer than other 2), length ranged 0.70 -0.90 cm with a mean of 0.81 cm and standard deviation 0.07 cm. Style length ranged 0.04-0.50 cm with a mean of 0.48 cm and standard deviation 0.04 cm. Pollen diameter ranged 7.80-10.92 µm with a mean 9.05 µm and standard deviation 1.57 µm. Pollen of *O. cernua* collected from tomato was round (Plate III and Table 2).

Table 2. Measurements of different plant parts of *Orobanche* attacking tomato

Parameter	Range	Mean	Standard deviation
Shoot height (cm)	14.00-32.0	23.13	5.27
Maximum stem girth (cm)	1.70-4.0	3.26	0.85
Maximum canopy diameter (cm)	1.40-2.0	1.68	0.25
Leaf length (cm)	1.00-1.5	1.27	0.20
Maximum leaf width (cm)	0.40-0.7	0.58	0.10
Number of sepals	2.00-2.0	2.00	-
Sepal length (cm)	0.70-1.0	1.88	0.11
Petal length (cm)	1.30-2.0	1.68	0.25
Stigma diameter (cm)	0.10-0.2	0.16	0.05
Ovary length (cm)	0.30-0.7	0.57	0.13
Ovary diameter (cm)	0.20-0.30	0.27	0.03
Stamen length (cm)	0.70-0.9	0.81	0.07
Style length (cm)	0.40-0.5	0.48	0.04
Pollen diameter (µm)	7.80-10.92	9.05	1.57

Comparison of *O. aegyptiaca* and *O. cernua*

Morphological differences between two *Orobanche* specimens collected from mustard and tomato are shown in Table 3. *Orobanche* collected from mustard showed higher shoot length, canopy diameter, sepal number, petal length, ovary length, ovary diameter, and style length. Flowers were with 2 bractlets, calyx divided at the base posteriorly, and

entire anteriorly, stamen number was 4 (all equal), with wooly anthers. On the other hand, *Orobanche* collected from tomato had higher stem girth, leaf length, leaf width, sepal length, stigma diameter and stamen length. Flowers were without bractlets, calyx divided at the base anteriorly as well as posteriorly, stamen number was 4 (2 longer than other 2), with glabrous anthers. The morphological characters clearly showed that the two *Orobanche* observed on two hosts species were distinctly different species. The morphological characters were compared with a standard key published in China for identifying them up to species level (Lie Dang Shu 1998). According to the key, *Orobanche* from mustard was identified as *Orobanche aegyptiaca* L. and the parasite attacking tomato was identified as *Orobanche cernua* L. Previous reports published by Bangladesh National Herbarium also reveal that *Orobanche aegyptiaca* and *Orobanche cernua* occur in Bangladesh (Begum and Huq 1983). The pests are widely distributed in Southern Europe, Asia and Africa. *Orobanche aegyptiaca* is also known as *O. indica*, and *Phelipaea aegyptiaca* (Musselman 1980, 1986).

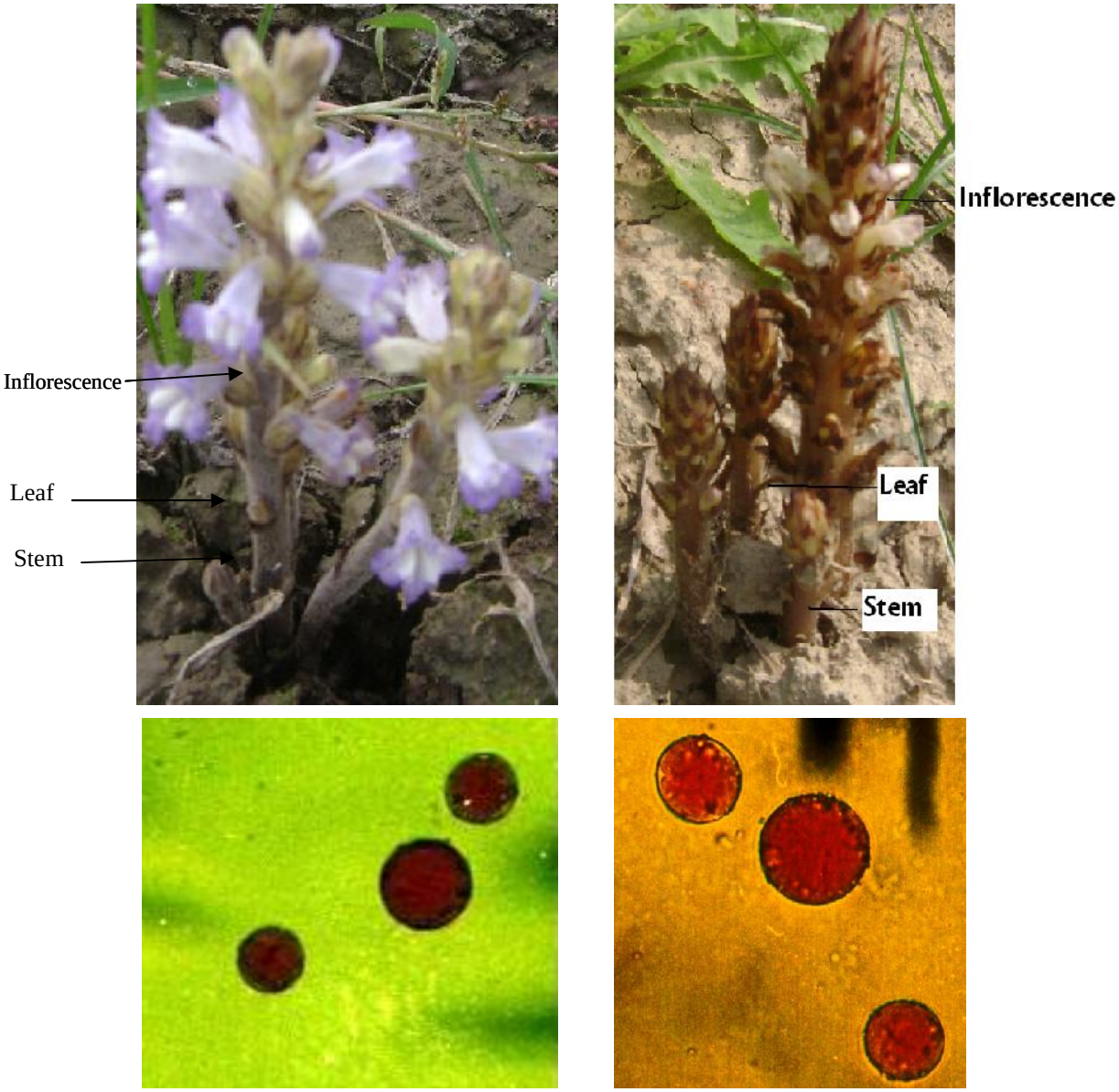


Plate III. Photograph showing stems, leaf, inflorescence, flowers and pollens of *O. aegyptiaca* (left) and *O. cernua* (right)

Table 3. Comparison of different morphological characters of *O. aegyptiaca* attacking mustard and *O. cernua* attacking tomato

Parameter	*O. aegyptiaca* host mustard	*O. cernua* host tomato
Shoot height (cm)	23.88±3.71	23.13±5.27
Maximum stem girth (cm)	1.69±0.35	3.26±0.85
Maximum canopy diameter (cm)	2.44±0.65	1.68±0.25
Leaf length (cm)	0.77±0.15	1.27±0.20
Maximum leaf width (cm)	0.41±0.09	0.58±0.10
Number of sepals	4.00±0.00	2.00±0.00
Sepal length (cm)	1.01±0.16	1.88±0.11
Petal length (cm)	1.73±0.02	1.68±0.25
Stigma diameter (cm)	0.12±0.04	0.16±0.05
Ovary length (cm)	0.65±0.14	0.57±0.13
Ovary diameter (cm)	0.35±0.05	0.27±0.03
Stamen number	4 (all equal)	4 (2 longer)
Stamen length (cm)	0.70±0.08	0.81±0.07
Style length (cm)	0.48±0.04	0.48±0.04
Pollen diameter (µm)	8.05±1.09	9.05±1.57
Flower	With 2 braclets,	Without braclet
Calyx	Divided at the base posteriorly, and entire anteriorly	Divided at the base posteriorly and anteriorly
Stamen	with wooly anther	with glabrous anther

LITERATURE CITED

Alam M. M., Begum, F., Hossain, M. S., Hossain, M. D. and Hoque, M. Z. 2008. Screening of rapeseed-mustard lines for resistance to broomrape (*Orobanche* spp.). Regional Agricultural Research Station, BARI. Ishurdi, Pabna.

Begum, M., and Huq, A. M. 1983. Flora of Bangladesh. Loranthaceae. Bangladesh National Herbarium. Bangladesh Agricultural Research Council. 33: p.1.

Dhanapal G.N., Struik, P. C., Udayakumar, M., and Timmermans, P. C. J. M. 2009. Management of broomrape (*Orobanche* spp.): A review. J. Agron. & Crop Sci. 175: 335-359.

Diaz, S. J., Norambuena, M. H. and Lopez-Granados, F. 2006. Characterization of the holoparasitism of *Orobanche ramosa* on tomatoes under field conditions. Instituto de Investigaciones Agropecuarias, Centro Regional de Investigacion Carillanca, Agric. Tecn. 66(3): 223-234. URL: http://www.inia.cl/at/agritec.htm.

Islam, M. M., Satter, M. A, Rahman, M. M., Qayum, M. A, Alam, S. M. N., Alam, M. S., and Mustafi, R. A. 2004. Hand Book on Agricultural Technology [in Bangla]. Bangladesh Agricultural Research Institute (BARI), Gazipur. p. 128.

Lie Dang Shu. 1998. Flora of China. 18: 231-239.

Linke, K. H., Sauerborn, J., and Saxena, M. C. 1989. *Orobanche* Field Guide. ICARDA-FLIP, Aleppo, Syria. 42 pp.

Musselman, L. J. 1980. The biology of *Striga*, *Orobanche*, and other root-parasitic weeds. Annu. Rev. Phytopathol. 18: 463-489.

Musselman, L.J. 1986. Taxonomy of Orobanche. in: S.J. ter Borg (ed.) Proceedings of a Workshop on the Biology and Control of Orobanche, LH/VPO, Wageningen, The Netherlands. pp. 2-10.

Nun, N. B., and Mayer, A. M. 1993. Preconditioning and germination of *Orobanche* seeds: Respiration and protein synthesis. Phytochemistry 34(1): 39-45.

Patrick, M. 1994. The Biology and Distribution of Parasitic Plants within the Orobanchaceae: An Overview of Secondary Plant Compound Involvement Colorado State University. Fort Collins, Colorado 80523.

Stewart, G. R., and Press, M. C. 1990. The physiology and biochemistry of parasitic angiosperms. Annu. Rev. Plant Phys. Plant Mol. Biol. 41: 127-151.

Visser, J., and Dorr, I. 1986. The Haustorium (CH 7). *In*: L. J. Musselman (ed.), Parasitic Weeds in Agriculture. Vol. I, *Striga*. CRC Press, Inc., Boca Raton, FL.