SEED TREATMENT AND FOLIAR SPRAY WITH FUNGICIDES TO CONTROL SEEDLING DISEASES AND CERCOSPORA LEAF SPOT OF MUNGBEAN

A. A. Khan[1], M. M. Hossain[2] and I. H. Mian[1]

[1] Professor and [2] Ph.D. student, Department of Plant Pathology
Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706
Email of first author: E- mail: ashraf@bsmrau.edu.bd

ABSTRACT

A. A. Khan, M. M. Hossain and I. H. Mian. 2015. Seed treatment and foliar spray with fungicides to control seedling diseases and cercospora leaf spot of mungbean. Bangladesh J. Plant Pathol. 31(1&2): 31-38.

An investigation was undertaken to find out the influence of seed treatment and foliar spray with fungicide in controlling of seedling infection and Cercospora leaf spot and production of healthy seeds of mungbean. Three seed treating fungicides namely Provax-200, Metataf and Cupravit and three foliar fungicides such as Dithane M-45, Rovral 50 WP and Bavistin 50 WP were selected for the investigation. These were used alone and in different combinations. Provax-200 found effective to increase the emergence and to reduce seedling diseases. Highest seedlings emergence (55.64%) and the lowest seedling infection were recorded in seed treatment with Provax-200. All treatments with fungicides significantly reduced incidence and severity (PDI) of Cercospora leaf spot over control. *Cercospora* leaf spot incidence and PDI were observed lower in treatment, seed treatment with Provax + spaying of Bavistin. Seed treatment with Metataf + spaying of Bavistin and seed treatment with Cupravit + spaying of Bavistin was also promising in reducing Cercospora leaf spot incidence and PDI. The highest seed yield was recorded from the treatment; seed treated with Provax and foliar spray with Bavistin. Harvested seeds under this treatment yielded the lowest prevalence of *Aspergillus* spp. and *Fusarium oxysporum*.

Key words: Seed treatment, foliar spray, fungicides, mungbean.

INTRODUCTION

Mungbean [*Vigna radiata* (L.) Wilczek] is one of the important food legumes in Bangladesh. It is extensively cultivated in the country during Kharif season in the gap period between Boro and Aus rice. Yield of legumes in farmer's fields is usually less than 1 t ha^{-1} against the potential yield of 2 to 4 t ha^{-1} (Ramkrishna *et al*. 2000). Such yield gap of legumes indicates a great opportunity to increase the productivity of mungbean at farm level. Diseases are known to play a vital role in decreasing the yield and quality of the crop seeds. The crop suffers from at least 20 different diseases in Bangladesh. Of which 12 are caused by fungi (Bakr 1994). Cercospora leaf spot caused by *C. cruenta* is one of the major pulse diseases considering high incidence, wide distribution and detrimental effect on yield (Talukdar 1974). It can reduce yield up to 58% (Lal *et al*. 2001). Any variety with acceptable level of resistance against the disease is not available (Saifullah *et al*. 2005). Seed is the vital input in agriculture. From the pathological point of view, quality of seed is very important to get disease free seedling and plant, and finally to achieve a satisfactory yield (Fakir *et al*. 2002).

Seed treatment is an important measure for the control of seed transmitted diseases. It is unquestionable that proper seed treatment can substantially improve the quality of seed and seedling with satisfactory increase in the yield through increase of plant stand. Among the recommended practices, seed treatment with fungicides is the cheapest method of plant disease control. Treatments of seeds with fungicides have been proved to be quite effective in reducing seed-borne infection (Saraswathi *et al*. 1989, Dubey and Singh 2005, Barua *et al*. 2007).

In Bangladesh, use of seed treating fungicides like Vitavax-200 and Bavistin 50 WP may be useful to control seed-borne fungal pathogens. Disease management in the field is also an important prerequisite for the production of quality and healthy seeds. Foliar diseases of mungbean may be effectively controlled by spraying appropriate fungicides. According to Huq *et al*. (1991), Haque *et al*. (1994), Iqbal *et al*. (1995), Mian *et al*. (2000), Khunti *et al*. (2005), Rathore (2006), Saxena and Tripathi (2006) spraying of fungicides is the best way to control *Cercrospoa* leaf spot.

The present paper reports the results of an investigation conducted to evaluate the efficacy of three seed treating fungicides and another three foliar fungicides to control seedling infection and *Cercospora* leaf spot (*C. cruenta*) of mungbean with a view to select effective ones to control the disease.

MATERIALS AND METHODS

The experiment was conducted in the Research farm of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur during 2011-2013 to screen six fungicides against seedling infection and Cercospora leaf spot (*C. cruenta*) of mungbean with a view to select effective ones to control the disease. Three fungicides namely Provax-200 (Carboxin), Metataf (Metalaxyl) and Cupravit (Copperoxichloride) were tested as pre-sowing seed treating chemicals @ 2.5% (w/w). Other three namely Dithane M-45 (Mancozeb) @ 2.5 (w/v), Rovral 50 WP (Iprodione) @ 2.0% (w/v) and Bavistin 50 WP (Carbendazim) @ 2.0% (w/v) were tested as foliar spray applied at 15, 30 and 45 days after planting (DAS).

Only seed treatment with Provax-200, Metataf or Cupravit as well as only foliar spray with Dithane M-45, Rovral 50 WP or Bavistin 50 WP and seed treatment with a fungicide followed by foliar with one fungicide were tested in 15 treatments viz. Provax-200, Metataf, Cupravit, Dithane M-45, Rovral 50 WP, Bavistin 50 WP, Provax-200+ Dithane M-45, Provax-200+ Rovral 50 WP, Provax-200+ Bavistin 50 WP, Metataf+ Dithane M-45, Metataf+ Rovral 50 WP, Metataf+ Bavistin 50 WP, Cupravit+ Dithane M-45, Cupravit+ Rovral 50 WP and Cupravit + Bavistin 50 WP. A control treatment was maintained where seeds were not treated and no spray was applied.

Mungbean (*Vigna radiata*) variety BU mung-2 was grown following standard methods. The experiment was laid out in a randomized complete block design with 3 replications. The unit plot size was 1.5 m X 2.0 m and distance between plots was 1.0 m. Treated seeds were planted at a depth of 5 cm, maintaining 10 cm seed to seed and 30 cm row to row distances. Seed rate was 50 kg ha^{-1}. Weeding, irrigation and other intercultural operations were done as and when needed.

Data on seedling emergence were recorded 7 days after sowing (DAS) and expressed as percentage based on total number of seeds sown. Seedling mortality caused by fungal pathogens was also recorded after two weeks of sowing and expressed in percentage. The incidence of Cercospora leaf spot (CLS) was recorded three times starting from 30 DAS at 15 days interval. The incidence of CLS was recorded based on the basis visible symptoms as suggested by Bakr (1991) and expressed in percentage. The severity of CLS was indexed on a 0-9 scale (Singh *et al.* 1982)), where 0=no visible symptoms on leaves, plant growth, pod formation normal, 1=0.1- 5.0% foliage is affected by small pinhead sized brown lesions, 3=5.1- 10% foliage is covered with round brown spots, 5=10.1-25% foliage is covered with bigger leaf spots, 7=25.1-50% foliage is covered with enlarging, coalescing leaf spots and 9=50.1-100% foliage is covered with very large, coalesced spots. The severity was expressed in percent disease index (PDI) which was computed using a standard formula (Mian *et al.* 2000).

The mungbean was harvested at full ripening stage. The harvested crop was sun dried and threshed manually. Seeds were sun dried, cleaned and stored at 4C for further use. Before harvesting 10 plants were selected in each plot for collection of seed yield data. Data on pod number plant^{-1}, pod length (cm), seed number pod^{-1}, 1000-seed weight (g) and seed yield (kg ha^{-1}) were recorded.

Seed health of the harvested mungbean seeds was tested following blotter method to detect the seed-borne fungal pathogens following the International Rules for Seed Testing (Anon. 1999). Four hundred seeds from each sample were taken randomly and placed on moist filter paper in sixteen replicated Petridishes at the rate of 25 seeds per plate. After seven days of incubation, the seeds were examined under steriobinocular microscope for the presence of seed-borne fungi and identified by observing their growth characters. In case of confusion, temporary mounts were prepared and examined under a compound microscope and identified the fungi using appropriate keys (Ellis 1993, Mathur and Kongsdal 2003). The prevalence of the pathogen was expressed in percentage based on total number of seeds plated. Data were analyzed for Analysis of Variance using MSTAT-C software and before analysis data were transformed following arcsin transformation method. Mean separation was done following Duncan's Multiple Range Test using the same computer program.

RESULTS AND DISCUSSION

Seedling emergence and **seedling disease**

At 7 days after sowing (DAS), the lowest seedling emergence of 41.12% was observed under control. Preplant seed treatment with Provax-200, Metataf and Cupravit caused significant increase in seedling emergence within the range of 50.64-55.64%l. The highest emergence was achieved with Provax-200 followed by Cupravit and Metataf. Efficacy of three fungicides to increase seedling emergence was statistically similar (Table 1).

At 14 DAS, seedling infection caused by soil borne fugal pathogens, *Sclerotium rolfsii*, *Rhizoctonia solani* and *Fusarium oxysporum* in a complex form were recorded. Maximum infection of 4.5% was recorded from control plot. It was reduced to 1.00-2.58% due to pre-sowing seed treatment with three fungicides. The reduction was significant over control. The lowest infection was recorded in Provax-200 treated seed plot followed by Cupravit and Metataf (Table 1).

Table 1. Effect of pre-sowing seed treatment with fungicide on seedling emergence and seedling infection of mungbean

Dose of seed treating fungicide (2.5% w/w)	Seedling emergence after 7 days sowing (%)	Seedling infection at 14 days after sowing (%)
Provax-200	55.64 a	1.00 c
Metataf	50.44 a	2.58 b
Cupravit	52.67 a	1.94 b
Control	41.12 c	4.50 a

Values are average of three observations and those within the same column with a common letter(s) do not differ significantly (P = 0.05)

Results of the experiment indicate that pre-sowing seed treatment with fungicides reduced seedling infection effectively. The findings are corroborated with the findings of other investigators (Saraswathi *et al.* 1989, Barua *et al.* 2007). Saraswathi *et al.* (1989) and Mortuza *et al.* (1995) also reported that field emergence of seedling increased due to seed treatment with fungicides compared to control. Seed treating fungicides might destroy the seed-borne fungi and enhanced germination. Barua *et al.* (2007) reported that the seed-borne fungal pathogens reduced seed germination but chemical seed treatment reduced seed infection.

Incidence of *Cercospora* leaf spot

Incidence of Cercospora leaf spot was 1.20, 4.70 and 18.62% under control at 30, 45 and 60 DAS, respectively. Combination of pre-sowing seed treatment and foliar spray with fungicides in different combinations reduced the disease incidence over control within the range of 0.23-0.78% at 30 DAS, 1.27-3.84% at 45 DAS and 6.41-14.60% at 60 DAS. The reduction was significant at all stages of data collection compared to control (Table 2). At 30 DAS, the lowest disease incidence was achieved with combination of seed treatment with Metataf and foliar spray with Bavistin 50 WP, which was statistically similar to foliar spray of Bavistin alone, Provax + Dithane M-45, Provax + Bavistin, Cupravit + Rovral and Cupravit + Bavistin. The highest incidence was recorded from Metataf followed by Cupravit, Metataf + Dithane M-45 and Rovral alone. Their differences were not significant. At 45 DAS, the lowest incidence of Cercospora leaf spot was observed in treatment Provax + Bavistin, which was statistically similar to the treatments, seed treatment with Metataf + foliar spray with Bavistin 50 WP, seed treatment with Cupravit + foliar spray with Rovral, seed treatment with Cupravit + foliar spray with Bavistin 50 WP and foliar spray with Bavistin 50 WP alone (Table 2).

The results of the experiment indicate that effect of seed treatment and foliar spray with fungicides on the reduction of incidence of Cercospora leaf spot of mungbean is appreciable. The combination of seed treatment with Provax and foliar spray of Bavistin is superior to other treatments in reducing disease incidence. Effectiveness of Bavistin 50 WP in controlling CLS has been reported by other workers. Mian *et al.* (2000) found efficacy of Bavistin 50 WP and Indofil 80 WP to control CLS. Bakr (1993) reported that foliar application of Bavistin 50 WP at 0.1% successfully controlled CLS of mungbean.

Severity (PDI) of CLS

At 30 DAS, the lowest PDI of CLS was recorded from the in treatment combination of seed treatment with Provax and foliar spray with Bavistin 50 WP, which was statistically similar to the treatments, seed treatment with Cupravit + foliar spray with Dithane M-45) and foliar spray with Bavistin. The highest PDI of CLS was recorded from the treatment, seed treatment with Cupravit, which was statistically similar to the treatments, seed treatment with Provax-200 + foliar spray with Dithane M-45 and seed treatment with Metataf + foliar spray with Rovral 50 WP. All other treatments also showed significant reduction in PDI compared to control (Table 2).

At 45 DAS, the lowest PDI of 3.47 was recorded in Provax + Bavistin 50 WP, which was statistically similar to the treatment Metataf + Bavistin, Cupravit + Bavistin and Provax + Rovral. The highest PDI of 12.20 was recorded in control. At 60 DAS, the lowest PDI of 10.14% was recorded from treatment Provax + Bavistin which was statistically similar to treatments Metataf + Bavistin, Cupravit + Bavistin, Provax + Rovral, Cupravit + Rovral, Cupravit + Dithane M-45, Provax-200 + Dithane M-45 and foliar spray of Bavistin. The maximum PDI of 26.96 was recorded in control. All treatments with fungicides caused significant reduction in the severity of CLS over control (Table 2).

Table 2. Effect of pre-sowing seed treatment and foliar spray of seed crop with fungicide on incidence and severity of *Cercospora* leaf spot of mungbean

Treatment	Incidence (%) at different days after sowing			Severity (%) at different days after sowing		
	30	45	60	30	45	60
Seed treatment with Provax-200	0.51d (4.083)	2.89 c (9.783	12.66 bcde (20.846)	1.28 bc (6.496)	8.49 cd (16.940)	20.73 bc (27.086)
Seed treatment with Metataf	0.78 b (5.067)	3.71 bc (11.101)	13.42 bcd (21.490)	1.33 bc (6.615)	10.86 b (19.238)	23.18 b (28.779)
Seed treatment with alone Cupravit	0.70 bc (4.810)	3.20 bc (10.305)	14.60 b (22.461)	1.70 a (7.498)	9.32 c (17.775)	22.96 b (28.631)
Foliar spray with Dithane M-45	0.61 cd (4.480)	3.34 bc (10.535)	13.72 c (21.738)	0.87 def (5.343)	7.10 ef (15.450)	18.40 c (25.401)
Foliar spray with Rovral	0.67 bc (4.695)	3.07 bc (10.086)	12.95 bcde (21.092)	1.20 bcd (6.297)	7.29 de (15.661)	21.67 b (27.741)
Foliar spray with Bavistin alone	0.36 e (3.425)	1.63 d (7.342)	9.89 fgh (18.330)	0.73 fg (4.891)	5.24 gh (13.237)	13.24 de (21.340)
Seed treatment with Provax-200 + foliar spray with Dithane M-45	0.27 e (2.962)	2.83 c (9.690)	11.80 cdef (20.094)	1.52 ab (7.082)	5.14 gh (13.100)	12.89 de (21.043)
Seed treatment with Provax-200 + foliar spray with Rovral 50 WP	0.56 cd (4.303)	3.35 bc (10.546)	11.48 def (19.802)	1.22 bcd (6.334)	4.79 ghi (12.642)	11.89 de (20.173)
Seed treatment with Provax-200 + foliar spry with Bavistin 50WP	0.25e (2.866)	1.27 d (6.478)	6.41 i (14.662)	0.41 g (3.671)	3.47 i (10.731)	10.14 e (18.571)
Seed treatment with Metataf + foliar spray with Dithane M-45	0.68 bc (4.720)	3.84 b (11.296)	10.89 efg (19.266)	1.08 cde (5.957)	5.98 fg (14.151)	14.75 d (22.585)
Seed treatment with Metataf+ foliar spray with Rovral 50 WP	0.55 cd (4.253)	2.89 c (9.793)	9.93 fgh (18.368)	1.43 abc (6.875)	5.60 gh (13.692)	13.92 d (21.909)
Seed treatment with Metataf+ foliar spray with Bavistin 50 WP	0.23 e (2.767)	1.58 d (7.228)	9.24 gh (17.693)	0.83 ef (5.237)	4.45 hi (12.174)	11.38 de (19.715)
Seed treatment with Cupravit + foliar spray with Dithane M-45	0.57 cd (4.318)	3.30 bc (10.462)	10.34 fgh (18.760)	0.70 fg (4.799)	5.02 gh (12.943)	12.64 de (20.828)
Seed treatment with Cupravit + foliar spray with Rovral-200	0.29 e (3.087	1.51 d (7.058)	8.50 h (16.954)	1.25 bc (6.412)	4.93 gh (12.825)	12.44 de (20.650)
Seed treatment with Cupravit + foliar spray with Bavistin 50 WP	0.27e (2.962)	1.53 d (7.112)	9.17 gh (17.630)	1.17bcde (6.218)	4.65 ghi (12.449)	11.82 de (20.109)
Control	1.20 a (6.281)	4.70 a (12.525)	18.62 a (25.563)	1.52 ab (7.089)	12.20 a (20.446)	26.96 a (31.281)

* Values are average of three observations and those within the same column with a common letter(s) do not differ significantly (P = 0.05). *Figures within parentheses are arcsin transformed value.

All the treatments controlled the pathogen effectively and reduced disease incidence and severity. Among the treatments, combination of seed treatment with Provax + foliar spray of Bavistin 50 WP performed better than other treatments to reduce incidence and severity of the CLS. Dubey and Singh (2005) reported that dry seed treatment and foliar sprays with Carbendazim gave maximum grain yield along with minimum PDI of Cercospora leaf spots. Khunti *et al.* (2005) found Carbendazim as a highly effective fungicide for the management of Cercospora leaf spot and powdery mildew. Singh *et* *al.* (1994) reported that Cercospora leaf spots could be effectively controlled with Carbendazim because of longer residual toxicity as compared to Mancozeb. The findings of the present study support the findings of Khunti *et al.* (2005) and Singh *et al.* (1994).

Yield and yield contributes

Pod length, seed number per pod and 1000-seed weight varied 7.85-9.00 cm, 8.61-9.64 and 43.33-46.64 g under different treatments including control with means of 8.25 cm, 9.08 and 45.64 g and standard deviation of 0.29 cm, 0.37 and 0.92 g, respectively. However, their variations were not significant and data are not shown in tabular form.

The number of pods plant^{-1} ranged from 37.23 to 53.33 under different fungicidal treatments including control. Effect of only seed treatment with Provax, Metataf and Cupravit, only foliar spray with Dithane M-45, Rovral, and Bavistin and combination of seed treatment with Metataf and foliar spray with Rovral on number of pods per plant was not significant compared to control. Other treatments with seed treatants as well as foliar spray with fungicides significantly increased pod number per plant compared to control. The highest number of pods plant^{-1} was recorded from the treatment, Provax + Bavistin 50 WP which was statistically similar to the treatments where fungicides were applied as foliar spray (Table 3).

Grain yield under different treatments including control ranged from 1465.13 to 1862.42 kg ha^{-1}. The highest yield was recorded from the treatment Provax + Bavistin 50 WP, which was statistically similar to other treatments where foliar spray alone and in combination with seed treatments was used. The yield in these treatments was significantly higher over control. The maximum yield increase over control was recorded from the treatment Provax + Bavistin 50 WP followed by the treatment Metataf + Bavistin, Provax + Rovral, Cupravit + Bavistin (Table 3).

Mian *et al.* (2000) reported that increase in the number of pods plant^{-1}, pod length, seeds pod^{-1} and 1000-seed weight might have resulted in maximum yield of mungbean. Haque *et al.* (1994) reported that three or four sprays with Bavistin was effective to control CLS and to increase yield of mungbean. Ayub *et al.* (1993) also reported that Bavistin, Bavistin + Dithane M-45, Calixin, Copac, Rovral and Tilt were effective against CLS and increased the seed yield of mungbean. Results of the present study reveal that seed treatment with Provax and foliar spray with Bavistin 50 WP thrice reduces the incidence of Cercospora leaf spot (CLS) and increases seed yield appreciably.

Seed health of mungbean

All the fungicidal treatments significantly reduced the prevalence of fungi associated with harvested seeds compared to control. Seed-borne fungal pathogens were *Aspergillus* spp. (*A. flavus* and *A. niger*) and *Fusarium oxysporum*. The lowest prevalence of *Aspergillus* spp. was recorded in treatment Provax + Bavistin, which was statistically similar to all other treatment combinations of seed treatment and foliar spray except Metataf + Dithane. The highest *Aspergillus* spp. was recorded from control (Table 3).

The lowest prevalence of *F. oxysporum* was obtained with the treatment Provax + Bavistin, which was statistically similar to the treatment Cupravit + Bavistin. Significantly the highest prevalence of *F. oxysporum* was recorded under control. Effects of foliar spray with Dithane M-45, Rovral and Bavistin 50WP were statistically similar but significantly higher than seed treatment with Provax, Metataf and Cupravit alone (Table 3).

The significantly highest prevalence of *Aspergillus* spp. and *F. oxysporum* was recorded in control treatment than any other treatments indicating significant effect of fungicidal seed treatment and foliar spray on the control of *Aspergillus* spp. and *F. oxysporum*. The results of this study indicate that there was a significant effect of fungicidal seed treatment and foliar spray independently on the reduction of seed infection but combination of seed treatment + foliar spray performed better. Effectiveness of Vitavax-200 in controlling seed-borne fungi has also been reported by Barua (2004), who reported that seed treatment + foliar spray with Vitavax-200 successfully controlled 85.5 to 90.1% seed-borne fungi of *M. phaseolina* and *Fusarium* sp.. Seed-borne infection could be reduced if infected seeds are treated with Oxathiin @ 2.5 g kg^{-1} of seed (Agarwal and Verma1975). Results of the present study reveal that seed treated with Provax + foliar spray with Bavistin 50 WP reduced the prevalence of seed-borne fungi in harvested mungbean seeds and thus improved seed health.

The findings of the present investigation reveal that seed treatment with Provax and three foliar spray of seed crop with Bavistin 50 WP is the best treatment to reduce the seedling infection, incidence and severity of *Cercospora* leaf spot (CLS) and to increase yield. The treatment also reduces the prevalence of seed-borne fungi in harvested seeds and improved seed health.

Table 3. Effect of pre-sowing seed treatment and foliar spray of seed crop with fungicide on yield and health of mungbean seeds

Treatment	Pods number plant^{-1}	Yield (kg ha^{-1})	% seed-borne infection	
			**Aspergillus* spp.	*Fusarium oxysporum*
Seed treatment with Provax	39.73 bcd	1612.08 bc	18.25 b	8.00 b
Seed treatment with Metataf	38.37 cd	1612.36 bc	18.25 b	8.00 b
Seed treatment with Cupravit	38.53 cd	1617.10 bc	18.00 bc	7.58 b
Foliar spray with Dithane M-45	45.45 abcd	1655.9 abc	14.75 def	5.42 c
Foliar spray with Rovral	43.47 abcd	1647.9 abc	16.75 bcd	5.83 c
Foliar spray with Bavistin	47.68 abcd	1766.86 ab	15.92 bed	4.67 cd
Seed treatment with Provax + Foliar spray with Dithene M-45	51.23 a	1752.37 ab	11.42 fg	4.50 cd
Seed treatment with Provax + Foliar spray with Rovral	50.98 ab	1794.59 ab	11.92 efg	4.58 cd
Seed treatment with Provax + Foliar spray with Bavistin	53.33 a	1862.42 a	10.00 g	3.17 e
Seed treatment with Metataf + Foliar spray with Dithene M-45	50.25 ab	1777.36 ab	14.83 cde	4.67 cd
Seed treatment with Metataf + Foliar spray with Rovral	48.03 abcd	1712.55 ab	11.75 efg	4.58 cd
Seed treatment with Metataf + Foliar spray with Bavistin	51.13 ab	1837.13 ab	11.08 g	4.58 cd
Seed treatment with Cupravit + Seed treatment with Cupravit	49.10 abc	1729.30 ab	11.67e fg	4.83 cd
Seed treatment with Cupravit + Foliar spray with Rovral	51.02 ab	1747.25 ab	12.67e fg	4.58 cd
Seed treatment with Cupravit + Foliar spray with Bavistin	51.80 a	1792.18 ab	11.50 efg	3.92 de
Control	37.23 d	1465.13 c	30.00 a	12.67 a
CV%	12.55	9.85	7.67	10.79

*Values within the same column having a common letter(s) do not differ significantly ($P = 0.05$).

* *Aspergillus* spp. includes *A. flavus* and *A. niger*.

LITERATURE CITED

Agarwal, V. K. and Verma, H. S. 1975. Discoloration of seeds. Seed Tech. News. 5: 1-2.

Anonymous. 1999. International Rules for Seed Testing. Seed Sci. and Technol. Proc. Int. Seed Test. Assoc. 173 pp.

Ayub, A., Dey, T. K., Hossain, M. M. and Alam, K. B. 1993. Efficacy of fungicides against leaf spot (*Cercospora cruenta*) of mungbean. Bangladesh J. Plant Pathol. 21: 67-69.

Bakr, M. A. 1991. Management of important disease of major pulses. Advances in pulse research in Bangladesh: Proceedings of the Second National Workshop on Pulse 6-8 June, 1998. ICRISAT, India. pp. 121-125.

Bakr, M. A. 1993. Plant protection of mungbean in Bangladesh. Proceedings of the Seminar on Mungbean in South Asia. New Delhi, India. pp.177-186.

Bakr, M. A. 1994. Checklist of Pulses Disease in Bangladesh, Bangladesh J. Plant Pathol. 10(1& 2): 13-16.

Barua, J. 2004. Control of microflora of farmers stored seeds of mungbean and lentil. M. S. Thesis. Department of Plant Pathol. BAU. 78pp.

Barua, J., Hossain, M. M., Hossain, I., Rahman, A. A. M. S. and Sahel, M. A. T. 2007. Control of mycoflora of farmer's stored seeds of mungbean. Asian J. Plant Sci. 6: 115-121.

Dubey, S. C. and Singh, B. 2005. Integrated management of diseases of curd bean. Indian Phytopathol. 58: 345-346.

Ellis, M. B. 1993. Demataceous hyphomycetes. CAB. International Wallingford Oxen. OXIOBDE UK. pp. 95-98.

Fakir, G. A., Hossain, I., Ahmed, M. U., Asad-ud-Doullah, M. and Alam, M. 2002. Quality of framer's Boro and T. Aman rice seeds collected before sowing from Bogra, Rajshahi and Rangpur districts of Bangladesh. Presented in review of rice seed health improvement (PETRRA) project, held on 21-22 April at BRRI, Gazipur, Bangladesh.

Haque, M. S., Rahman, M. L. and Malek, M. A. 1994. Effect of fungicides and number of spray on cercospora leaf spot of cowpea. Bangladesh J. Plant Pathol. 10(1&2): 3-4.

Huq, M. I., Ali, R. and Ahmed, H. U. 1991. Efficacy of three foliar fungicides in controlling *Cercospora* leaf spot of mungbean. Bangladesh J. Agril. Res. 16(2): 163-165.

Iqbal, S. M., Zubair, M. and Malik, B. A. 1995. Control of *Cercospora* leaf spot of mungbean by foliar fungicides. Pakistan J. Phytopathol. 7(1): 84-85.

Khunti, J. P., Bhoraniya, M. F. and Vora, V. D. 2005. Management of powdery mildew and Cercospora leaf spot of mungbean by some systemic fungicides. Leg. Res. 28(1): 65-67.

Lal, G., Kim, D., Shanmugasundaram, S. and Kalb, T. 2001. Mungbean production. AVRDC. 6 p.

Mathur, S. B. and Kongsdal, O. 2003. Common laboratory seed health testing methods for detecting fungi. DGISP for developing countries, Thorvaldsanvej 57, DK-1871 Frederiksberg, Copenhagen, Denmark. 425 pp.

Mian, M. S., Hossain, M. D. and Jalaluddin, M. 2000. Effect of fungicides and urea on Cercospora leaf spot of mungbean. Bangladesh J. Plant Pathol. 16: 23-26.

Mortuza, M. G., Alam, S. M. K., Reza, M. M. A. and Hossain, M. M. 1995. Effect of seed treatment on fungal infection and Fusarium wilt of chickpea. Bangladesh J. Agric. Res. 27(2): 216-226.

Ramkrishna, A., Gowda, C. L. L. and Johansen. 2000. Management factors affected legumes production in the Indo-Gangetic plain. In: legumes in rice and wheat cropping systems of the Indo-Gangetic plain-constrains and opportunities. pp. 155-165.

Rathore, B. S. 2006. Management of diseases of green gram with fungicides. J. Mycol. Plant Pathol. 36(2): 138-141.

Saifulla, M., Mahes, M., Nagesha, G. K. and Byregoda, M. 2005. Screening of mungbean genotypes against major diseases. Plant Dis. Res. 20:90.

Saraswathi, S., Reddy, A. G. R. and Singh, B. G. 1989. Effect of seed treatment with fungicides and *Rhizobium* spp. on seed germination, nodulation, growth, dry matter, nitrogen content and yield of mungbean. Field Crops Abst. 42(1): 986-990.

Saxena, P. and Tripathi, H. S. 2006. Fungicidal management of cercospora leaf spot of mungbean (*Vigna radiata*). J. Mycol. Plant Pathol. 36(2): 336-337.

Singh, B. R., Sing, M., Yadav, M. D. and Dingar, S. M. 1982. Yield loss in mungbean due to Cercospora leaf spot and yellow mosaic. Sci. and Cult., C. S. Azad University. Agric. and Tech. Kanpur, India. 48(12): 435-436.

Singh, S. K., Gupta, B. R. and Verma, V. S.1994. Combined management of anthracnose and Cercospora leaf spots in mungbean. Indian J. Mycol. and Plant Pathol. 24(2): 139-140.

Talukdar, M. J. 1974. Plant diseases of Bangladesh. J. Agric. Res. 1: 61-86.

IDENTIFICATION OF GARLIC LEAF BLIGHT: FIRST RECORD IN BANGLADESH

M. A. Wadud[1], T. K. Dey[2], M. R. Humauan[3] and B. K. Gowsami[4]

[1]Scientific Officer, Spices Research Center, Shibgonj, Bogra, [2]Director, Pulse Research Center, [3]Scientific Officer, RARS Ishurdi, Pabna, and [4]Director, Planning and Evaluation, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh.

E-mail of corresponding author: wadudbari@yahoo.com

ABSTRACT

M. A. Wadud, T. K. Dey, M. R. Humauan and B. K. Gowsami. 2015. Identification of garlic leaf blight: First record in Bangladesh. Bangladesh J. Plant Pathol. 31 (1&2): 39-41.

Recently, a new disease appeared on garlic plants grown under field conditions of Bangladesh, showing leaf blight like symptoms killing the entire leaves progressively from the tip. An investigation was conducted to identify the disease and its causal pathogen. The disease was identified as *Stemphylium* blight and the causal pathogen as *Stemphylium*. sp. The disease was confirmed through Koch's postulate. This is the first record of the disease in Bangladesh.

Key words: Leaf blight, garlic, *Stemphylium* sp.

Garlic (*Allium sativum* L.) is the second most widely consumed allium in the world. It is one of the major spices in Bangladesh. In the country, annual garlic production area is about 66,000 ha, where the production is 430000 metric tons and at an average yield of 6.55 mt/ha during 2013-2014 crop season (Krishi Diary, 2015). Total production as well as per hectare yield of garlic in Bangladesh is very low compared to other garlic producing countries like China where the production is 59 million metric tons annually with an average yield of 12.3 mt/ha (Krishi Diary, 2015).

In Bangladesh, recently, a new disease appears on garlic plants, showing leaf blight like symptoms killing the leaves progressively from the tip (Fig. 1). It is one of the major diseases of garlic in the country. It seems to be responsible for low production and yield of the crop. In 2015, garlic plants in several commercial fields of Bogra, Natore, Rajshahi and Faridpur districts, and at the research field of Spices Research Centre of Bangladesh Agricultural Research Institute (BARI), Bogra exhibited symptoms of the blight on the garlic leaves. Early symptoms are observed as white spots, 1-3 mm diameter, which enlarges to produce sunken purple lesions, extending whole leaves withered as described by Zheng *et al.* (2008) from China. In China, leaf blight of garlic is caused by different species of *Stemphylium*. However, the causal agent of the leaf blight like disease appears in Bangladesh has not yet been identified.

The present piece of research was undertaken to identify the causal pathogen of garlic leaf blight disease in the country.

The experiment was conducted at Spices Research Centre, BARI, Bogra, Bangladesh during 2014-15 to identify the causal pathogen of garlic leaf blight disease. To isolate the causal fungus associated with garlic leaf blight like symptoms infected leaves of garlic (Fig. 1) were collected from the field and cut into small pieces. The pieces were surface sterilized with 0.5% sodium hypochlorite solution for 1 minute, rinsed with sterilized distilled water for three times, soaked with sterilized filter paper and placed on Petri dishes containing sterilized moist Whitman filter paper No. 2. The leaf pieces in Petri dishes were incubated for 7 days at room temperature (28±2C). After incubation, profuse sporulation of the causal fungus developed on the incubated leaf pieces as observed under a stereo dissecting microscope and identified as *Stemphylium* sp. based on morphological characteristics (Ellis 1971).

To confirm the causal of the fungus of the disease, Koch's postulate was performed by inoculating healthy garlic plants with spore suspension of the isolated fungus. Spores of *Stemphylium* sp. grown in Petri dishes were collected and suspended in 100 ml beaker containing sterile distilled water (Fig. 2). The concentration of spore was more than 70 per microscopic field. Three months old apparently healthy garlic plants grown in earthen pots (Fi9g. 3) were inoculated with the spore suspensions using a camel hair brush under sterile conditions. After inoculation, plants were covered with polyethylene bags to maintain proper humid condition. After 48 hr, the bags were removed and plants were kept under field conditions until symptoms appeared. The blight symptoms on inoculated plants (Fig. 4) were observed after 4 days of inoculation which was similar to the blight symptom appeared in the field (Fig. 1). The fungus was reisolated from lesions of the inoculated plants to complete Koch's postulate.

The results of the Koch's postulate confirmed that leaf blight of garlic occurs in Bangladesh which is caused by *Stemphylium* sp. This is the first record of *Stemphylium* leaf blight disease of garlic in the country. However, Rao and Pavgi (1975) first reported the disease from India. Shishkoff and Lorbeer (1987, 1989) also reported that leaf blight of garlic is caused by *Stemphylium* in the USA. Available reports reveal that the disease is widespread in Asia (Rao and Pavgi 1975, Zeng *et al.* 2008). Recently it has been reported on garlic from South Africa (Aveling and Naude 1992), Spain (Melero 1993) Brazil (Mehta 1998) and Australia (Suheri and Price 2000).

Figure 1. Symptoms of leaf blight of naturally infected garlic caused by *Stemphy-lium* sp.

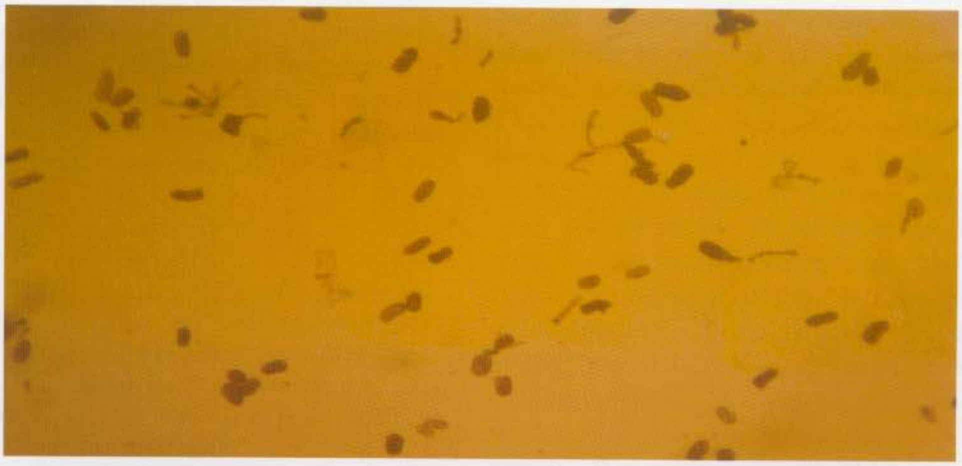

Figure 2. Conidia of *Stemphylium* sp.

LITERATURE CITED

Aveling, T. A. S. and Naude, S. P. 1992. First report of *Stemphylium vesicarium* on garlic in South Africa. Pl. Dis. 76:426.

Ellis, M. B. 1971. Dematiaceous Hypomycete. Kew, London, UK. Commonwealth Mycological Institute, Kew, England.

Krishi Diary. 2015. Agriculture Information Service, Khamarbari, Farmgate, Dhaka-1215. p. 15.

Mehta, Y. R. 1998. Severe outbreak of *Stemphylium* leaf blight, a new disease of cotton in Brazil. Pl. Dis., 82: 333–336.

Melero, E. V. J. M. (1993). First report in Spain of two leaf spots of garlic caused by *Stemphylium vesicarium*. Pl. Dis. 77:952.

Rao, N. N., and Pavgi, M.S. 1975. *Stemphylium* leaf blight of onion. Mycopathologica 56:113–118.

Shishkoff, N. and Lorbeer, J.W. 1987. A new leaf disease of onion in New York caused by *Stemphylium vesicarium*. Phytopathology 77: 1713

Shishkoff, N. and Lorbeer, J. W. 1989. Etiology of *Stemphylium* leaf blight of onion. Phytopathology 79:301-304

Suheri, H., and Price, T.V. 2000. Stemphylium leaf blight of garlic (*Allium sativum*) in Australia. Australian. Plant Path. 29:192–199.

Zheng, L., Huang, J. B., and Hsiang, T. 2008. First report of leaf blight of garlic (*Allium sativum*) caused by *Stemphylium solani* in China. Plant Pathol. 57: 380.

Figure 3. Healthy garlic seedlings which were used for inoculation.

Figure 4. Symptoms of *Stemphylium* leaf blight of garlic development after 4 days of inoculation.