

Efficacy of Some Selected Copper Fungicides Against Seed Borne Bacteria of Maize

¹K. Roy, ²N. Sultana, ²N. Akhter, ³M.Z.R. Bhuiyan and ⁴Karima Akter

¹MS Student, Department of Plant Pathology, Sher-e-Bangla Agricultural University,
Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh

²Department of Plant Pathology, Sher-e-Bangla Agricultural University,
Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh

³Department of Plant Pathology, Sher-e-Bangla Agricultural University,
Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh

⁴Quarantine Pathologist (Rules, Policy and Laboratory), Plant Quarantine Wing, DAE, Bangladesh

Abstract: The experiment was carried out at the Seed Pathology Laboratory and Plant Disease Diagnostic Laboratory of Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh during January-August 2012 to determine the efficacy of some selected Copper fungicides against seed borne bacteria of maize variety NK-40. Three bacterial genera viz. *Acidovorax* sp., *Burkholderia* sp. and *Ralstonia* sp. were isolated from maize seeds. Moreover, three Cu-fungicides viz. Sulcox 50WP, Champion 50WP and Cupravit 50WP were used against these bacteria. Effects of fungicides were studied using nutrient agar plate method, water agar test tube method and rolled paper towel method. Bioassay of these fungicides against different bacteria were done and observed that Sulcox 50WP @ 0.3%, Champion 50WP @ 0.4% and Cupravit 50WP @ 0.3% showed remarkable inhibition zone against *Acidovorax* sp., *Burkholderia* sp. and *Ralstonia* sp., respectively. In nutrient agar plate method, germination varied from 94.83%-98.67%, where the effect of Cu-fungicides was insignificant. In water agar test tube method, the highest number of normal seedlings (69.0%) were recorded when seeds were treated with Cupravit 50WP @ 0.3% and the lowest number of normal seedlings were recorded in control (44.0%). The lowest number of abnormal seedlings and diseased seedlings were recorded when the seeds were treated with Cupravit 50WP @ 0.3%. In rolled paper towel method, Cupravit 50WP @ 0.3% showed best performance regarding germination, shoot length, root length and vigor index. The vigor index varied from 885.50-2488.40, where the highest count was recorded in Cupravit 50WP @ 0.3% and lowest in control.

Key words: Efficacy • Seed borne bacteria • Copper fungicides • Maize

INTRODUCTION

Maize (*Zea mays* L.) is one of the most leading cereal crops in the world next to wheat and rice [1,2]. Its cultivation covering an area of 3.12 lakh ha of land and total production of about 21.78 lakh tones [3]. In Bangladesh, it has good potential as a cereal crop due to its low cost of production, wide adaptability and diversified uses. Average yield ha⁻¹ which is lower as compared to production of developed countries of the world. Annual national requirement approximately 2.5 million tones but only 24% maize area is covered by our

own seed. Many factors involved in yield loss of maize of which environmental conditions, genetical variation of hybrids, antagonistic action of different diseases, insects and some nutritional scarcity are important points of consideration [4]. Among these factors, diseases play a significant role and seed borne diseases create a great threat to the production of maize in Bangladesh. As many as 490 seed borne diseases are known to attack 756 different crop plants in Bangladesh [5] and maize suffers from 28 diseases in seedling stage [6] among them 11 are seed borne in nature [7]. On the other hand a total of 112 diseases are known to occur in maize crops [8] and among

them more than 70 are seed borne. Important seed borne diseases of maize are leaf spot, leaf blight, collar rot, kernel rot, scutellum rot, seedling blight, anthracnose and head smut [9]. Maize seeds are infected by three major category of pathogens namely fungi, bacteria and viruses that affect seed health and quality [10]. Seed treatment with chemicals is the best way to keep good seed health condition. Bacterial disease can be removed by using some antibiotics and some Cu-fungicides. During the last five years, several antibiotics and synthetic compounds have been developed for disease control. Copper oxychloride, Cupravit performed well against bacterial leaf blight of rice with 43.25% and 48.19% disease incidence and gave the highest yield of 3.63 and 3.58 t/ha, respectively [11]. Streptomycin, deoxy, dehydro streptomycin, streptocycline, choramphenicol and cellocidin are commonly used antibiotics to control bacterial plant pathogens [12]. On the other hand, when the Cu-fungicides are used in combination with other fungicides or antibiotics, they also give good results. In Bangladesh, little or no attention has been given yet for the management of seed and seedling diseases. From this point of view, the present study has been undertaken to determine the efficacy of some selected Cu-fungicides against seed borne bacteria of hybrid maize and their transmission behavior from seed to seedling of hybrid.

MATERIALS AND METHODS

The experiment was conducted at the Seed Pathology Laboratory and Plant Disease Diagnostic Laboratory of Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh during January-August 2012. Hybrid maize variety NK-40 was used in this experiment. Blotter method and nutrient agar plate method were used for the detection of seed borne bacteria. The collected seed samples of maize were analyzed for the presence of major seed borne bacterial pathogens following the rules of ISTA [13] by agar plate method. In this method, 400 seeds were tested and the plated seeds were usually incubated for 7 days at 30°C under 12hrs alternating cycles of light and darkness. After incubation, bacterial ooze that come out from incubated seeds were examined and streaked on new Nutrient Agar (NA) plates to get single colony. Isolated bacteria were purified by streaking using a wire loop, incubated at 30°C for 48 hours. Identification of bacteria was done based on different biochemical characteristics and salt tolerant test. After purification the bacteria streaked on NA slants and incubated for 48 hours. Then these slants were kept in refrigerator at 4°C as stock culture.

Several biochemical tests were done following Bergey's manual of determinative bacteriology such as KOH test [14], gram reaction, oxidase test, catalase test, citrate utilization test, pectolytic test and gelatin liquefaction tests were done. For pathogenecity test, the pseudostem is inoculated by injecting bacterial cell suspension 10^8 cfu/ml using a syringe and kept in a net house and observed upto 7 days of inoculation. After the appearance of symptoms, the bacteria were re-isolated by using dilution plate technique. To determine the efficacy of Cu-fungicides against seed borne bacteria each of the isolate grown in test tube in shaker incubator at 150 rpm and incubated at 30°C for 48 hrs. After 24 hours of growth, nutrient agar plates were swabbed with bacterial isolate. Wells, 4 mm in diameter were punched into the NA medium and different concentration of Cu-fungicides were added in the wells. The plates were then incubated at 30°C upto 4 days of incubation and the inhibition zone around the wells measured and recorded. 400 seeds were treated with Sulcox 50WP @ 0.3%, Champion 50WP @ 0.4% and Cupravit 50WP @ 0.3% by dipping for one hour. Then the dried seeds were tested in water agar test tube method [15] and rolled paper towel method [16]. The recorded data were analyzed by using MSTAT-C software. The difference between the treatment means were judged by Least Significance Difference (LSD) Test following the procedure as described by [17].

RESULTS

Three bacterial genera were identified from maize variety NK-40 viz. *Acidovorax* sp., *Burkholderia* sp. and *Ralstonia* sp. (Fig. 1a,b,c). Bacterial incidence on seeds of hybrid maize variety NK-40 were varied from 1.25 to 2.25%, where *Acidovorax* sp. incidence was 2.25% and incidence of *Burkholderia* sp. and *Ralstonia* sp. were 1.5% and 1.25%, respectively (Table 1).

Biochemical test of isolated bacteria were done and the results were showed in Table 2. In gram staining, all three genera were gram negative. Colonies on YDC (differential) medium, *Acidovorax* sp. produced dark beige color, *Burkholderia* sp. produced purple color and *Ralstonia* sp. produced beige to light brown color. These genera were catalase positive. In starch hydrolysis test, these genera showed positive results. In oxidase test, color was changed into blue within 30 seconds. After incubation with gelatin liquification, tubes were kept in the refrigerator for 20 minutes and liquification observed. In citrate utilization test and pectolytic test, the results were positive. In case of motility test, bacteria grew away from the stab line and indicated that the bacteria were motile.

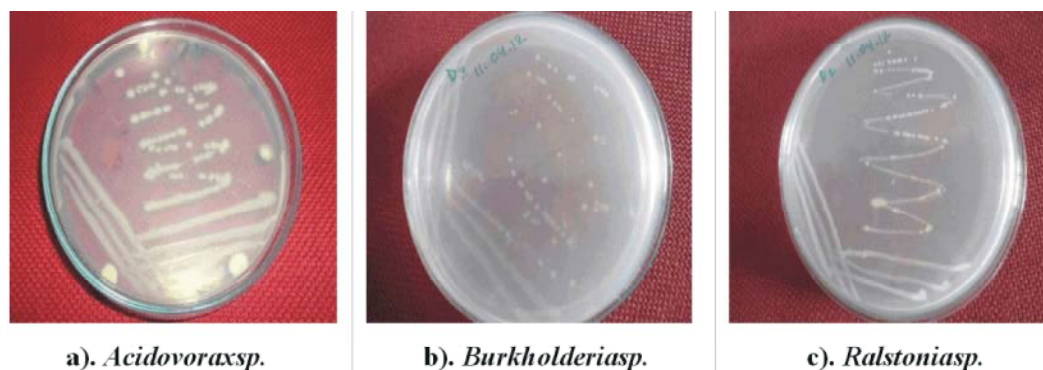


Fig. 1: Pure culture of maize seed borne bacterial genera on NA medium.

Table 1: Prevalence of seed borne bacteria of hybrid maize variety NK-40 in agar plate method.

Bacteria	Incidence (%)
<i>Acidovorax</i> spp.	2.25
<i>Burkholderia</i> spp.	1.5
<i>Ralstonia</i> spp.	1.25

Table 2: Tests used to differentiate genera of plant pathogenic prokaryotes that found on the seeds of hybrid maize variety NK-40.

Tests	Isolated bacteria		
	<i>Acidovorax</i> sp	<i>Burkholderia</i> sp	<i>Ralstonia</i> sp
Gram staining	-	-	-
Colonies color on YDC	dark beige	purple	beige to light brown
Catalase test	+	+	+
Starch hydrolysis	+	+	+
Oxidase test	+	+	+
Gelatin liquification test	+	+	+
Citrate utilization test	+	+	+
Pectolytic test	+	+	+
Motility test	+	+	+

Table 3: Growth observation of different isolate of hybrid maize variety NK-40 seeds at 1-7% NaCl nutrient broth (salt tolerance test).

Isolates	Concentration						
	1%	2%	3%	4%	5%	6%	7%
<i>Acidovorax</i> sp.	++	+++	-	-	-	-	-
<i>Burkholderia</i> sp.	+	++	++++	-	-	-	-
<i>Ralstonia</i> sp.	++	+++	-	-	-	-	-

Table 4: Efficacy of Cu-fungicides on seedlings of hybrid maize variety NK-40 by nutrient agar plate method.

Treatments	% Germination	% Seed Infection	% Dead Seed
Control	94.83	3.88 a	2.03 b
Sulcox 50 WP @ 0.3%	97.58	3.25 b	1.28 c
Champion 50 WP @ 0.4%	98.13	2.97 b	2.28 a
Cupravit 50 WP @ 0.3%	98.67	2.23 c	1.40 c
LSD _(0.05)	NS	0.429	0.141
CV%	2.25	6.97	4.12

In column, means containing same letter indicate significantly similar under LSD at 5 % level of significance.

Table 5: Efficacy of Cu-fungicides on seedlings of hybrid maize variety NK-40 on germination (%), normal and abnormal seedling (%), diseased seedling (%) and dead seed (%) (water agar test tube method).

Treatments	Germination (%)	Normal seedlings (%)	Abnormal seedlings (%)	Diseased seedlings (%)	Dead seed (%)
Control	85.33 b	44.67 d	34.67 a	13.00 a	7.07 a
Sulcox 50 WP @ 0.3%	89.00 ab	56.67 c	29.33 b	9.00 b	4.80 c
Champion 50 WP @ 0.4%	91.67 a	62.33 b	25.67 b	8.00 c	2.96 d
Cupravat 50 WP @ 0.3%	93.00 a	69.00 a	20.00 c	5.00 d	6.00 b
LSD _(0.05)	5.212	5.577	3.723	0.502	0.712
CV (%)	2.91	4.85	6.80	2.86	6.84

In column, means containing same letter indicate significantly similar under LSD at 5 % level of significance.

Table 6: Efficacy of Cu-fungicides on seedlings of hybrid maize variety NK-40 on germination %, shoot length, root length and vigor index (Rolled paper towel method).

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Vigor index
Control	91.00 b	5.72 d	4.00 c	885.50 d
Sulcox 50 WP @ 0.3%	92.83 b	9.16 c	6.33 b	1438.40 c
Champion 50 WP @ 0.4%	97.17 a	12.06 b	11.43 a	2281.20 b
Cupravat 50 WP @ 0.3%	98.67 a	13.97 a	11.24 a	2488.40 a
LSD _(0.05)	3.811	0.778	1.630	6.258
CV (%)	2.01	3.81	9.89	5.32

In column, means containing same letter indicate significantly similar under LSD at 5 % level of significance.

Table 7: Efficacy of Cu-fungicides on *Acidovorax* sp. of hybrid maize variety NK-40 at different days after incubation.

Treatments	Inhibition zone after 24 hrs (cm)	Inhibition zone after 48 hrs (cm)	Inhibition zone after 72 hrs (cm)	Inhibition zone after 96 hrs (cm)
Control	0.00 d	0.00 c	0.00 c	0.00 c
Sulcox 50 WP @ 0.3%	1.77 a	1.70 a	1.67 a	1.60 a
Champion 50 WP @ 0.4%	1.07 c	1.23 b	1.23 b	1.03 b
Cupravat 50 WP @ 0.3%	1.43 b	1.63 a	1.90 a	1.70 a
LSD _(0.05)	0.203	0.313	0.3362	0.176
CV (%)	6.20	9.37	9.40	5.29

In column, means containing same letter indicate significantly similar under LSD at 5 % level of significance.

Table 8: Efficacy of Cu-fungicides on *Burkholderia* sp. of hybrid maize variety NK-40 at different days after incubation.

Treatments	Inhibition zone after 24 hrs (cm)	Inhibition zone after 48 hrs (cm)	Inhibition zone after 72 hrs (cm)	Inhibition zone after 96 hrs (cm)
Control	0.00 c	0.00 c	0.00 c	0.00 c
Sulcox 50 WP @ 0.3%	1.36 b	1.47 b	1.47 b	1.53 b
Champion 50 WP @ 0.4%	1.57 b	1.58 b	1.77 b	1.67 b
Cupravat 50 WP @ 0.3%	1.97 a	2.07 a	2.17 a	2.23 a
LSD _(0.05)	0.344	0.313	0.359	0.259
CV (%)	8.99	8.31	8.95	6.24

In column, means containing same letter indicate significantly similar under LSD at 5 % level of significance.

Table 9: Efficacy of selected Cu-fungicides on *Ralstonia* sp. of hybrid maize variety NK-40 at different days after incubation.

Treatments	Inhibition zone after 24 hrs (cm)	Inhibition zone after 48 hrs (cm)	Inhibition zone after 72 hrs (cm)	Inhibition zone after 96 hrs (cm)
Control	0.00 c	0.00 c	0.00 c	0.00 c
Sulcox 50 WP @ 0.3%	1.30 a	1.27 a	1.20 b	1.13 b
Champion 50 WP @ 0.4%	1.10 b	1.20 ab	1.20 b	1.23 b
Cupravat 50 WP @ 0.3%	1.17 ab	1.17 b	1.37 a	1.67 a
LSD _(0.05)	0.143	0.072	0.160	0.304
CV (%)	5.61	2.75	5.30	9.92

In column, means containing same letter indicate significantly similar under LSD at 5 % level of significance.

Salt Tolerance test on Nutrient Broth: All the isolates of the bacteria grew well at 2% salt stress. But *Acidovorax* sp. and *Ralstonia* sp. failed to grow at 3-7%. In case of *Burkholderia* sp. grew well at 3% salt stress, but failed to grow at 4-7% salt stress (Table 3).

Efficacy of Cu-fungicides against seed borne bacteria of hybrid maize variety NK-40 was determined by nutrient agar plate method (Table 4). The germination is varied from 94.83 to 98.67%, where numerically the lowest germination (94.83%) was recorded in control and the highest (98.67%) from the seeds treated with Cupravit 50WP @ 0.3% followed by Champion 50WP @ 0.4% and Sulcox 50WP @ 0.3%. The highest seed infection (3.88%) was recorded in control and the lowest (2.23%) for Cupravit 50WP @ 0.3%. The highest number of dead seed (2.03%) was recorded in control and the lowest (1.28%) for seeds were treated with Sulcox 50WP @ 0.3%.

The effect of Sulcox 50WP @ 0.3%, Champion 50WP @ 0.4% and Cupravit 50WP @ 0.3% on percent germination, number of normal seedlings, number of abnormal seedlings, number of diseased seedlings and percent dead seeds of hybrid maize variety NK-40 was tested by water agar test tube method (Table 5). The lowest germination (85.33%) was recorded in control and the highest (93.0%) was recorded from the seeds treated with Cupravit 50WP @ 0.3% followed by Champion 50WP @ 0.4% and Sulcox 50WP @ 0.3%, which showed 89.0% and 91.67% germination, respectively. The highest number of normal seedlings (69.0%) was observed when the seeds treated with Cupravit 50WP @ 0.3% and the lowest (44.67%) was observed in control. The effect of Sulcox 50WP @ 0.3% and Champion 50WP @ 0.4% were statistically identical, where the number of abnormal seedlings were recorded 29.33% and 25.67%, respectively. The highest diseased seedlings (13.0%) were recorded in control and the lowest (5.0%) was recorded when the seeds treated with Cupravit 50WP @ 0.3%. The highest number of dead seed (7.07%) was recorded in control and the lowest (2.96%) was observed from Champion 50WP @ 0.4%.

Efficacy of Sulcox 50WP @ 0.3%, Champion 50WP @ 0.4% and Cupravit 50WP @ 0.3% on Shoot length, vigor index, percent germination and root length of hybrid maize variety NK-40 are presented (Table 6). The effect of Champion 50WP @ 0.4% and Cupravit 50WP @ 0.3% were statistically similar, where the germination were 97.17% and 98.67%, respectively followed by Sulcox 50WP @ 0.3% and control. Significantly, the highest root length (13.97 cm) was observed Cupravit 50WP @ 0.3% treated seeds followed by Champion 50WP @ 0.4% (12.06

cm) and the lowest (5.72 cm) was recorded in control. The highest shoot length (11.43 cm) was recorded in Champion 50WP @ 0.4% treated seeds which was statistically identical with Cupravit 50WP @ 0.3% (11.24 cm) followed by Sulcox 50WP @ 0.3% (6.33 cm) and lowest (4.00 cm) was recorded in control. The highest vigor index (2488.40) was recorded from Cupravit 50WP @ 0.3% treated seeds followed by Champion 50WP @ 0.4% (2281.20) and the lowest (885.50) was found from control.

Comparative Efficacy of Various Fungicide on different Bacteria of Hybrid Maize: Significant variation was observed among the fungicides used against *Acidovorax* sp. (Table 7). At 24 hrs of incubation, the highest inhibition zone (1.77 cm) was recorded in Sulcox 50 WP @ 0.3% followed by Cupravit 50WP @ 0.3% (1.43 cm). At 48, 72 and 96 hrs of incubation, the highest inhibition zone was recorded in Sulcox 50 WP @ 0.3% and Cupravit 50WP @ 0.3%, which was statistically identical to Champion 50WP @ 0.4%. Significant variation was also observed among the fungicides used against *Burkholderia* sp. At 24 hrs of incubation, the highest inhibition zone (1.97 cm) was found in Cupravit 50WP @ 0.3% followed by Sulcox 50WP @ 0.3% and Champion 50WP @ 0.4%, which was statistically identical. At 48, 72 and 96 hrs, the highest inhibition zone was recorded by Cupravit 50WP @ 0.3% followed by Champion 50 WP @ 0.4% and Sulcox 50WP @ 0.3%, which was statistically identical (Table 8). At 24 hrs of incubation, the highest inhibition zone of *Ralstonia* sp. (1.30 cm) was recorded by Sulcox 50WP @ 0.3% followed by Cupravit 50WP. At 48 hrs of incubation, the highest inhibition zone (1.27 cm) was recorded by Sulcox 50WP 0.3% followed by Champion 50WP. At 72 and 96 hrs of incubation, the highest inhibition zone was recorded by Cupravit 50WP @ 0.3%, followed by Champion 50WP @ 0.4% and Sulcox 50WP @ 0.3% (Table 9).

DISCUSSIONS

In this study, the seed health test was conducted by blotter method and revealed three species of seed borne bacteria viz. *Acidovorax* sp., *Ralstonia* sp. and *Burkholderia* sp. The CIMMYT reported the seed borne bacteria of maize and the pathogens were *Acidovorax avenae* sp. *avenae*, *Burkholderia andropogonis*, *Clavibacter michiganensis* sp. *nebraskensis*, *Erwinia chrysanthemi* pv. *zetae*, *Pantoea stewartii* and *Pseudomonas syringae* pv. *lapsea*. [18] reported that *Burkholderia* spp. was found on sorghum, corn, mucuna, trifolium, dolichos, vicia. Similar findings were also

reported by [19], Yuan [20]. Tahat and Sijam [21] studied on *Ralstonia* spp. (race 3 biovar 2) and stated that *Ralstonia* spp. is a bacterial wilt causal agent of many plant species and infect potatoes, eggplant, peppers, tomatoes, ginger, corn and a few weed species including bittersweet, nightshade and stinging nettle. *Acidovorax* sp. and *Ralstonia* sp. grew well at 2% salt stress and *Burkholderia* sp. grew well at 3% salt stress. Similar findings are also reported by Schaad *et al.* [22], Cowan [23]. In Nutrient agar plate method, the incidence of *Acidovorax* sp., *Burkholderia* sp. and *Ralstonia* sp. was 2.25%, 1.5% and 1.25%, respectively.

There were three Cu-fungicides viz. Sulcox 50WP, Champion 50WP and Cupravit 50WP. Among the treatments, the effect of seed treatment with different Cu-fungicides on germination, seed infection, normal seedlings, abnormal seedlings, dead seed and vigor index were recorded and Cupravit 50WP @ 0.3% performed the best result. Ritchie [24] reported on the bacterial spot pathogen is sensitive to Copper. There are many factors that affect the efficacy of copper in controlling bacterial plant pathogens. Ellis and Bradley [25] studied about Copper fungicide formulations were available to organic growers that can effectively kill fungi and bacteria. Milijasevic *et al.* [26] tested three Copper-based compounds against *Clavibacter michiganensis* sp. *michiganensis* in tomato seedlings and observed Cu-hydroxides were the most prominent in reducing bacterial population where Mancozeb did not improve the effects. Spraying with Cu-fungicides alternatively with Streptomycin (250 ppm) was found effective in controlling bacterial leaf blight of rice [27]. Islam *et al.* [28] used Streptomycin sulphate, Thiovit 80WP, Sulfuric acid, Dithane M-45 and Cupravit either alone or in combination for controlling bacterial blight and on yield of cotton and observed highest germination (86.31%) when seed treatment with Streptomycin sulphate (0.15%) and foliar spray with Cupravit (0.2%) + Streptomycin sulphate (150 ppm). The lowest disease index (21.24%) was found in that treatment subsequently after three foliar sprays at 104 DAS. This treatment reduced the disease intensity and increased the yield of seed cotton with 26.02%.

CONCLUSIONS

From the findings, it may be concluded that Sulcox 50WP, Champion 50WP and Cupravit 50WP has promising potentiality in controlling different seed borne bacteria of maize with increasing seedling stand. In the world, Cu-fungicides has bright prospect for controlling bacterial diseases of maize.

REFERENCES

1. Aldrich, S.R., W.O. Scott and E. R. Leng, 1975. Modern corn production. 2nd edition. United States of America. pp: 1-5.
2. Achakzai, A.K.K. and Z.A. Bazai, 2007. Effect of water stress on seedling growth of maize cultivars: In mannitol plus culture solution. Int.J. Biol. Biotech., 4(1): 37-42.
3. DAE, 2012-2013. Annual report on maize production in Bangladesh. pp: 46-47.
4. Leon, C.D., 1984. Maize disease a guide for field identification. Maize program. CIMMYT. pp: 2-84.
5. Fakir, G.A., 2000. An annotated list of seed-borne disease in Bangladesh. Seed Pathology, Bangladesh Agricultural University, Mymensing.
6. Bari, M.A. and M.S. Alam, 2004. Major diseases of wheat and maize and their control. A Bengali Booklet published from the Division of Plant Pathology, BARI, Joydebpur, Gazipur, 2: 12-16.
7. Fakir, G.A., 2001. List of seed-borne diseases of important crops occurring Bangladesh. Seed Pathology Lab., Dept. of Plant Pathology, Bangladesh Agricultural University, Mymensing.
8. USDA, 1960. Index of maize diseases in the United States. Agricultural Hand book No. 165: pp: 531.
9. Richardson, M.J., 1990. An Annotated List of Seed-Borne Diseases. 4th Edn., The International Seed Testing Association, Zurich, Switzerland.
10. Avinder, R. and B.K. Rai, 1991. Seed mycoflora of *Zea mays* in tribal area. Indian Phytopathol., 44(4): 526.
11. Khan, T.Z., S. Yasin, M. Ayub, J. Anwar and M. Ahmad, 2005. Effect of different chemicals and antibiotics on BLB (*Xanthomonas oryzae*) of rice. Proction International Seminer Rice. Rice Research Institute, Kala Shah Kaku. pp: 275-278.
12. Tagami, U. and S. Yoshimura, 1967. Forecasting and control of bacterial leaf blight of rice in Japan. Protection Syrnpon Tropical Agriculture Research. Trop. Agric. Res. Series No. 1, Ministry of Agriculture and Forestry, Japan.
13. ISTA, 1996. International Rules for Seed Testing. Rules Amendments. Seed Sci. and Tech., 24: 39-42.
14. Suslow, T.V., M.N. Schroth and M. Isaka, 1982. Application of a rapid method for Gram differentiation of plant pathogenic and saprophytic bacteria without staining. Phytopathol., 72: 917-918.
15. Khare, M.N., S.B. Mathur and P. Neergaard, 1977. A seedling symptom test for detection of *Septorianodorum* in wheat. Seed Sci. Tech., 5: 613-617.

16. Warham, E.J., 1990. Effect of *Tilletia indica* infection on viability, germination and vigor of wheat seed. Plant Dis., 74: 130-135.
17. Gomez, K.A. and A.A. Gomez, 1984. Statistical procedure for agricultural research. Second Edn. Intl. Rice Res. Inst., John Wiley and Sons. New York. pp: 1-340.
18. Hebbbar, K.P., A.G. Davey, J. Merrin, T.J. McLoughlin and P.J. Dart, 1992. *Pseudomonas cepacia*, a potential suppressor of maize soil-borne diseases-seed inoculation and maize root colonization. Soil Biol. Biochem., 24: 999-1007.
19. Goto, T., K. Nishiyama and K.I. Ohata, 1987. Bacteria causing grain rot of rice. Ann. Phytopathol. of Soc. Jap., 53: 141-149.
20. Yuan X., 2004. Identification of bacterial pathogens causing panicle blight of Rice in Louisiana. MS Thesis. The Department of Plant Pathology and Crop Physiology. Louisiana. pp: 1-97.
21. Tahat, M.M. and K. Sijam, 2010. *Ralstonia* spp.: The bacterial wilt causal agent. Asian J. of Plant Sci., 9: 385-393.
22. Schaad, N.W., J.B. Jones and W. Chun, 2000. Laboratory guide for identification of plant pathogenic bacteria. 3rd edition. APS Press. St. Paul, Minnesota. pp: 78.
23. Cowan, S.T., 1974. Cowan and Steel's Manual for the Identification of Medical Bacteria 2nd edition. S. T. Cowan. Cambridge University Press, Great Britain.
24. Ritchie, D., 2004. Copper-containing fungicides/bactericides and their use in management of bacterial spot on peaches. Plant Dis., 4: 731-734.
25. Ellis, B.W. and F.M. Bradley, 1992. The Organic Gardener's Handbook of Natural Insect and Disease Control. Rodale Press. Emmaus, PA. pp: 534.
26. Milijasevic, S., B. Todorovic, I. Potocnik, E. Rekanovic and M. Stepanovic, 2009. Effects of Copper-based Compounds, Antibiotics and a Plant Activator on Population Sizes and Spread of *Clavibacter michiganensis* subsp. *michiganensis* in Greenhouse Tomato Seedlings. Pestic. Phytomed. (Belgrade). 24: 19-27.
27. Ahmad, M., M. Akhtar and M. Anwar, 2005. Basmati rice: Progress, issues and prospects for Pakistan. Protection International Seminar Rice, Oct 2-3, 2005. Rice Research Institute, Kala Shah Kaku. pp: 1-7.
28. Islam, M.Z., K.M. Khalekuzzaman, G. M. M. Rahman, M.T. Islam and M.H. Mosharraf, 2003. Effect of chemicals in controlling bacterial blight of cotton. Asian J. of Plant Sci., 2: 539-543.