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Biochemical Characterization of *Xanthomonas axonopodis* pv. *malvacearum* Isolated from Infected Cotton Plant and It's *in vitro* Sensitivity against Some Selected Chemicals

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Authors' contributions

This work was carried out in collaboration between all authors. Author NS designed the study, performed the statistical analysis and wrote the protocol. Author SS carried out the research and wrote the first draft of the manuscript. Authors NS and FMA supervised the study and managed the analyses of the study. Author SS managed the literature searches. Author FMA edited and corrected the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of our study is to isolate, identify and pathogenicity test of *Xanthomonas axonopodis* pv. *malvacearum* (*Xam*) causing bacterial blight of cotton and to determine the comparative efficacy of some selected chemicals and antibiotic in controlling *Xam in vitro.*
Place and Duration of the Study: This study was carried out at the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh from January, 2012 to December, 2013.
Methodology: *Xanthomonas axonopodis* pv. *malvacearum* was isolated from infected leaves, stems, branches, cotyledons and bolls of cotton. Biochemical characterization and *in vitro* sensitivity

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were evaluated as per the standard methods as described in materials and methods.
Results: The bacterium was gram negative, rod shaped and showed positive results in KOH solubility, starch hydrolysis, catalase, citrate utilization, motility indole ureas agar (MIU), gelatin liquefaction test and oxidase test. It produced, slightly raised, blond to bright yellow colour, mucoid colonies on NA medium; circular, flattened or slightly raised, yellow to bright yellow colour, mucoid colonies on YDCA medium and light yellow, mostly circular, slightly flattened on SX medium. *In vitro* evaluation of selected chemicals revealed that Streptomycin sulphate was highly effective against *Xam.* In *in vitro* condition, the highest inhibition zone (35.33, 36.17, 35.17, 33.5 and 32.33 mm after 24, 48, 72, 96 and 120 hrs., respectively) was observed in Streptomycin sulphate @ 0.15% treated plates.
Conclusions: Streptomycin sulphate showed higher effectivity against *X. axonopodis* pv. *malvacearum* compared with selected fungicides.

Keywords: Xanthomonas axonopodis pv. *malvacearum; cotton; biochemical characterization; in vitro test; chemicals; antibiotics.*

1. INTRODUCTION

Bangladesh, the second largest apparel producer after China, is also the third raw cotton importer in the world. The Cotton Development Board has set a target of production of 670,000 bales of cotton by 2015 to reduce import of cotton, the basic raw material for the textile sector [1]. Annual requirement of raw cotton for local textile industry would be 4.0 million bales in future. Country's present cotton production is around 1.0 lakh 29 thousand bales which is a small portion of the total annual requirement of local spinning mills [2]. There are many constraints of production of cotton in Bangladesh. Among them disease is the most serious one [3]. More than 60 diseases were recorded in cotton [4]. Of the major diseases, Bacterial Blight (BB) caused by *Xanthomonas axonopodis* pv. *malvacearum* is an important and destructive disease of cotton affecting yield and fiber quality seriously [5]. In the United States this pathogen caused 73,000 bales loss as a result of foliar and stem phase of bacterial blight [6]. Exact losses caused by this disease are not known in Bangladesh. Bacterial blight is a common disease affecting the growth, development and yield of cotton [7]. The disease affects all the aerial parts of plant and known as angular leaf spot, vein blight, black arm and boll rot depending on the plant part infected [8,9]. Bacterial blight severity is higher at high ambient temperatures (86-97°F) and high relative humidity conditions. The pathogen spread most effectively by splashing water, particularly rain water. Bacteria can enter the plant through natural openings like stomata, nectarines, or through wounds. In Bangladesh for controlling bacterial blight, farmers are using copper fungicides (Cupravit 50 WP), Diathane M-45, and sulfer but not able to manage the disease properly due to the systemic infection nature of the bacteria [10]. That is why we are trying to test the sensitivity of bacteria against the antibiotics as well as against the existing chemicals. Adjustment of sowing date, regular spraying of antibiotics and fungicides, flooding, sanitation etc. may reduce the incidence of the disease [11]. No research work has been critically done in Bangladesh on biochemical characterization, *in vitro* sensitivity of the pathogen, progressive symptoms development, control measures etc. [3]. The present research work was therefore carried out biochemical characterization of *X. axonopodis* pv. *malvacearum* and it's sensitivity against some selected chemicals.

2. MATERIALS AND METHODS

2.1 Isolation, Identification and Characterization of Causal Organism

2.1.1 Isolation

Infected leaves were collected from experimental field of Cotton Research, Training and Seed Multiplication Farm, Sreepur, Gazipur (24°12´N 90°28´E). Lesions with green healthy portion of diseased leaves were cut into small pieces. Surface sterilized with 5% sodium hypochlorite solution for 2-3 minutes and washed them three times with sterile water. After surface sterilization, the cut pieces were kept for 30 minutes in 2 ml of sterile water for bacterial streaming (moving out of bacterial populations from the affected leaf tissues after the tissues served in water). One ml of this stock solution was transferred with the help of sterile pipette into the second test tube containing 9 ml sterile water and shaken thoroughly resulting 10^{-1} dilution. Similarly, final

dilution was made up to 10^{-4}. After preparing different dilution, 0.1 ml of each dilution was spreaded over plate containing Nutrient Agar (NA) medium (bacto agar- 15 g/L, peptone- 5 g/L and beef extract- 3 g/L). For each dilution, three replicated plates were prepared as described by Goszczynska and Serfontein, [12]. Inoculated plates were incubated at 30°C. The plates were observed after 24 hrs and 48 hrs. Then single colony grew over NA plate was restreaked on another plate (NA) with the help of a sterile loop to get pure colony. After purification, bacterium was streaked over NA slant and kept in incubator at 30°C for 48-72 hrs. Finally the culture tubes were kept at refrigerator at 4°C for further use.

2.1.2 Identification and characterization

Gram's Staining and KOH solubility test were performed to know the Gram reaction nature of the strain of the bacteria.

2.1.3 Biochemical characters

Different biochemical chemical tests such as starch hydrolysis, citrate utilization test, motility indoleureas agar (MIU) test, catalase test, oxidase test, pecteolytic test, gelatin liquefaction test and salt tolerance test were performed as per the standard methods [13,14].

2.1.4 Cultural characters

Most of pathovars of *Xanthomonas* can be differentiated by growth and colony morphology on different media [13]. Growth characteristics of the pathogen were studied by using various differential, selective and semi-selective media. The bacterium was inoculated over NA, YDCA and SX medium and incubated at 30°C for at least 24 hrs and observed the colony characters.

2.2 Tobacco Hypersensitivity Reaction

To determine the pathogenic nature of the isolates, hypersensitivity reaction was performed on tobacco (*Nicotiana rustica*) plants by injection infiltration technique [15]. An aliquot of the inoculums suspension of bacterium (approximately 10^8 cfu per ml) was prepared in sterile distilled water from a 24 hours culture plate. Then the lower surface of a mature tobacco leaf was infiltrated by pressing a syringe containing the suspension against the leaf and distilled water was used as a negative control. Then it was observed for 72 hours.

2.3 Pathogenicity Test

Cotton plants (CB-9) were grown on pots under nethouse condition to test pathogenicity of *X. axonopodis* pv. *malvacearum* following standard method [16]. Bacterial cells were grown overnight in NA broth and resuspended in sterile distilled water to a concentration of approximately 10^8 cfu per ml. An aliquot of the inoculum suspension was injected forcedly into the lower surface of the leaf using a sterile syringe and distilled water was used as negative control. Inoculated plants were maintained at field temperature and were observed for 15 days. Data based on visual symptoms were recorded. *X. axonopodis* pv. *malvacearum* was reisolated from the infected plants and confirmation test was performed.

2.4 *In vitro* Evaluation of Some Selected Chemicals against the Bacteria

In vitro evaluation of some selected chemicals (Table 1) against the bacterium was done by well diffusion method to measure inhibition zone [17]. Cotton swabs on wooden applicator sticks were prepared. The swabs were kept in a dry tube and were sterilized in the autoclaved [18]. Two test tubes, each containing 10 ml nutrient broth, were taken and inoculated with 48 hours old pure culture of bacteria grown on NA plates. Another test tube containing 10 ml nutrient broth was taken as control. Test tubes were transferred in shaker incubator maintaining 30°C temperature and 150 rpm for 24 hrs. After shaking, the broth culture was spreaded uniformly on NA plate with the help of sterile cotton swabs. One hole of 4 mm in diameter was punched into the same

Table 1. Selected chemicals, their trade name, active ingredient and concentration used in bioassay

Trade name	Active ingredient	Chemical name	Concentration (%)	
Cupravit50 WP	Copper Oxychloride	Copper chloride oxide hydrate	0.2	0.4
Indofil M-45	Mancozeb80 WP	N-(2,6 dimethyl phenyl)-N (methoxyacetyl)-alanine methyl ester ($C_{14}H_{21}NO_4$)	0.2	0.4
Streptomycin Sulphate	Streptomycin sulphate	Streptomycin sulphate	0.015	0.15

NA plate at center. Then 100 µl volumes of chemical suspension at different concentrations [Cupravit 50 WP (0.2 and 0.4%), Indofil M-45(0.2 and 0.4%) and Streptomycin Sulphate (0.015 and 0.15%)] were added into the holes. Three replications were maintained for each concentration. Sterile water was used instead of chemical as control. The plates were then incubated at 30°C in incubation chamber. Zone of inhibition around the holes were measured and recorded after every 24 hrs for 5 consecutive days.

3. RESULTS

3.1 Colony of *Xanthomonas axonopodis* pv. *malvacearum*

Colonies of *Xanthomonas axonopodis* pv. *malvacearum* on NA plates were yellow, convex, mucoid and circular after 48 hours of incubation (Plate 1A).

3.2 Morphological Characteristics

Under the compound microscope *Xanthomonas axonopodis* pv. *malvacearum* was rod shaped with round ends, cells appeared singly and also in pairs, gram negative bacteria (red colour) and capsulated. The cells were readily stained with common stains such as crystal violet (Plate 1B). The Bacterium creates thread at KOH solubility test (Plate 1C).

3.3 Pathogenicity Test

The result of pathogenicity test revealed that the bacteria showed typical angular leaf blight symptoms and after isolation gave the same colonial color texture as what these was inoculated. Therefore, these seem to be pathogenic (Plate 4).

3.4 Colony Morphology on Different Growth Media

Colonies of *X. axonopodis* pv. *malvacearum* on NA medium appered as circular, mucoid, convex, yellow to orange color (Plate 5A). Circular, flattened or slightly raised, yellow to bright yellow color, mucoid colonies were found on YDCA medium (Plate 5B) Bacterium exhibited very poor growth with light yellow, mostly circular, small, flattended, mucoid on SX Medium (Plate 5C).

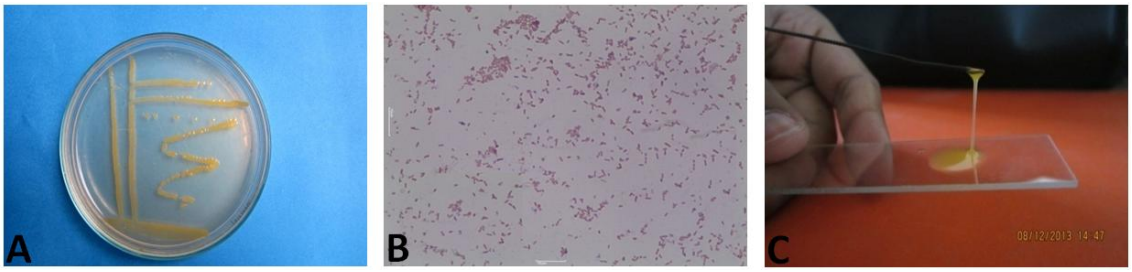

Plate 1A. Pure culture of *Xanthomonas axonopodis* pv. *malvacearum* on NA plate, B. Microscopic view of *Xanthomonas axonopodis* pv. *malvacearum* after gram's staining at 125x magnification, C. KOH solubility test for *Xanthomonas axonopodis* pv. *malvacearum*

Table 2. Biochemical characteristics of *Xanthomonas axonopodis* pv. *malvacearum*

Biochemical tests	Results	According to Schaad (1992) [13] and Salle (1961) [14]
Starch hydrolysis test (Plate 2A)	+	+
Catalase test (Plate 2B)	+	+
Oxidase test (Plate 2C)	+	+
Citrate utilization test (Plate 2D)	+	+
Motility indole urease agar (MIU) test (Plate 2E)	+	+
Gelatin liquefaction test (Plate 2F)	+	+
Tobacco hypersensitivity (Plate 3)	+	+

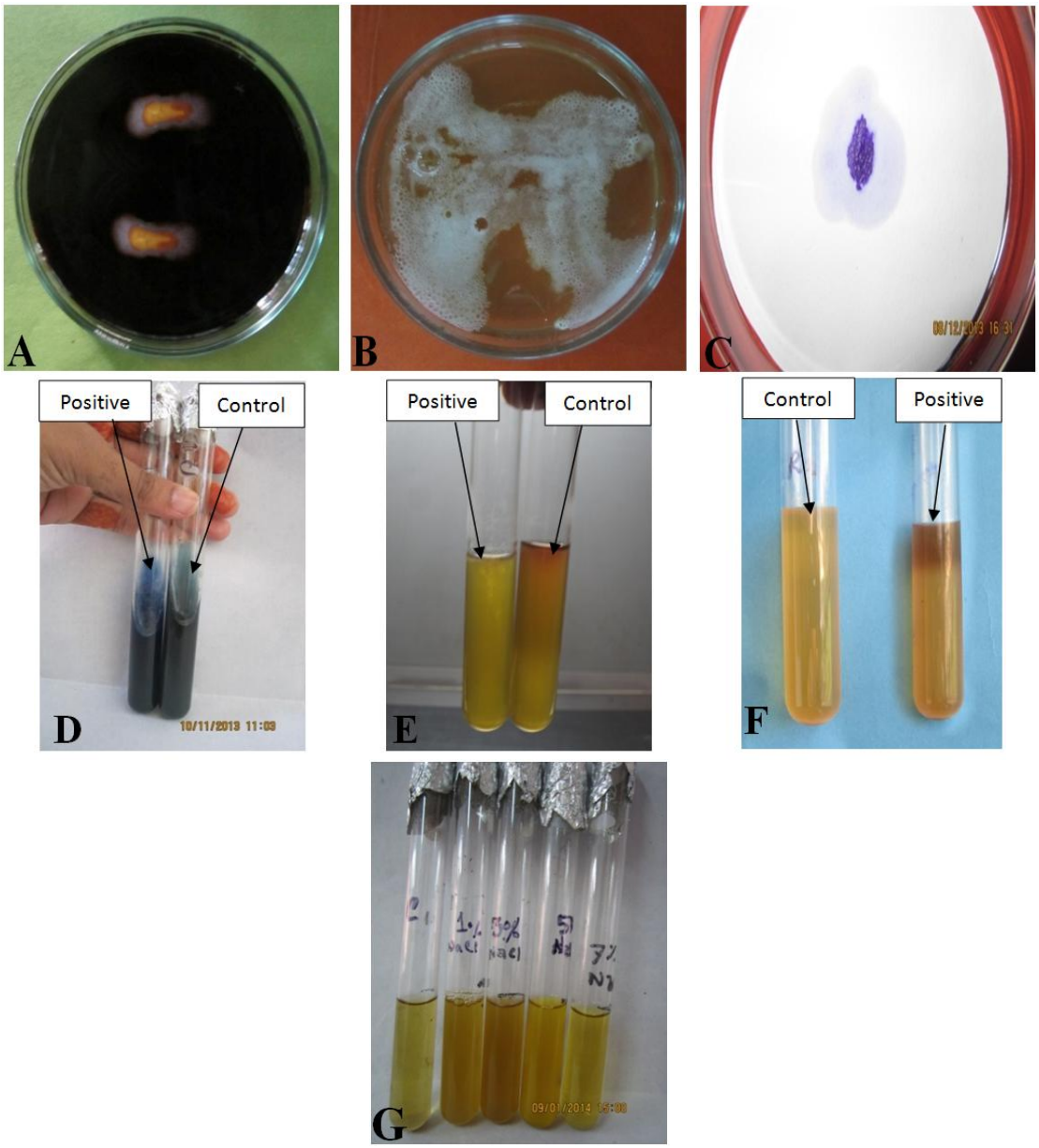

Plate 2. Different biochemical tests of *X. axonopodis* pv. *malvacearum.* A. Starch hydrolysis test (positive); B. Catalase test (positive); C. Oxidase test (positive); D.Citrate utilization test (positive); E. Motility indole urease agar (MIU) test; F. Gelatin liquefication test (positive); G. Salt tolerance test (positive)

Table 3. Salt tolerance test for *Xanthomonas axonopodis* pv. *malvacearum* in nutrient broth

Time	Salt tolerance (Plate 2G)					
	1%	2%	3%	4%	6%	7%
24 hr	+	+	-	-	-	-
48 hr	+	+	+	-	-	-
72 hr	+	+	+	-	-	-

3.5 Efficacy of Selected Chemicals against *Xanthomonas axonopodis* pv. *malvacearum* at Different Days After Incubation

A significant variation of inhibition zone was observed against the selected chemicals. The highest inhibition zone (35.33, 36.17, 35.17, 33.5 and 32.33 mm after 24, 48, 72, 96 and 120 hr., respectively) was observed against Streptomycin sulphate @ 0.15%. On the other hand, no inhibition zone was observed in control condition (Fig. 1. and Plate 6). All the selected chemicals give the highest result at 48 hrs. After incubation which was gradually decrease on the successive hrs.

Plate 3. Tobacco hypersensitivity test. A. Inoculation of bacterial suspension into the lower surface of tobacco leaf with a sterile syringe; B. Positive reaction and initiation of infection (*Xanthomonas axonopodis* pv. *malvacearum* inoculated)

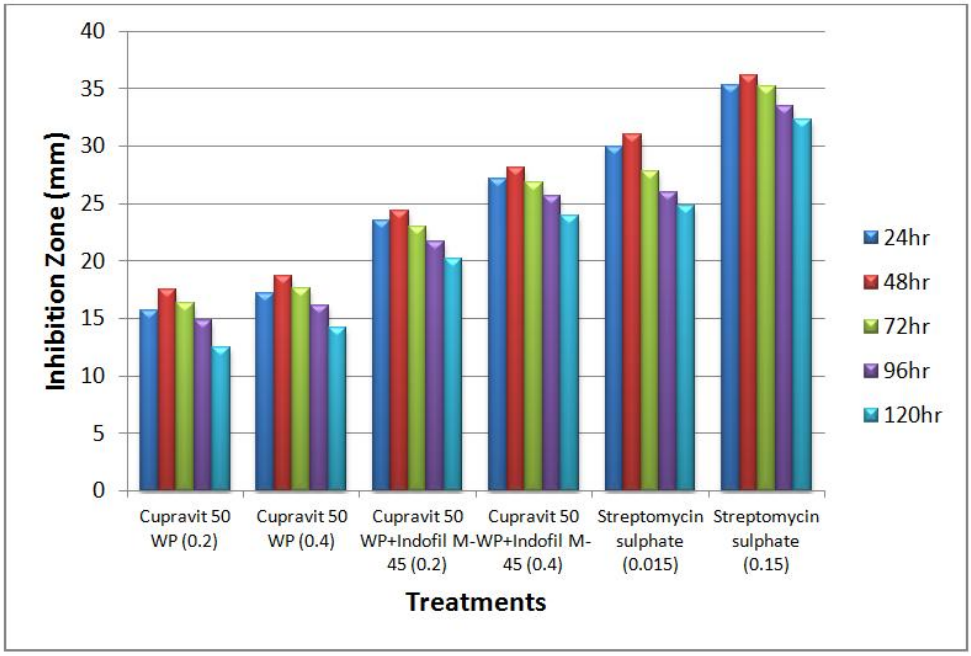

Fig. 1. Efficacy of selected chemicals against *Xanthomonas campestris* pv. *malvacearum* at different days after incubation

T1 = Cupravit 50 WP @0.2% ; T2 = Cupravit 50 WP @0.4%; T3 = Cupravit 50 WP @0.2% + Indofil M-45 @0.2% ; T4 = Cupravit 50 WP @0.4% + Indofil M-45 @0.4%; T5 = Streptomycin sulphate @0.015%; T6 = Streptomycin sulphate @0.15%

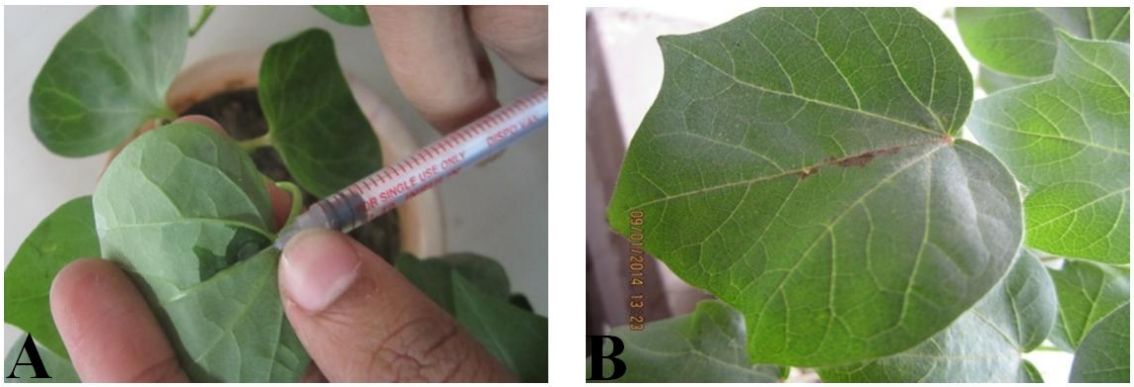

Plate 4. Pathogenicity test, A. Inoculation of bacterial suspension into the lower surface of cotton leaf with a sterile syringe; B. Symptom of bacterial blight on inoculated leaf of cotton

Plate 5. Cultural characteristics of *Xanthomonas campestris* pv. *malvacearum* on different growth media. A. On nutrient agar (NA) medium; B. On yeast extract dextrose calcium carbonate agar (YDCA) medium; C. On SX agar medium

4. DISCUSSION

The causal organism of BB was isolated from the infected leafs showing typical symptoms of bacterial blight. For confirmation of bacterial blight oozing test was performed. *Xanthomonas axonopodis* pv. *malvacearum* produced yellow, convex, mucoid, colonies of bacterium on nutrient agar medium after 48 hours of incubation at 30°C. The pathogen has also been reported by many researchers throughout the world [19,20,21,22,23]. *Xanthomonas* produce mycoid, circular, convex, yellow, round, glistening and raised colonies on nutrient agar mdeium [24].

Xanthomonas axonopodis pv. *malvacearum* was rod shape, gram negative bacteria. It showed positive reactions in Starch hydrolysis test, Catalase test, Oxidase test, Citrate utilization test, Motility indole urease agar (MIU) test, Gelatin liquefaction test and Salt tolerant test. Similar results has also been reported [19,7,20,24,25,26,27,28]. In the present study, it was observed that *X. axonopodis* pv. *malvacearum* produce circular, flattened or slightly raised, yellow to bright yellow color, mucoid colonies on YDCA medium and light yellow, mostly circular, small, flattended, mucoid on SX Medium. *Xanthomonas* gave yellow, circular, and viscous bacterial colonies on yeast extract nutrient agar (YDCA) after 48-72 h which is supported Hamid et al. [7] and Jabeen et al. [29]. In SX Medium the bacterium gave light yellow to slightly azure, mostly circular, small, flattended, mucoid colonies. A similar result has also been reported by many researchers [19,20,21,30,31]. With the present study, it was observed that *X. axonopodis* pv. *malvacearum* can tolerate up to 3% salt concentration after 72 hours of incubation which is supported by researchers [20,32]. The present study revealed that after infiltration the lower surface of a mature tobacco leaf with *X. axonopodis* pv. *malvacearum,* the infiltrated area became dry and necrotized within 72 hours which confirmed the pathogenic nature of the bacteria. A same result has also been reported [19,20,33,34].

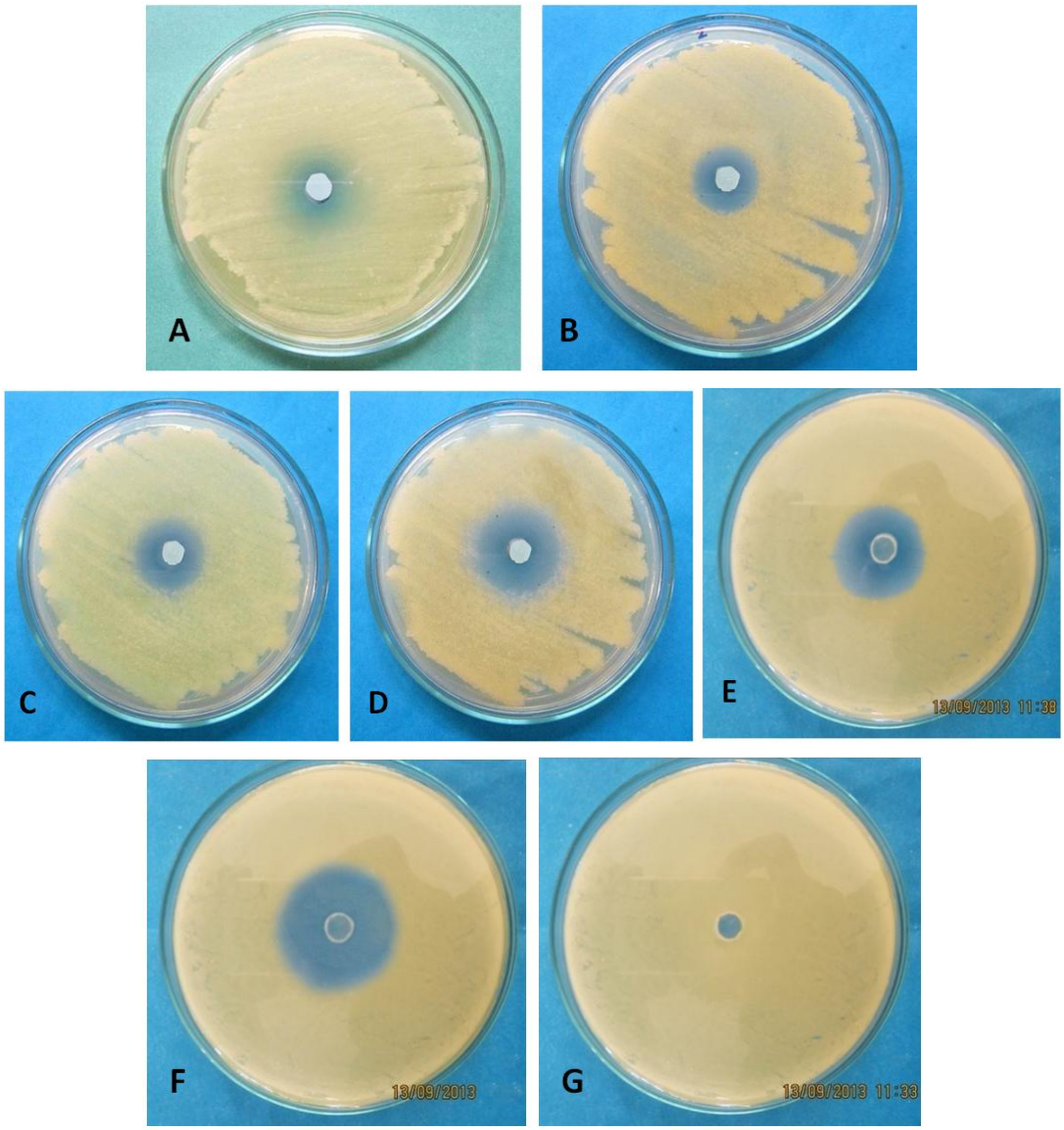

Plate 6. Bioassay of some selected chemicals against the bacteria, A. Cupravit 50WP (0.2%), B. Cupravit 50WP (0.4%); C. Cupravit 50WP + Indofil M-45 (0.2%); D. Cupravit 50WP + Indofil M-45 (0.4%); E. Streptomycin sulphate (0.015%); F. Streptomycin sulphate (0.15%); G. Control

Among the selected chemicals viz.Cupravit 50 WP, Indofil M-45 and Streptomycin sulphate evaluated in the present *in vitro* investigation, Streptomycin sulphate @0.15% (36.17 mm) and Streptomycin sulphate @0.015% (31.00 mm) exhibited significantly superior efficacy in inhibiting the growth of *Xanthomonas axonopodis* pv. *malvacearum* while Cupravit 50 WP + Indofil M-45 @0.4% (28.17 mm) and Cupravit 50 WP + Indofil M-45 @0.2% (24.33 mm) were found moderately effective and Cupravit 50 WP @0.4% (18.67 mm) and Cupravit 50 WP @0.2% (17.5 mm) were less effective. The results obtained in the present study were in agreement with the reports of earlier workers [20,35,36]. Menegium et al. [37] reported that copper as well as mixture of copper with mancozeb were effective in controlling *Xanthomonas* spp.

5. CONCLUSIONS

Xanthomonas axonopodis pv. *malvacearum* was isolated and identified from cotton leaf on the basis of pathogenicity test, tobacco

hypersensitivity test, morphological, biochemical test and growth characters on differential media viz. YDCA medium and SX Medium. *In vitro* evaluation of selected chemicals revealed that Streptomycin sulphate was highly effective against *X. axonopodis* pv. *malvacearum*. Streptomycin sulphate @ 0.15% amended culture resulted the highest inhibition zone of 36.17 mm after 48 hrs. Streptomycin sulphate @ 0.15% can be tested against different races of reference isolates of *Xam* before field application.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bhuyan MOU. Bangladesh targets to produce 670,000 bales of cotton by 2015. Published at The Daily New Age. Available:http://www.newagebd.com/detail.php?date=2012-12-01&nid=31911#.Uaq1npij_KQand http://print.thefinancialexpress-bd.com/old/more.php?news_id=126548&date=2012-04-13 (Accessed 1 December 2012)
2. Cotton Development Board. Achievements of CDB at present Govt. 2009- Dec 2015. Available:http://www.cdb.gov.bd/
3. Islam MZ, Khalequzzaman KM, Rahman GM, Islam MT, Hossain MM. Effect of chemicals in controlling bacterial blight of cotton. Asian J Plant Sci. 2003;2(7):539-543.
4. Anonymous. Compendium of cotton diseases. Edited by: Q. M. Watkins. American Phytopathological Society; 1981.
5. Hussain T, Tahir M. Chemical control of bacterial blight of cotton. Pak J Phytopath. 1993;5(1-2):119-121.
6. Watkinson GM. Compendium of cotton diseases. The American Phytopathological Society. 1981;25.
7. Hamida MI, Iqbala MA, Usman M, Ghazanfara YI, Akhtara N. Correlation of environmental conditions with bacterial blight disease of *cotton* (*Gossypium hirsutum* L.). Pak J. Phytopath. 2012;24(1): 39-43.
8. Hillocks RJ. Cotton disease research in Tanzania. Int J. Pest Manage. 1981;27(1): 1-2.
9. Hosagoudar GN. Studies on foliar diseases of cotton with special reference to Bt cotton. MS Thesis. University of Agricultural Science. Dharwad; 2007.
10. Cotton Development Board. Annual report and workplan. 2015;67.
11. Singh PR, Verma PJ. Control of bacterial blight of cotton. Pesticides. 1973;7:16-17.
12. Goszczynska T, Serfontein JJ. Milk–tween agar, a semiselective medium for isolation and differentiation of *Pseudomonas syringae* pv. *syringae*, *Pseudomonas syringae* pv. *phaseolicola* and *Xanthomonas axonopodis* pv. *phaseoli*. Jmicrobiol Methods. 1998;32(1):65-72.
13. Schaad NW. *Xanthomonas.* In: Laboratory guide for identification of plant pathogenic bacteria. 2nd ed. Lucknow: International Book Distributing Co. 1992;165.
14. Salle AJ. Fermentation of carbohydrates and related compounds. In: Laboratory manual on fundamental principles of bacteriology. 5th ed. New York: McGraw Hill Book Company, Inc. 1961;94-98.
15. Klement Z, Goodman RN. The hypersensitive reaction to infection by bacterial plant pathogens. Annu. Rev Phytopathol. 1967;5(1):17-44.
16. Lin HC, Chang H, Tzeng KC. Characterization of novel strains of citrus canker bacterium from citrus in Taiwan. 2008;57(4):265-278.
17. Anonymous. Indian Pharmacopoeia. 3rd ed. New Delhi: Controller of publications, Ministry of Health and family welfare, Government of India; 1996.
18. Vandepitte J, Engback K, Piot P, Heuck CC. Basic microbiology procedures in clinical bacteriology. Geneva, Switzerland: World Health Organization. 1991;85.
19. Roni NI. Prevalence of seed borne pathogens and their effect on seedlings of selected imported hybrid rice varieties. MS Thesis. Sher-e-Bangla Agricultural University, Dhaka; 2013.
20. Rashid MO. Prevalence of canker on seedlings of citrus (*Citrus* spp.) in selected areas of Bangladesh and it's management. MS Thesis. Sher-e-Bangla Agricultural University, Dhaka; 2012.
21. Vudhivanich S. Efficacy of thai herbal extract for growth inhibition of *Xanthomonas axonopodis* pv. *citri.*, the bacterial canker of citrus. Kasetsart J. (Nat Sci.). 2003;37:445-452.

22. Kale KB, Kolte SO, Peshney NL. Economics of chemical control of citrus canker caused by *Xanthomonas campestris* pv. *citri* under field conditions. Indian Phytopathol. 1994;47(3):253-255.
23. Qui CH, Ni BQ. A preliminary test on the control of citrus canker with mono-molecular forming substance (MMFS) and other chemicals. Fujian Agril. Sci. Technol. 1988;1:19.
24. Chand R, Kishun R. Studies on bacterial blight (*Xanthomonas campestris* pv. *punicae*) of pomegranate. Indian Phytopathol. 1991;44(3):370-372.
25. Hossain MM. Seed-borne fungi and bacteria of cotton and their control. MS Thesis. Bangladesh Agricultural University, Mymensingh; 2001.
26. Yenjerappa ST. Epidemiology and management of bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* (Hingorani and Singh), Doctoral dissertation, University of Agricultural Science, Dharwad; 2009.
27. Akhtar MA, Khan IU. Bacterial blight of cotton and its control. Pakistan J Agri. Res. 1988;9(2):202-204.
28. Chand JN, Pal V. Citrus canker in India and its management. In: Problems of citrus diseases in India. New Delhi: Surabhi Printers and Publishers. 1982;21-26.
29. Jabeen R, Iftikhar T, Batool H. Isolation, characterization, preservation and pathogenicity test of *Xanthomonas oryzae* pv. *oryzae* causing BLB disease in rice. Pak J. Bot. 2012;44(1):261-265.
30. Balestra GM, Sechler A, Schuenzel E, Schaad NW. First report of citrus canker caused by *Xanthomonas citri* in Somalia. Plant Dis. 2008;92(6):981.
31. Braithwaite M, Leite RP, Smith JJ, Boa E, Saddler GS. First report of citrus canker caused by *Xanthomonas campestris* pv. *citri* on *Citrus sinensis* in Bolivia. Plant Pathol. 2002;51(3):383.
32. Vernière C, Hartung JS, Pruvost OP, Civerolo EL, Alvarez AM, Maestri P, Luisetti J. Characterization of phenotypically distinct strains of *Xanthomonas axonopodis* pv. *citri* from Southwest Asia. Eur. J. Plant Pathol. 1998;104(5):477-487.
33. Swarup S, De Feyter R, Brlansky RH, Gabriel DW. A pathogenicity locus from *Xanthomonas citri* enables strains from several pathovars of *X. campestris* to elicit cankerlike lesions on citrus. Phytopathology. 1991;81(7):802-809.
34. Klement Z, Farkas GL, Lovrekovich L. Hypersensitive reaction induced by phytopathogenic bacteria in tobacco leaf. Phytopathology. 1964;54(4):474-477.
35. Behlau F, Amorim L, Belasque J, Bergamin Filho A, Leite RP, Graham JH, Gottwald TR. Annual and polyetic progression of citrus canker on trees protected with copper sprays. Plant Pathol. 2010;59(6):1031-1036.
36. Shahid MM, Sahi ST, Ghazanfar MU, Haq MI, Haq I, Haq YI, Iftikhar Y, Sarwar MS, Ahmad T. Evaluation of different toxicants against *Xanthomonas campestris* pv. *citri* (Hasse) Dows. Int J. Agri Bot. 2005;7(1): 121-124.
37. Meneguim L, Rinaldi DA, Santos AC, Rodrigues LS, Silva MR, Canteri MG, Leite Júnior RP. Sensibility of *Xanthomonas axonopodis* pv. *citri* to copper and mancozeb. Fitopatol Bras. 2007;32(3):247-252.

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