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# Effect of Partially UV-Blocking Films on *Xanthomonas axonopoides* Pv. *Citri* Causing Citrus (*Citrus aurantifolia*) Canker

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**Abstract:** Citrus canker caused by *Xanthomonas axonopoides* pv *citri* is currently the most devastating disease impacting world citrus production. An experiment was conducted to testify the hypothesis that partially UV-blocking film has the ability to suppress the development of canker disease of citrus. The quantified variables were latent period of the pathogen, temperature, relative humidity, partially UV-blocking conditions, disease incidence and disease severity. The mini houses were constructed and covered with four types of polyolefin films that have the ability to block solar UV-irradiation shorter than UV-A of (<400 nm), (<360 nm), (<350 nm) and (<340 nm) and the results were compared with UV-transmitting and outdoors. The result showed that the leaves under <400 nm and <360 nm UV-blockings took less time (8 days) to express the symptom than outdoors (13 days). The lowest incidence (66.7%) was recorded in outdoors and highest incidence (100%) was recorded under <400 nm at 45 days after inoculation. Similarly, lowest severity (10%) was recorded in outdoors and the highest severity (43.3%) was recorded under <400 nm at 45 days after inoculation. Disease incidence and severity gradually decreased as the UV blocking rates decreased. Solar radiation with contains combination of different UV-radiation may be detrimental for multiplication of the bacteria *Xanthomonas axonopoides* pv *citri.*

**Key words:** Citrus canker · Disease management · Incidence and severity · Partially UV-blocking

## INTRODUCTION

Citrus (*Citrus aurantifolia*) belonging to the family Rutaceae is one of the most important nutritious fruit crops of the world as well as Bangladesh. In Bangladesh, the total acreage under citrus cultivation is about 5,995 ha while the total production is around 136,756 mt [1]. Various factors are responsible for lower citrus production in Bangladesh. Among them, plant disease is one of the major influential factors. Different species of citrus grown in the world suffers from more than 100 diseases [2]. In Bangladesh, twelve diseases are known to occur in different species of citrus where citrus canker is considered as the most important disease. The export of citrus from Bangladesh is seriously hampered due to this disease. Citrus canker is distributed over thirty countries of the world [3]. This disease is caused by *Xanthomonus axonopodis* pv. *citri*, which is a rod-shaped gram-negative bacterium [4]. Plants infected with citrus canker have characteristic lesions on leaves, stems and fruit with raised, brown, water-soaked margins, usually with a yellow halo or ring effect around the lesion. Older lesions have a corky appearance, still in many cases retaining the halo effect [5]. Temperatures between 15 to 20 °C and 35 to 40 °C are conducive for infection and development of citrus canker disease, respectively [6]. Relative humidity between 75 to 85% is also favorable for infection and development of this disease [7]. Management practice of this devastating disease is still limited. Application of some agricultural chemicals, especially copper compounds were proven effective against this disease [3, 8] but they are hazardous for both human health and environment [9]. Therefore, public concern is focused on alternative methods for pest control [10]. Ultraviolet (UV) radiation is an important stress factor for bacterial communities and may be an

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effective way to reduce this disease [11]. UV radiation is divided into three spectral regions, viz. UV-C (100-280 nm), UV-B (280-320 nm) and UV-A (320-400 nm). On a photon basis, UV-A radiation contains less energy than UV-B radiation; however, the fraction of UV-A region (95%) in the solar UV radiation is far greater than that of the UV-B region (5%). So, biological damage of plants can be by the energy of UV radiation [12, 13]. The effects of UV-A are considered to be mostly indirect, that is, mediated by reactive oxygen species (ROS) formed via photodynamic reactions involving intracellular or extracellular photo-sensitizers [14]. These ROS can react with cellular constituents, most notably proteins and lipids, leading to altered membrane permeability and/or disruption of trans-membrane ion gradients that can eventually cause cell death. The growth of *Penecillium digitatum* was reduced in citrus fruit under UV irradiation [15]. It is found that UV- A has some detrimental effects on microorganisms. It can kill the microorganism up to a desirable level. UV-A killed a lot of *Esherechia coli* within nine hours and reduces the synthesis of SOD, catalase and enzyme activity of the bacteria [16]. When *E. coli* is subjected to continuous, low-influence UV-A irradiation, it responds by changing the activity levels of hydroperoxidases, glutathione reductase and manganese superoxide dismutase. That causes a delay growth rate of bacteria. When *E. coli* is given a UV-A dose of 50 W/m, extensive protein oxidation occur, which may contribute to the inhibition of key cellular enzymes, leading to cellular dysfunction, DNA damage and eventually death [17].

UV- irradiation can contribute for the reduction of postharvest losses caused by citrus black spot and reduce the use or doses of fungicides on disease control [18]. The effect of partially UV-blocking shorter than UV-A on citrus canker is still unknown. Therefore, the present study was undertaken to investigate the effect of partially UV-blocking films shorter than UV-A on citrus canker disease.

## MATERIALS AND METHODS

**Preparation of Mini House:** Effect of partially UV-blocking shorter than UV-A on citrus canker was observed during 10 August to 30 September in 2014. Laboratory experiments were conducted in Plant Disease Diagnostic Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh and field experiments were conducted in the Horticultural farm, Department of Horticulture, Sher-e-Bangla Agricultural University, Dhaka.

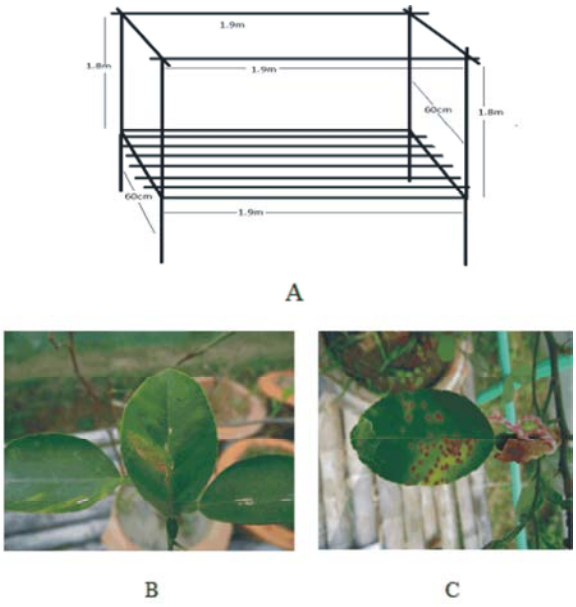


Plate 1: A-Measurements of the mini house. B- Minimum disease severity in $T_6$ at 45 DAI; C- Maximum disease severity in $T_1$ at 45 DAI.

Fifteen plastic mini houses of 1.8 m × 1.9 m × 0.6 m (L×B×H) with different PO films (0.13-mm thickness) were prepared for experimentation (Plate 1. A). Mini houses were covered with different PO films which can block UV-radiations shorter than 400 nm (<400 nm, T1), 360 nm (<360 nm, T2), 350 nm (<350 nm, T3) and 340 nm (<340 nm, T4), respectively (PO film collected from Mitsubishi Plastics Agri Dream, Tokyo, Japan) and the results were compared with that of UV-transmitting ($T_5$) and outdoors ($T_6$). A bamboo bench was used above three feet of the soil level for each tunnel to protect soil heat. The first 60 cm (33%) of the tunnel from the soil level remained open to control the heat of the tunnels and allow the invasion by insects. Earthen pots with citrus plants (one year old) were transferred in those mini houses and three plants were put under each tunnel.

**Measurement of Environmental Conditions:** Temperature, humidity, visible and UV-light irradiations were measured in daily basis during the experiment. Both temperature and humidity were recorded at 8:00, 12:00 and 24:00, while visible and UV-light irradiations were measured at 12.00.

**Cultivation of Citrus Seedling and Inoculation with Bacteria:** Citrus seedling (1 year old) collected from Krishibid Nursery, Bangladesh were used for this assay. The seedlings were planted in 25 × 25 $cm^2$ earthen pots

(one plantpot$^{-1}$). Sterile loamy soil and sand (2:1) were used as potting media. Seedlings were irrigated with tap water once a day. There is no additional nutrient was supplied during the study period. Seedlings were inoculated with bacterial suspension (5 µl, OD: 0.5) containing $10^8$ CFU/ml by injection method using 1 ml syringe after 15 days of transplantation. Five leaves of each plant were inoculated. Plants were covered with polythene bag after inoculation for 24 hours to maintain suitable moisture condition. Then the plants were transferred under the UV-blocking films to observe the post inoculation effect of UV-A on citrus canker.

**Isolation and Identification of the Causal Organism:** Leaves were collected from infected citrus (*Kagji lebu*; *C. aurantifolia*) field and surface sterilized by 0.1% $HgCl_2$ solution for 30 seconds and then thoroughly washed with distilled water thrice to remove any trace amounts of $HgCl_2$ [19]. The excess moisture was removed by placing these pieces in between two folds of sterilized blotter paper. Then the causal bacterium, *Xanthomonas axonopodis* pv. *citri* was isolated using the techniques described by Goszczynska and Serfontein [20]. Colonies of bacteria were purified on NA and SX media plates. The causal organism of citrus canker, *Xanthomonas axonopodis* pv. *citri* was identified by following gram's staining reaction [21], potassium hydroxide (3% KOH) test [22], starch hydrolysis test [23], catalase test [24] and oxidase test [25].

**Pathogenicity Test:** A bacterial suspension (5 µl, OD: 0.5) containing $10^8$ colony forming units per ml (CFU/ml) was inoculated into the lower surface of citrus leaf with a sterile syringe by injection method and observed for 15 days [26]. Visual symptoms were recorded and examined. To confirm Koch's postulates, bacteria re-isolated from diseased leaves were streaked on NA plate and re-identified using the methods outlined by Lin *et al*., [27].

**Recording of Data:** Data on disease incidence and severity were collected after 15, 30 and 45 days of inoculation. Assessment of disease incidence and severity was calculated using the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{No. of diseased leaf among the inoculated leaf in each plant}}{\text{No. of total inoclated leaf in each plant}}$$

$$\text{Disease severity (\%)} = \frac{\text{Amount of diseased in the inoculated leaf in each plant}}{\text{Amount of total disease in the inoculated leaf in each plant}}$$

**Experimental Design:** The experiment was laid out following randomized complete block design (RCBD) design with three replications. Six treatments were used in the experiment including control.

**Statistical Analysis:** Data were subjected to analysis of variance and significant differences of the means among treatments were analyzed using MSTAT-C software (East Lansing, MI, USA).

## RESULTS AND DISCUSSIONS

**Isolation and Identification:** The isolated bacteria identified as *Xanthomonas axonopodis* pv. *citri* according to morphological, biochemical characters of the bacterium as per standard microbiological procedures (Plate 2). Typical, yellow, convex, mucoid, colonies of *Xanthomonas axonopodis* pv. *citri* was found on NA plates after 48 hours of incubation at 30±1 ºC (Plate 2A). Chand and Kishun [28] reported that *Xanthomonas* produces mucoid, circular, convex, yellow, round, glistening and raised colonies on nutrient agar medium.

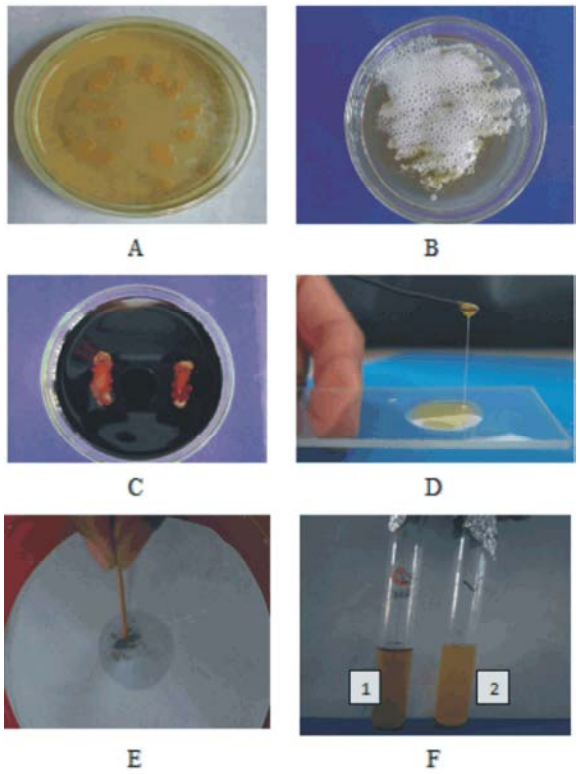


Plate 2: Biochemical test of *Xanthomonus.* A-colony of bacteria; B- Catalase test; C-Starch hydrolysis; D – KOH test; E-Oxidase test; and F-Gelatin liquefaction test (1-positive, 2-negative).

Table 1: Biochemical tests to identify *Xanthomonas axonopodis* pv. *citri*

| Name of tests | Reaction |
|---|---|
| Gram Staining | - |
| KOH solubility test | + |
| Starch hydrolysis test | + |
| Catalase test | - |
| Oxidase test | + |
| Motility indole urease agar (MIU) test | + |
| Gelatine liquefaction test | + |
| Tobacco hypersensitivity test | + |

- = yes, + = No

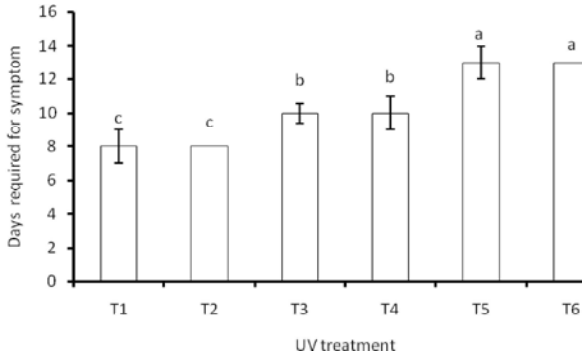


Fig. 1: Days required for symptom expression in citrus plant after inoculation.
$T_1$– <400nm, $T_2$- <360nm, $T_3$- <350nm, $T_4$- <340nm, $T_5$- UV-transmitting, $T_6$- Outdoors.
Different lowercase letters above the vertical bars indicate significance at P<0.05.

The bacterium was rod shaped with rounded ends, cells appeared singly and also in pairs, gram negative (red color) and capsulated under the compound microscope at 100 times magnification with oil immersion. It produced a mucoid thread when lifted with the loop (Plate 2 D), showed catalase activity, bubbles were formed after adding 3% $H_2O_2$ (Plate 2 B), formed dark purple colour on oxidase disk (Plate 2 E), gelatin was liquefied (Plate 2 F), starch was hydrolyzed (Plate 2 C) and the findings are summarized in Table 1.

**Symptom Expression:** After inoculation of bacteria, the plants were periodically observed for symptom expression. The latent period (time between inoculation and symptom expression) of the bacteria varied from treatment to treatment. The symptoms of citrus canker disease were first expressed eight DAI in treatments $T_1$ and $T_2$. Both the treatments $T_3$ and $T_4$ required ten days to produce symptoms. $T_5$ and $T_6$ treatments needed thirteen days to express symptoms (Fig. 1). Initially the symptoms were water soaked small yellow in color which in later became corky appearance with broad yellow halo zone (Plate 1 C).

**Incidence and Severity of Citrus Canker under Different Treatments:** Disease incidence and severity of citrus canker were observed at different DAI under different treatments (Table 2; Fig. 2, 3). Incidence of canker of citrus varied significantly under different UV intensities ranged from 58.3 to 100% at 15 DAI and 60 DAI (Table 2). The highest incidence 83.3% was recorded in treatment $T_1$ at 45 DAI and lowest incidence (58.3%) was recorded in treatment $T_6$ at 15 DAI. Statistically similar incidence (66.7, 83.3 and 83.3 %) at 15, 45 and 60 DAI was recorded in $T_3$ and $T_4$. Statistical significant differences were recorded among the other treatments.

Severity of citrus canker also varied significantly under different ultraviolet intensities varied from 1.8 to 43.3% at 15 DAI and 60 DAI (Table 2). The highest severity (43.3%) was recorded in treatment $T_1$ at 45 DAI

Table 2: Incidence and Severity of citrus canker under different ultraviolet intensity

| | Disease incidence (%) | | | Disease severity (%) | | |
|---|---|---|---|---|---|---|
| Treatment | 15 DAI | 30 DAI | 45 DAI | 15 DAI | 30 DAI | 45 DAI |
| <400nm | 91.7 a | 100.0 a | 100.0 a | 4.1 a | 16.7 a | 43.3 a |
| <360nm | 83.3 ab | 91.7 ab | 91.7 ab | 3.5 a | 11.5 b | 18.3 b |
| <350nm | 66.7 ab | 83.3 abc | 83.3 abc | 3.2 ab | 9.6 bc | 14.2 bc |
| <340nm | 66.7 ab | 83.3 abc | 83.3 abc | 3.2 ab | 9.3 bc | 13.8 bc |
| UV- | 58.3 b | 75.0 bc | 75.0 bc | 2.1 bc | 8.1 c | 10.8 c |
| Outdoor | 58.3 b | 66.7 c | 66.7 c | 1.8 c | 7.0 c | 10.0 c |
| Significance | * | * | * | ** | ** | ** |

*ns-non significant, *- significant at P=0.05, **- significant at P=0.01. Different lowercase letters beside the mean value indicate significant at P=0.05 or 0.01.*

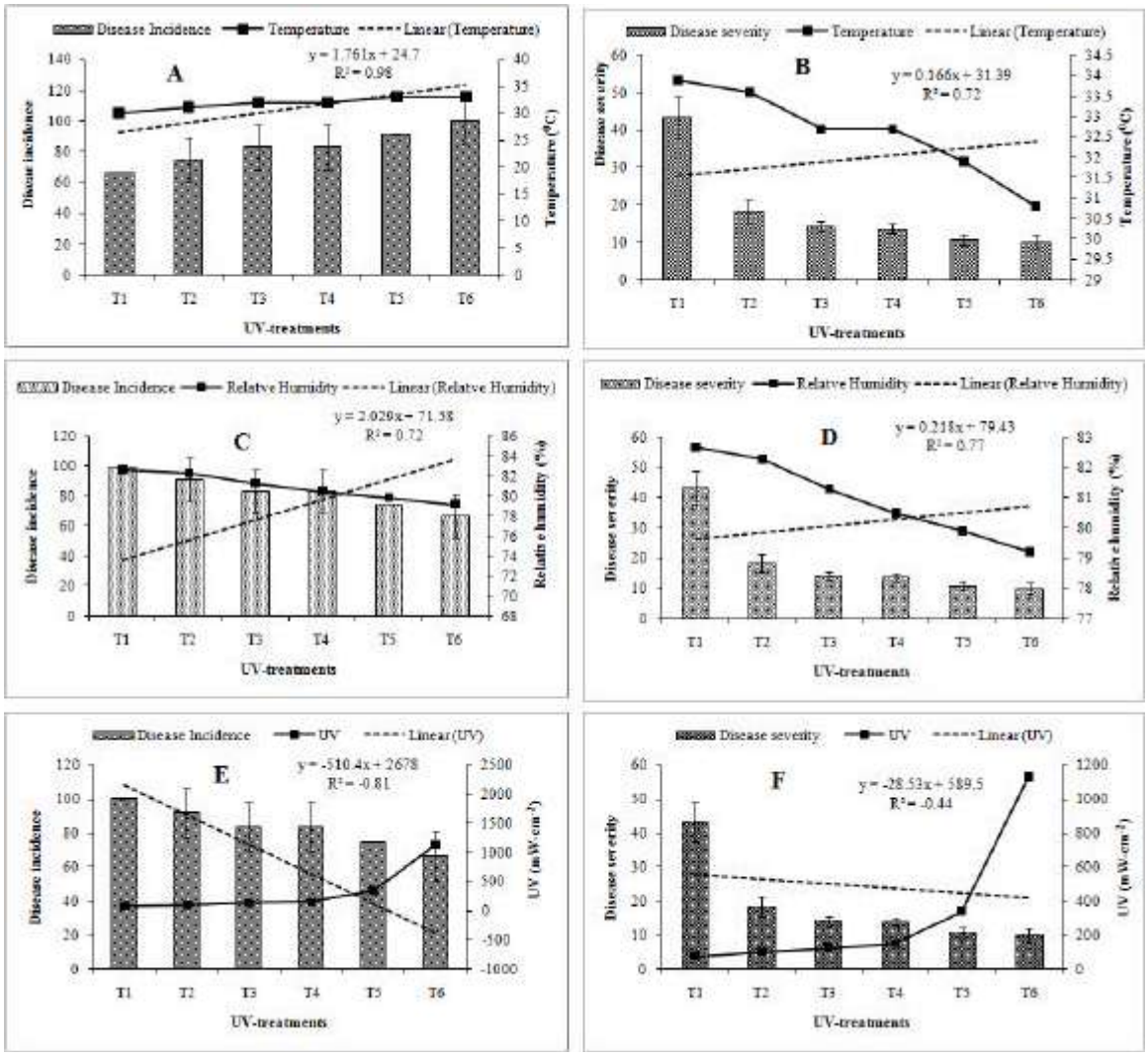


Fig. 2: Correlation of temperature, relative humidity (RH%) and UV intensity with disease incidence and severity. A–correlation between temperature and incidence; B– correlation between temperature and severity; C–correlation between humidity and incidence; D–correlation between humidity and severity; E– correlation between UV intensity and incidence; and F–correlation between UV intensity and severity. $T_1$– <400nm, $T_2$- <360nm, $T_3$- <350nm, $T_4$- <340nm, $T_5$- UV-transmitting, $T_6$- Outdoors.

and lowest incidence (1.8%) was recorded in treatment $T_6$ at 15 DAI. Statistically similar severity (3.2, 9.3 and 13.8%) at 15, 45 and 60 DAI was recorded in treatment $T_3$ and $T_4$. However, statistical significant difference was found in treatment $T_3$ and $T_6$. Statistical significant difference in UV intensity was recorded among all the treatments (Table 3).

**Effect of Environmental Conditions under Different Treatments:** Temperature, relative humidity and UV intensity varied significantly under different treatments. Statistically similar temperatures, 33.9, 33.6 and 32.7, 32.7°C were found in treatment $T_1$, $T_2$ and $T_3$, $T_4$ respectively. Statistical significant difference was found in treatment $T_5$ and $T_6$. Statistically similar humidity (approx. 80%) was found in treatment $T_1$, $T_2$ and $T_4$, $T_5$.

**Relationship Between Weather Factors and Incidence as Well as Severity of Citrus Canker:** Correlation coefficient and regression equation were calculated to find out the effect of temperature, relative humidity and UV intensity (W-cm$^{-2}$) on disease incidence and severity of citrus canker (Table 4). Plants treated with different wavelengths of UV radiation showed gradually decreasing disease incidence and severity with decreased

Table 3: Effect of Partially UV-blocking films on temperature, relative humidity (RH%) and UV intensity during the experiment.

| | Temperature ( °C) | | | Relative humidity (RH%) | | | Visible light intensity ($W.cm^{-2}$) | | UV intensity ($mW-cm^{-2}$) | |
|---|---|---|---|---|---|---|---|---|---|---|
| UV Treatment | 8:00 | 12:00 | 24:00 | 8:00 | 12:00 | 24:00 | | % of outdoors | | % of outdoors |
| <400nm | 24.7 a | 30.80 d | 22.4 a | 71.8 a | 79.2 d | 81.5 a | 20.1 c | 78.3 | 76.8 f | 8.2 |
| <360nm | 24.3 a | 31.90 c | 21.9 a | 70.7 a | 79.9 c | 80.3 a | 20.3 bc | 79.2 | 101.3 e | 10.8 |
| <350nm | 23.8 a | 32.70 b | 22.3 a | 71.7 a | 80.5 c | 80.3 b | 18.8 d | 73.3 | 128.5 d | 13.7 |
| <340nm | 23.3 a | 32.70 b | 21.7 a | 70.5 a | 81.3 b | 80.5 c | 20.5 bc | 80.0 | 151.9 c | 16.3 |
| UV-transmitting | 24.1 a | 33.60 a | 22.1 a | 71.2 a | 82.3 a | 79.9 c | 21.6 b | 84.2 | 340.5 b | 36.4 |
| Outdoor | 23.9 a | 33.90 a | 21.9 a | 70.1 a | 82.7 a | 79.2 d | 25.3 a | 100 | 935.1 a | 100 |
| Significance | ns | ** | ns | ** | ** | ** | ** | | ** | |

*ns- non significant, * - significant at P=0.05, **- significant at P=0.01. Different lowercase letters beside the mean value indicate significant at P=0.05 or 0.01.*

Table 4: Linear correlation analysis on the effect of temperature, relative humidity and UV intensity on the incidence and severity of citrus canker

| | Slope (b) | | Correlation co-efficient ($R^2$) | | Probability (p) | | Intercept | |
|---|---|---|---|---|---|---|---|---|
| Climatic factors | Incidence | Severity | Incidence | Severity | Incidence | Severity | Incidence | Severity |
| Temperature | 0.09 | 0.06 | 0.98 | 0.72 | 0.001 | 0.08 | 24.70 | 31.39 |
| Relative humidity | 0.11 | 0.08 | 0.97 | 0.77 | 0.007 | 0.05 | 71.58 | 79.43 |
| UV | -28.26 | -14.52 | -0.81 | -0.44 | 0.03 | 0.36 | 2677.97 | 589.58 |

temperature and relative humidity. Negative correlation coefficient found among temperature, RH% and UV intensity ($R^2$= 0.98, 0.97, -0.81 and 0.72, 0.77, -0.44 for diseases incidence and severity, respectively) (Fig. 2 A-F). Correlation co-efficient and linear regression analyses were performed to determine the relationship between different components of climatic factor (temperature, RH% and UV) and the incidence as well as severity of citrus canker. From the correlation studies it was revealed that the temperature and RH% was positively but UV was negatively correlated to both the incidence and severity of citrus canker (Table 4).

Citrus production and export is being threatened in Bangladesh due to devastating outbreak of citrus canker disease. Still now no effective chemical control measure is available for this disease. Nowadays chemical control is discouraging due to its residual effects and environmental hazardousness. Solar radiations that contain different UV radiations have profound effect on different microorganisms [29-32]. This study focused on the effect of UV-A on citrus canker disease development. The experiment revealed that the *Xanthomonas* bacterium is sensitive to ultraviolet light. Disease incidence and severity were higher in treatment T1, where only visible light were passed and all kinds of UV radiation were blocked by the film. On the other hand, the lowest disease incidence and severity were measured in treatment outdoor in which no UV protecting film was used and plants got the complete solar radiation (UV-A+UV-B). As different wavelengths of UV-A was blocked gradually by the treatment $T_1$ to $T_4$ resulted gradual decrease of disease incidence and severity. The result also revealed that outdoor and UV transmitting treatments required longer period to express symptom after inoculation which gradually decreased to treatment $T_1$. This finding indicates the multiplication rate of the bacteria in host plant may be interrupted by UV light. The multiplication rate of bacteria may increase when they are imposed under ultraviolet light and vice-versa. UV-A may cause indirect damage by producing ROS at cellular level and UV-B may cause direct damage by breaking DNA of bacteria. The result also revealed that treatments $T_2$, $T_3$, $T_4$ had lower incidence and severity than $T_1$ but higher incidence and severity than $T_6$ indicating that partially UV-A blockings had less influence on the multiplication rate of *Xanthomonas* than outdoor which got full solar radiation.

The result also revealed that temperature and relative humidity in combined with UV intensity may have potential role in disease development. In treatment $T_1$ both the temperature and relative humidity were higher than $T_6$ treatment that may have positive role for disease development. Moreover, in treatment $T_2$ and $T_1$ both the temperature and humidity were non-significant but UV

intensity, disease incidence and severity were statistically significant. The findings indicate that, higher UV intensity has potential role to reduce disease incidence and severity of citrus canker. Furthermore, temperature and humidity showed positive correlation and UV intensity showed negative correlation with disease incidence and severity. Thus, disease incidence and severity increase with high temperature, humidity and low UV intensity and vice-versa. From the findings of this study, it can conclude that higher UV-A radiations are suitable to avoid canker disease of citrus in tropical and subtropical regions. However, further similar investigations are needed to clarify the individual effect of UV-B against the disease.

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## REFERENCES

1. BBS. 2012. Bangladesh Bureau of Statistics. Statistical Year Book of Bangladesh. Statistics Division, Ministry of Planning, Government of the Peoples Republic of Bangladesh. pp: 375.
2. Klotz, L.J., 1973. Color Hand Book of Citrus Disease. University of California, Div. Agric. Sci., Pp: 122.
3. Das, A.K., 2003. Citrus canker – A review. J. Appl. Hort., 5(1): 52-60.
4. Graham, J.H., T.R. Gottwald, J. Cubero and D.S. Achor, 2004. *Xanthomonas axonopodis* pv. *citri*: factors affecting successful eradication of citrus canker. Mol. Plant Pathol., 5(1): 1-15.
5. Gottwald, T.R., J.H. Graham and T.S. Schubert, 2002. Citrus canker: The pathogen and its impact. Online: Plant Health Progress doi: 10.1094/PHP-2002-0812-01-RV.
6. Pria, M.D., R.C.S. Christiano, E.L. Furtado, L. Amorim and A. Bergamin-Filho, 2006. Effect of temperature and leaf wetness duration on infection of sweet oranges by Asiatic citrus canker. Plant Pathology, 55(5): 657-663.
7. Rashid, M., M.S.M. Chowdhury and N. Sultana, 2014. Prevalence of canker on seedlings of citrus (*Citrus spp*.) in selected areas of Bangladesh and its management. The J. Plant Pathol., 114: 177-187.
8. Graham, J.H. and R.P.J. Leite, 2004. Lack of control of citrus canker by induced systemic resistance Compounds. Plant Disease, 88(7): 745-750.
9. Hossain, S.M., A.M. Hossain, M.A. Rahman, M.M. Islam, A.M. Rahman and M.T. Adyel, 2013. Health Risk Assessment of Pesticide Residues via Dietary Intake of Market Vegetables from Dhaka, Bangladesh. Foods. 2(1): 64-75; doi: 10.3390/foods2010064
10. Sutton, T.B., 1996. Changing options for the control of deciduous fruit tree diseases. Annual Review of Phytopathol., 34: 527-547.
11. Moran, M.A. and R.G. Zepp, 2000. UV radiation effects on microbes and microbial processes. In Microbial ecology of the oceans; Kirchman, D. L., Ed.; Wiley: New York, pp: 201-228.
12. Frederick, J.E. and D. Lubin, 1988. The budget of biologically active ultraviolet radiation in the Earth- atmosphere system. J. Geophys. Res., 93: 3825-3832.
13. Moran J., A. Dahlback and R.B. Setlow, 1999. Epidemiological support for an hypothesis for melanoma induction indicating a role for UVA radiation. Photochem. Photobiol., 70(2): 243-247.
14. Santos, A.L., C. Moreirinha, D. Lopes, A.C. Esteves, I. Henriques, A.M. Almeida, M. Rosario, Domingues, V. Delgadillo, A. Correia and A. Cunha 2013. Effects of UV Radiation on the Lipids and Proteins of Bacteria Studied by Mid-Infrared Spectroscopy. Environ. Sci. Technol., 47: 6306-6315.
15. Arcas, M.C., J.M. Botia, A.M. Ortuño and J.A. Del, 2000. UV irradiation Alters the Levels of Flavonoids Involved in the Defence Mechanism of *Citrus aurantium* Fruits against *Penicillium digitatum*. European J. Plant Pathol., 106(7): 617-622.
16. Idil, O., C. Darcan, T. Ozen and R. Ozkanca, 2013. The Effect of UV-A and Various Visible Light Wavelengths Radiations on Expression Level of *Escherichia coli* Oxidative Enzymes in Seawater. Jundishapur J. Microbiol., 6(3): 226-32.
17. Hoerter, J.D., A.A. Arnold, D.A. Kuczynska, A. Shibuya, C.S. Ward, M.G. Sauer, A. Gizachew, T.M. Hotchkiss, T.J. Fleming and S. Johnson, 2005. Effects of sublethal UVA irradiation on activity levels of oxidative defense enzymes and protein oxidation in *Escherichia coli*. J. Photochem. Photobiol., 81(3): 171-80.
18. Maria C., Canale, A., Eliane, Benato, C., Patricia L. Marineia, Haddad, F. Sergio and Pascholati, 2011. *In vitro* effect of UV-C irradiation on *Guignardia citricarpa* and on postharvest control of citrus black spot. Trop. Plant Pathol., 36(6): 43-47.

19. Behlau, F., J.J. Belasque, A. Bergamin-Filho and R.P.J. Leite, 2007. Citrus canker incidence and severity on 'Pera Rio' sweet orange under chemical control and windbreak protection. Fitopatologia Brasileira, 32(4): 311-317.
20. Goszczynska, T. and J.J. Serfontein, 1998. Milk Tween Agar, a semiselective medium for isolation and differentiation of *Pseudomonas syringae* pv. *syringae*, *Pseudomonas syringae* pv. *phaseolicola* and *Xanthomonas axonopodis* pv. phaseoli. Journal, Microbiological, Methods, 32(1): 65-72.
21. Gerhardt, P., 1981. Manual of methods of General Bacteriology. American Society of Microbiology, Washington DC, pp: 127.
22. 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.
23. Cowan, S.T., 1974. Cowan and Steel's manual for the identification of medical bacteria, 2nd ed. Cambridge University Press, Cambridge.
24. Schaad, N.W., 1992. *Xanthomonas*. In: Laboratory Guide for Identification of Plant Pathogenic Bacteria. 2nd ed. International Book Distributing Co. Lucknow, pp: 165.
25. Kovacs, N., 1956. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature, 178: 703.
26. Patel, M.K. and A.C. Padhya, 1964. Sodium arsenite, copper sulphate spray for the control of citrus canker. Curr. Sci., 33: 87-88.
27. Lin, H.C., H. Chang and K.C. Tzeng, 2008. Characterization of novel strains of citrus canker bacteria from citrus in Taiwan. J. Taiwan Agric. Res., 57: 265-278.
28. Chand, R. and R. Kishun, 1991. Studies on bacterial blight (*Xanthomonas campestris* pv. *punicae*) of pomegranate. Indian Phytopathol., 43: 370-371.
29. Pérez, M.T. and R. Sommaruga, 2007. Interactive effects of solar radiation and dissolved organic matter on bacterial activity and community structure. Environ Microbiol., 9(9): 2200-10.
30. Paul, H., T.P. María and R. Sommaruga, 2011. Contrasting effects of ultraviolet radiation on the growth efficiency of freshwater bacteria. Aquat Ecol., 45(1): 125-136.
31. Sundin, G.W. and J.L. Jacobs, 1999. Ultraviolet radiation (UV-R) sensitivity analysis and UVR survival strategies of a bacterial community from the phyllosphere of field-grown peanut (*Arachis hypogeae* L.). Microb. Ecol., 38(1): 27-38.
32. Coohill, T.P. and J.L. Sagripanti, 2008. Overview of the inactivation by 254 nm ultraviolet radiation of bacteria with particular relevance to biodefense. Photochem. Photobiol., 84(5): 1084-1090.