Effect of partially UV-blocking films on purple blotch of onion

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Abstract. The purple blotch of onion is the most important disease in Bangladesh as well as worldwide. UV-blocking films and pathogens were used in this experiment to testify the ability of UV radiations to suppress the purple blotch of onion disease development. Disease incidence and disease severity were measured from the pathogens, temperature, relative humidity, partially UV-blocking conditions. 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. Different pathogenic combination of *Alternaria porri* + *Stemphylium vasicarium*, *A. porri* and *S. vasicarium* was also inoculated to find out the pathogenic effect. The lowest disease incidence (43.2%) and severity (2.4%) was recorded in T6 (outdoors) and highest disease incidence (83.6%) and severity (41.8%) was recorded in T1 (<400 nm) under different UV treatments. The maximum incidence (80.5%) and severity (39.2%) was recorded in P1 (*A. porri* + *S. vasicarium*) and the minimum incidence (48.9%) and severity (4.7%) was recorded in P3 (*S. vasicarium*) under pathogen treatment. The highest incidence (91.6%) and severity (44.3%) was recorded in treatment T1P1 at 21 days after incubation (DAI) and the lowest incidence (34.7%) and severity (1.3%) was recorded in treatment T6P3 under combination of UV and pathogen treatments. Disease incidence and severity gradually decreased as the UV blocking rates decreased. Solar radiation which contains combination of different UV radiation intensity may be detrimental for multiplication of the pathogens.
Key Words: microclimate, pathogen, leaf spot, disease management, solar radiation.

Introduction. Onion (*Allium cepa*) is an important spices crop commercially grown in many countries of the world. It is used as important and popular vegetables in Australia, Belgium, India, Japan, United Kingdom, USA and many other countries. Out of 15 important vegetables and spice crops listed by FAO, onion stands second in terms of annual world production (Ali 2008). It ranks first in production among the spices crops cultivated in Bangladesh (BBS 2008). It is also used as the condiments for flavoring a number of foods and medicines (Hassan et al 2006). Besides being used as salad and vegetables, onion is generally used as spice in most of the Asian countries. Onion has great economic importance due to its medicinal and dietetic values (Chakraborty et al 2015). Global vegetable production of nearly 36 million tons onion per annum, next to tomatoes and cabbages bears importance (FAO 2012). The production of onion is nearly 1.159 million metric tons from 33,5000 acres of land. In Bangladesh requirement of onion per year is around 1.95 million metric tons per year (BBS 2012). The production of onion in Bangladesh is 8.95 t ha^{-1} (AIS 2011) which is very lower in comparison of other onion producing countries.

Onions are attacked by ten diseases in Bangladesh caused by various pathogens (Ahmed & Hossain 1985). Purple blotch of onion is noted as a major disease throughout the world including Bangladesh (Islam et al 2001). The fungus can cause a reduction in yield ranged from 30 to 50 % (Pascua et al 1997). The disease is characterized with small water-soaked lesions initially produce on leaves and seed stalk that quickly develop white centers. As lesions enlarge, they become zonate, brown to purple, surrounded by a yellow zone and extend upward and downward for some distance (Figure 1).

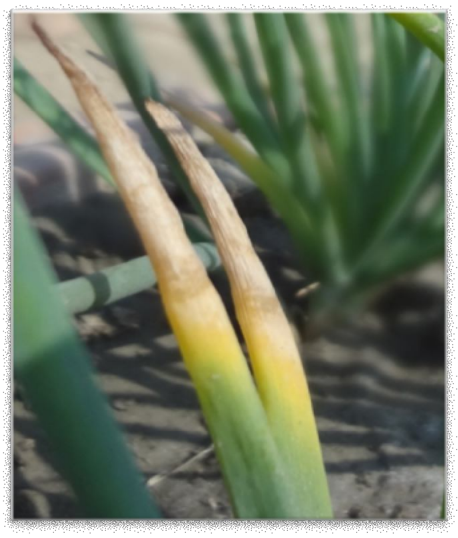


Figure 1. Typical symptom of purple blotch of onion (original).

Under humid condition, the surface of the lesion may be covered with brown to dark gray structure of fungus. A few large lesions have been formed in a leaf or seed stalk which may coalesce and girdle the leaf or seed stalks and tissues, distal to the lesions die. Usually the affected leaves or seed stalks fall down and die within 4 weeks if the environment favors the disease (Gupta et al 1986). Temperature and humidity are the most predominant factors for the development of purple blotch disease. The disease is favored by moderate temperature (24-30°C) and high relative humidity (Gupta & Pathak 1986; Everts & Lacy 1990; Rodriguez et al 1994).

Many research works have been conducted on the management of purple blotch of onion (Ashrafuzzaman & Ahmed 1976; Rahman et al 1988; Rahman 1990). Rovral 50 WP (0.2%), Dithane M-45 80 WP (0.2) and some other options are suggested as foliar spraying against the disease. But nowadays the fungicides are not working properly against the disease. Ultraviolet (UV) radiation is an important stress factor and may be an effective way to reduce this disease (Moran & Zepp 2000). 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 (Frederick & Lubin 1988; Moran et al 1999). 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 (Santos et al 2013). 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. Therefore, the present study was undertaken to investigate the effect of partially UV-blocking films shorter than UV-A on purple blotch of onion disease.

Material and Method. 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 on the Horticultural farm, Department of Horticulture, Sher-e-Bangla Agricultural University, Dhaka for observing the effect of partially UV blocking shorter than UV-A on purple blotch of onion.

Forty five plastic mini houses of 1.8×1.9×0.6 m (L×B×H) with different polyolefin (PO) films (0.13 mm thickness) were prepared for experimentation. 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 (T5) and outdoors (T6). 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 were transferred in those mini houses and three pots were put under each tunnel.

Measurement of environmental conditions. Temperature, humidity, visible and UV-light irradiations were measured in daily basis during the experiment. Temperature relative humidity and UV-light irradiations were recorded at 12:00.

Experimental design. The experiment was laid out following complete randomized design (CRD) with three replications and consisted two factors, namely factor A: UV treatment (level 6) viz., T1 - <400 nm, T2 - <360 nm, T3 - <350 nm, T4 - <340 nm, T5 - UV and T6 - outdoor and factor B: pathogen (level 3) viz., P1 - *Alternaria porri* + *Stemphylium vasicarium*, P2 - *Alternaria porri* and P3 - *Stemphylium vasicarium*.

Isolation and identification of the pathogen. The pathogen was isolated by tissue planting method from infected onion leaves (Hasan 2008). The surface of the working clean bench was sterilized with ethanol (70%). Then the infected onion samples were taken into the clean bench and cut into small pieces (0.5-1.0 cm) the cut pieces were sterilized in $HgCl_2$ solution (1:1000) for 1.5 minutes and then taken out with the help of sterile forceps and put on sterile distilled water to wash the samples and washing was repeated 3 times. After washing, the cut pieces were placed on sterilized blotter paper (Whatman No. 1) in Petri plates and also placed onto the PDA plates, incubated at 250ºC under near ultraviolet light following ISTA rules (ISTA 1996). Five to seven days after incubation the fungal culture were studied under stereoscope (Motic, SMZ-168) and compound microscope (Omano, OMTM-85) for identification of the desired pathogen.

Culture medium preparation and preservation of pathogen. Potato dextrose agar (PDA) was used for these experiments. The dehydrated PDA was hydrated in distilled water at 39 g L^{-1} in a conical flask (250 mL) and autoclaved at 121°C under 15 psi for 30 minutes. After autoclaved the media was kept few minutes for cool and added 25-30 drops of lactic acid then poured into sterile Petri plates. The axenic culture of the isolates from the PDA were transferred to PDA slants using hyphal tip culture method and preserved in refrigerator at 4±0.5°C for further use.

Plot preparation and sowing of onion bulbs. Air dried sandy loam soil and cow dung were mixed thoroughly at the ratio of 4:1 and filled in earthen pots (20 cm diameter). For testing the virulence levels of pathogens a local onion cultivar, Taherpuri was collected from Savar Bazar, Dhaka. Bulbs were sown at 4-5 cm depth in the pot soil.

Inoculation and incubation. The plants were inoculated by pathogens with 21×105 spore/mL at 30 days after planting. The conidia suspension of pathogens were prepared with sterilized water using 10 days old PDA culture incubated at 22-24°C under NUV light (12/12). The suspension was sieved through a double layered of cheese cloth to remove mycelia fragments and conidiophores. One drop of tween-20 (polyoxyethylene 20 sorbitan monolaurate) was added to the suspension to maintain uniform dispersion of conidia in suspension. The concentration of conidial suspension was adjusted to 21×105 per mL. The onion plants were inoculated by conidial suspension with hand sprayer and the inoculated plants were covered with polyethylene sheet to maintain high relative humidity (% RH) and also to prevent natural contamination with other fungal conidia or spores. The plants were kept in a net house at 25±2°C and humid condition was maintained by gently spraying sterilized distilled water.

Recording of data. Data on disease incidence and severity were collected after 7, 14 and 21 days of inoculation. Assessment of disease incidence and severity was calculated using the following formula:

Disease incidence (%) = No. of diseased leaf among the inoculated leaf in each plant/No. of total inoculated leaf in each plant

Disease severity (%) = Amount of diseased in the inoculated leaf in each plant/Amount of total diseased in the inoculated leaf in each plant

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 Discussion

Isolation and identification. The isolated pathogens were identified as *A. porri* and *S. vasicarium* according to their cultural and morphological characteristics. Olive green with concentric ring colony and muriform with short beak conidia was found for *A. porri* and pale brown colony with muriform, ovoid shape conidia was found for *S. vasicarium* on PDA plates after 7 days of incubation at 25±1°C (Figure 2 & 3).

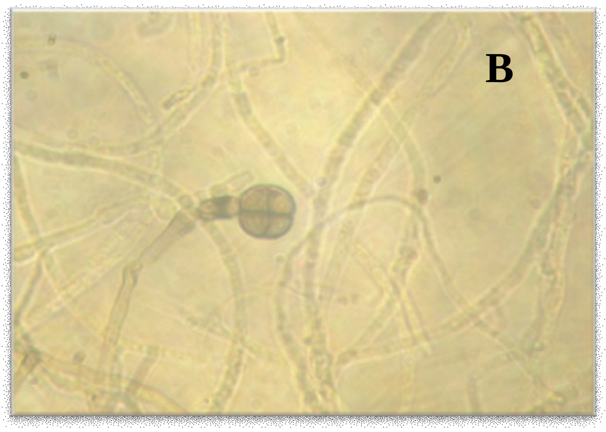
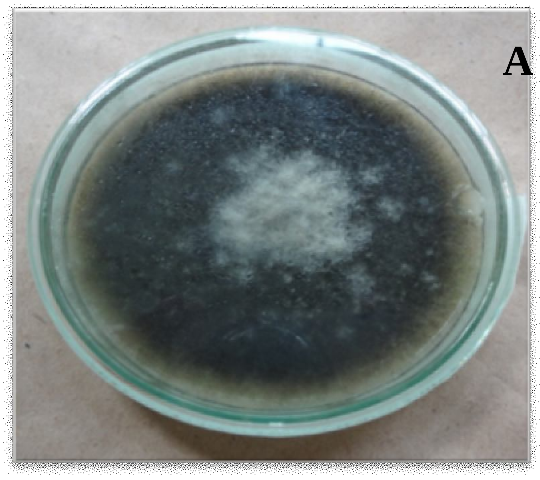


Figure 2. Identification of pathogen under compound microscope, (A) *Alternaria porri* (B) *Stemphylium vasicarium* (original).



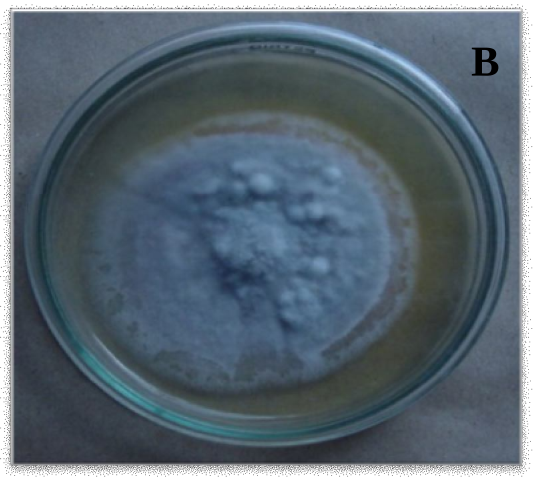


Figure 3. Pure culture of pathogens in PDA media, (A) *Alternaria porri* (B) *Stemphylium vasicarium* (original).

Incidence and severity of purple blotch of onion under different UV treatments. Disease incidence and severity of purple blotch of onion were observed at different days after incubation (DAI) under different UV treatments (Table 1). Incidence of purple blotch of onion varied significantly under different UV intensities ranged from 43.2 to 83.5% at 7 DAI and 21 DAI. The highest incidence 83.5% was recorded in treatment T1 at 21 DAI and lowest incidence 43.2% was recorded in treatment T6 at 7 DAI. Under different ultraviolet intensities severity also varied significantly ranged from 2.4 to 41.8% at 7 DAI and 21 DAI (Table 1). The highest severity 41.8% was recorded in treatment T1 at 21 DAI and lowest severity 2.4% was recorded in treatment T6 at 7 DAI.

Table 1

Incidence and severity of purple blotch of onion under different ultraviolet intensity

Treatment	*Disease incidence (%)*			*Disease severity (%)*		
	7 DAI	*14 DAI*	*21 DAI*	*7 DAI*	*14 DAI*	*21 DAI*
T_1	68.97 a	74.56 a	83.59 a	10.89 a	31.06 a	41.81 a
T_2	67.77 a	71.15 a	82.20 a	10.67 a	31.00 ab	41.61 a
T_3	54.19 ab	65.43 ab	73.56 ab	5.578 b	22.61 bc	37.44 a
T_4	54.14 ab	63.41 ab	72.76 ab	5.433 b	22.78 abc	37.28 a
T_5	44.24 b	53.13 b	64.10 b	2.744 c	15.06 c	30.67 b
T_6	43.20 b	52.41 b	63.47 b	2.433 c	15.11 c	30.22 b
Significance	**	**	*	**	**	*

T_1 - <400 nm, T_2 - <360 nm, T_3 - <350 nm, T_4 - <340 nm, T_5 - UV, T_6 - outdoor.

Incidence and severity of purple blotch of onion under different pathogen treatments. Disease incidence and severity of purple blotch of onion were observed by inoculation of different pathogen (Table 2). Incidence of purple blotch of onion varied significantly under different pathogen ranged from 48.9 to 80.5% at 7 DAI and 21 DAI. The highest incidence 80.58% was recorded in treatment P1 at 21 DAI and lowest incidence 48.9% was recorded in treatment P3 at 7 DAI. Under different ultraviolet intensities severity also varied significantly ranged from 4.7 to 39.2% at 7 DAI and 21 DAI (Table 2). The highest severity 39.2% was recorded in treatment P1 at 21 DAI and lowest severity 4.7% was recorded in treatment P3 at 7 DAI.

Table 2

Incidence and severity of purple blotch of onion under different pathogen

Treatment	*Disease incidence (%)*			*Disease severity (%)*		
	7 DAI	*14 DAI*	*21 DAI*	*7 DAI*	*14 DAI*	*21 DAI*
P_1	63.53 a	73.19 a	80.58 a	8.011 a	25.25 a	39.25 a
P_2	53.78 a	68.27 ab	74.21 ab	6.067 b	22.94 ab	36.36 ab
P_3	48.94 a	59.36 b	65.05 b	4.794 b	20.61 b	33.91 b
Significance	NS	*	*	*	**	*

P_1 - *Alternaria porri* + *Stemphylium vasicarium*, P_2 - *Alternaria porri*, P_3 - *Stemphylium vasicarium*.

Incidence and severity of purple blotch of onion under different combination of UV treatments and pathogen treatments. Disease incidence and severity of purple blotch of onion were observed by different combination of ultraviolet intensity and pathogen (Table 3). Incidence of purple blotch of onion varied significantly under different combination of ultraviolet intensity and pathogen ranged from 34.7 to 91.6% at 7 DAI and 21 DAI. The highest incidence 91.6% was recorded in treatment T1P1 at 21 DAI and lowest incidence 34.7% was recorded in treatment T6P3 at 7 DAI. Under different ultraviolet intensities severity also varied significantly ranged from 1.3 to 44.3% at 7 DAI and 21 DAI (Table 3). The highest severity 44.3% was recorded in treatment T1P1 at 21 DAI and lowest severity 1.3% was recorded in treatment T6P3 at 7 DAI.

Table 3
Incidence and severity of purple blotch of onion under different combination of ultraviolet intensity and pathogen

Treatment	Disease incidence (%)			Disease severity (%)		
	7 DAI	14 DAI	21 DAI	7 DAI	14 DAI	21 DAI
T_1P_1	73.80 a	81.58 a	91.67 a	13.83 a	33.17 a	44.33 a
T_1P_2	68.20 ab	75.19 ab	84.10 ab	10.17 b	31.33 ab	41.83 ab
T_1P_3	64.90 abc	64.01 bcd	75.00 bcd	8.667 bc	28.67 bc	39.27 bc
T_2P_1	73.80 a	80.15 a	91.07 a	13.50 a	33.00 a	44.33 a
T_2P_2	66.83 ab	71.27 abc	82.20 abc	10.17 b	31.33 ab	41.67 ab
T_2P_3	62.67 abc	62.13 bcd	73.33 bcd	8.333 bcd	28.67 bc	38.83 bc
T_3P_1	63.20 abc	68.23 abc	79.43 abc	6.833 cde	25.67 cd	40.33 bc
T_3P_2	51.77 abcd	64.00 bcd	75.00 bcd	5.233 efg	21.83 e	37.33 cd
T_3P_3	47.60 bcd	55.33 de	66.23 de	4.667 fgh	20.33 ef	34.67 de
T_4P_1	63.87 abc	68.23 abc	79.43 abc	6.633 def	25.67 cd	40.33 bc
T_4P_2	51.77 abcd	62.53 bcd	73.80 bcd	5.333 efg	22.50 de	37.17 cd
T_4P_3	46.80 bcd	54.04 de	65.03 de	4.333 ghi	20.17 ef	34.33 de
T_5P_1	54.17 abcd	60.68 cd	70.87 cd	3.667 ghi	17.00 fg	33.17 ef
T_5P_2	41.63 cd	53.76 de	64.90 de	3.167 hij	15.33 gh	30.17 fg
T_5P_3	36.93 d	45.48 e	56.53 e	1.400 j	12.83 h	28.67 g
T_6P_1	52.37 abcd	61.08 bcd	71.00 bcd	3.600 ghi	17.00 fg	33.00 ef
T_6P_2	42.50 cd	55.78 de	65.23 de	2.333 ij	15.33 gh	30.00 fg
T_6P_3	34.72 d	43.47 e	54.17 e	1.367 j	13.00 h	27.67 g
Significance	**	**	**	**	**	**

T_1 - <400 nm, T_2 - <360 nm, T_3 - <350 nm, T_4 - <340 nm, T_5 - UV, T_6 – outdoor.
P_1 - *Alternaria porri* + *Stemphylium vasicarium*, P_2 - *Alternaria porri*, P_3 - *Stemphylium vasicarium*.

Effect of environmental conditions under different UV treatments. Temperature, relative humidity and ultraviolet intensity varied significantly under different treatments. The maximum temperature 30.90°C was found from treatment T1 which was statistically similar with T2 (30°C) and T3 (29.60°C) whereas the minimum temperature 25.47°C was found from T6 which was statistically similar with T5 (26.43°C) (Table 4). The maximum relative humidity 69.67% was found from treatment T1 which was statistically similar with T2 (68.17%) whereas the minimum relative humidity 61% was found from T6. The maximum UV intensity 1567 mW/cm^{-2} was found from treatment T6 whereas the minimum UV intensity 173.1 mW/cm^{-2} was found from treatment T1.

Table 4
Effect of partially UV-blocking films on temperature, relative humidity and UV intensity during the experiment

Treatment	Temperature (°C)	Relative humidity (%)	UV (mW/cm^{-2})
T_1	30.90 a	69.67 a	173.1 d
T_2	30.00 a	68.17 ab	216.4 c
T_3	29.60 a	66.17 bc	223.1 c
T_4	27.40 b	66.17 bc	235.2 c
T_5	26.43 bc	64.00 c	710.7 b
T_6	25.47 c	61.00 d	1567 a
Significance	**	**	**

T_1 - <400 nm, T_2 - <360 nm, T_3 - <350 nm, T_4 - <340 nm, T_5 - UV, T_6 - outdoor.

Relationship between weather factors and incidence as well as severity of purple blotch of onion. Correlation co-efficient and regression equation were calculated to find out the effect of temperature, relative humidity and UV intensity (mW/cm^{-2}) on disease incidence and severity of purple blotch of onion (Table 5). Plants treated with different wavelengths of UV radiation showed gradually decreasing disease incidence and severity with decreasing temperature and relative humidity and with increasing UV radiation. Correlation co-efficient and linear regression analysis was performed to determine the relationship between different components of climatic factor (temperature, RH% and UV) and the incidence as well as severity of purple blotch of onion. Negative correlation coefficient were found among temperature (r^2 = -0.04) with disease incidence and RH% (r^2 = -0.81, -0.02) and UV intensity (r^2 = -0.88, -0.76) with both disease incidence and severity respectively but positive correlation coefficient was found among temperature (r^2 = 0.89) with disease severity (Figure 4, A-F).

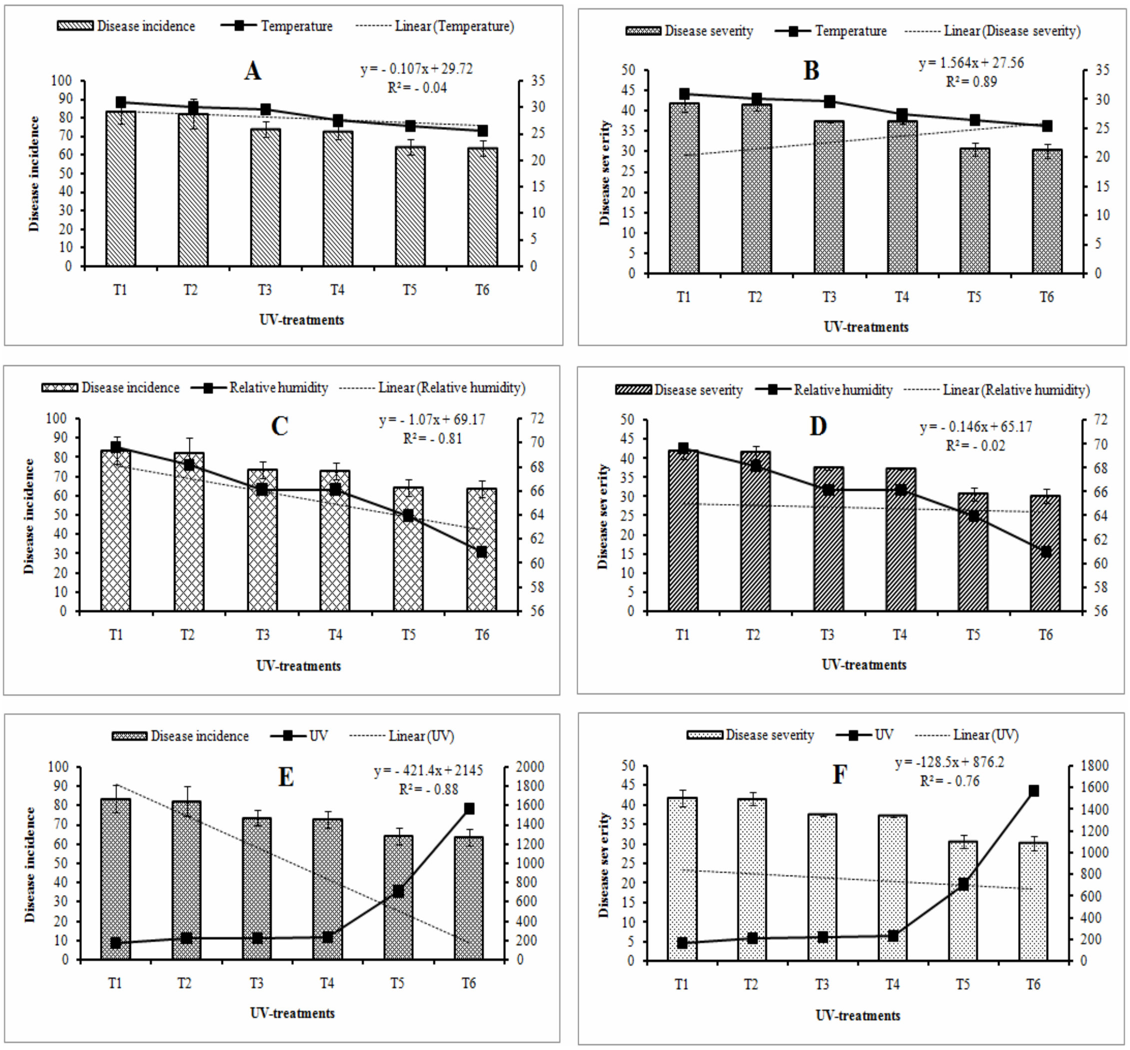


Figure 4. Correlation of temperature, relative humidity (RH%) and UV intensity with disease incidence and severity. A - correlation between temperature and incidence; B - correlation between temperature and severity; C - correlation between humidity and incidence; D - correlation between humidity and severity; E - correlation between UV intensity and incidence; and F - correlation between UV intensity and severity.
T1 - <400 nm, T2 - <360 nm, T3 - <350 nm, T4 - <340 nm, T5 - UV-transmitting and T6 - outdoors.

Table 5

Linear correlation analysis of the effect of temperature, relative humidity and UV on the incidence and severity of purple blotch of onion

Climatic factors	Slope (b)		Correlation co-efficient (r^2)		Probability (p)		Intercept	
	Incidence	*Severity*	*Incidence*	*Severity*	*Incidence*	*Severity*	*Incidence*	*Severity*
Temp.	- 0.089	0.057	- 0.04	0.89	0.001	0.028	29.72	32.3
RH	- 0.315	- 0.163	- 0.81	- 0.02	0.023	0.006	69.17	65.17
UV	- 298.4	- 22.23	- 0.88	- 0.76	0.005	0.224	2145.45	876.29

The highest incidence 83.5% and severity 41.8% was recorded in treatment T1 at 21 DAI and the lowest incidence 43.2% and severity 2.4% was recorded in treatment T6 at 7 DAI under different UV treatments. The maximum incidence 80.5% and severity 39.2% was recorded in treatment P1 at 21 DAI and the minimum incidence 48.9% and severity 4.7% was recorded in treatment P3 at 7 DAI under pathogen treatment. The highest incidence 91.6% and severity 44.3% was recorded in treatment T1P1 at 21 DAI and the lowest incidence 34.7% and severity 1.3% was recorded in treatment T6P3 at 7 DAI under combination of UV and pathogen treatments. Maximum disease incidence and severity were found in treatment T1 (<400 nm), where only visible light were passed and all kinds of UV radiation were blocked by the film. On the other hand, minimum disease incidence and severity were measured in treatment T6 (outdoor) in which no UV protecting film was used and plant got the complete solar radiation (UV-A+UV-B). UV-A was blocked gradually by the treatment T1 to T4 resulted decrease of disease incidence and severity. UV-A may cause indirect damage by producing ROS at cellular level and UV-B may cause direct damage by breaking DNA of pathogen. The highest disease incidence and severity were found in treatment P1 where both pathogens *A. porri* and *S. vasicarium* were present and the lowest disease incidence and severity were found in treatment P3 where only *S. vasicarium* was present. In case of combination of treatments maximum disease incidence and severity were found from T1P1 where only visible light were passed and all kinds of UV radiation were blocked by the film and both *A. porri* and *S. vasicarium* were present. Minimum disease incidence and severity were found from T6P3 where no UV protecting film was used and plant got the complete solar radiation and *S. vasicarium* was present. The results also revealed that temperature, relative humidity and UV intensity may have important role in disease development.

Disease incidence and severity were decreasing with the decreasing of temperature and relative humidity but disease incidence and severity were decreasing with the increasing of UV intensity. Higher temperature and relative humidity were found in treatment T1 but UV intensity was lower. Srivastava et al (1994) found that the high incidence (2.5-87.8%) of purple blotch in both the kharif and rabi onions, when high humidity prevailed, during the 5 years of the survey (1988-1993). Khare & Nema (1984) found that temperature between 22°C to 25°C and relative humidity 90% are the best for the development of purple blotch of onion. The findings indicate that, higher UV intensity has significant role to reduce disease incidence and severity of purple blotch of onion and UV intensity also showed highly negative correlation with both disease incidence and severity. UV intensity might be attack the conidia production and sporulation of the pathogen that why disease incidence and severity was found lower in higher UV intensity. Vakalounakis (1981) found that conidia are formed in colonies of *Alternaria cichorii* when exposed to UV radiation of wave lengths less than 340 nm but none are formed in colonies exposed to violet, blue-green, yellow, orange or red light. Sporulation in *A. cichorii* presents two distinct phases: an 'inductive phase' leading to formation of conidiophores and a 'terminal phase' leading to formation of conidia (Vakalounakis 1981; Vakalounakis & Christias 1986). The inductive phase is induced by near-UV radiation of wavelengths below 340 nm and proceeds most efficiently at high temperatures, while the terminal phase is inhibited by blue light (360-530 nm) and is operative at lower temperatures.

Conclusions. Purple blotch of onion is the major constraint for lowering the onion yield in worldwide including Bangladesh. The research was carried out to find out the impact of UV radiations and pathogens. It can conclude that higher UV-A radiations can reduce purple blotch of onion in tropical and subtropical area and it can also conclude that *A. porri* are more responsible to create purple of onion than *S. vasicarium*. By this research proper sustainable management for purple blotch of onion disease can be implemented and further similar investigations are needed to clarify the individual effect of UV-B against the disease.

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