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Screening of Selected Garlic Varieties Against White Rot Disease Caused by *Sclerotium cepivorum* at Dhaka City of Bangladesh

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Abstract: White rot caused by *Sclerotium cepivorum* fungus is a crucial soil borne disease on garlic damaging allium production globally including Bangladesh. In this study eight isolates of *Sclerotium cepivorum* were isolated from naturally infected garlic plants collected from the central farm and isolated in the central lab of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh to determine the prevalence of white rot disease incidence and severity and characterized for cultural and morphological variabilities of the isolated pathogen. Eight garlic varieties viz. BAU Rashun-1, BAU Rashun-2, BARI Rashun-1, BARI Rashun-2, BARI Rashun-3, BARI Rashun-4, Local Deshi and Local Indian were explored with three replication in the study. Results showed that the highest disease incidence and severity against white rot disease (33.33% and 89.33%) were appeared in the BARI Rashun-3 variety. Lowest disease incidence and severity (5.00% and 60%) were recorded on Local Indian variety. Almost all of the isolates had cottony fluffy growth on potato dextrose agar. The colony color varied from milky white to cottony watery white with regular, circular, smooth, cotton like sometimes striped surface texture was found. After 10 days of incubation all the isolates had suppressed growth on PDA media as it is a fast growing pathogen.

Keywords: Garlic White Rot, *Sclerotium cepivorum*, Disease Incidence, Disease Severity and Fungal Pathogen

1. Introduction

Garlic (*Allium sativum* L.) is an annual tuberous spice of the Alliaceae family next to onion that is inherent to Central and South Asia. For thousands of years garlic has been used as a functional food, spice and seasoning herb, as well as an effective, traditional medicine against different ailments including viral diseases. The highest garlic production countries in the world have been reported are China, India, South Korea, Egypt and USA [1]. Garlic is thought to be one of the most essential spice crops produced on a large scale in Bangladesh.

In spite of being a medicinal plant, garlic is attacked by various pathogenic agents that caused heavy economic losses to the crops. Among pathogenic agents particularly soil borne fungal pathogen *Sclerotium cepivorum* causes disease known as garlic white rot is a drastic yield reducing disease which prevalent in many Allium growing regions around the world [2-6]. The disease was first noticed in 1929 in Egypt [7] and then spread globally all over the world viz. Brazil [8], Netherlands, Spain, Switzerland, United Kingdom [9], Australia [10, 11], Canada [12], New Zealand [13], USA [14], Mexico [15], Iran [2] and Argentina [16].

Sclerotium cepivorum is the most devastating fungal pathogen and has critical concerns to the crops because once the sclerotia are in the soil; they can stable for up to 40 years in absence of host plant and for that it is difficult to control [17, 18]. The pathogen has caused great damage in different

garlic growing regions globally and causes serious reduction in the production of garlic crops. Earnshaw, Donald and Boland reported that white rot disease causes 100% yield loss in Mexico and Brazil [19]. Garlic and onion production were enforced to abandon by some growers of garlic growing regions in Minas Gerais and Sao Paulo states owing to presence of high levels of sclerotia in soil [20, 21]. In the cultivated field of garlic of Northern Showa of Ethiopia, owing to white rot disease incidence ranging from 37.28% to 42% [22]. Mycelium of *S. cepivorum* spread from plant Vieira, Lopes and Villata stated that white rot of garlic causes total crop losses when sclerotia levels are high in soil and environmental conditions favorable for disease development [25].

White rot is a major production threat of garlic in Bangladesh. The United Nations Food and Agriculture Organization stated that from 1,634,634 hectares of world area harvested garlic produce about 30,708,243 tonnes of garlic globally each year [26]. Total production of Garlic in the year of 2019 in Bangladesh is 466, 389 tonnes from 71734 ha land with an average yield of 65,016 hg/ha. Production of garlic is drastically reduced in presence of high amount of sclerotia in soil under favorable condition for disease development, inappropriate agronomic practices, imbalanced fertilizer, uneven irrigation and lac k of improved varieties [27]. The purpose of this current study was to assess the occurrence of white rot % disease incidence and severity of selected garlic varieties under natural condition and isolate and identify the pathogen for morphological characterization.

2. Materials and Methods

2.1. Description of the Experimental Site

A field experiment were conducted during the Rabi season of 2019-2020 at the central farm of Sher-e-Bangla Agricultural University and in the central Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh (23°41′N latitude and 90°22′E longitudes at the elevation of 8.6 m above the sea level, AEZ-28) on eight different varieties of garlic with three replications consists of 24 unit plots in Randomized complete block design (RCBD).

2.2. Source of Garlic Cloves

Eight several pure and disease-free garlic variety seed were culled from three different places. BAU Rashun-1 (V_1) and BAU Rashun-2 (V_2) varieties were brought from Bangladesh Agricultural University, Horticulture Department, Mymensingh. BARI Rashun-1 (V_3), BARI Rashun-2 (V_4), BARI Rashun-3 (V_5) and BARI Rashun-4 (V_6) varieties were collected from Bangladesh Agricultural Research Institute, Joydebpur, Gazipur and last two local varieties naming Local Deshi (V_7) and Indian Local (V_8) were collected from Siddik bazar, Dhaka, Bangledesh.

2.3. Method of Data Collection

The plant population in each plot was counted and average plant populations in three replications were considered as mean plant population density. Garlic varieties were observed on the basis of symptoms appeared on the above ground plants and disease incidence and severity was estimated by computing the number of plant showing white rot symptoms viz. yellowing, discoloration, rotten bulb, presence of sclerotia around the base of the plant etc.

2.3.1. (%) Disease Incidence

To estimate the percent disease incidence each plant was counted including infected one in the field and then expressed in percentage. For the determination of disease incidence of garlic the following formula given by [28] was used.

$$\% \text{ DI} = \frac{\text{Number of diseased plants}}{\text{Number of total plants observed}} \times 100 \quad (1)$$

2.3.2. % Disease Severity

Disease severity of white rot was computed by observing the level of damage by the pathogen using 0-5 scale and converted to percentage as follows by selecting 10 plants randomly from each plot and used for PDI (percent disease index) estimation.

Grade	Symptoms description
0	No infection
1	1-10% infection
2	11-20% infection
3	21-30% infection
4	31-50% infection
5	>50% infection

The percent disease index (PDI) was calculated according to the formula given by [29, 30].

$$\text{PDI} = \frac{\text{Total sum of numerical ratings}}{\text{Number of observations x Maximum disease rating}} \; x\; 100 \quad (2)$$

2.4. Isolation and Identification of Sclerotium Cepivorum

Naturally infected garlic plant showing white rot symptoms were collected from the field, placed in brown paper and were transferred to the central laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for isolation of the pathogenic agent. Isolation process was performed by cutting the diseased leaves into pieces with the help of sterilized scalpel, washed with distilled water and surface sterilized using 0.1 percent $MgCl_2$ solution (30 to 60 seconds) then were washed three times right away with double distilled water frequently to eradicate the certainty of mercuric chloride and dried with a towel on sterilized filter paper.

Disinfected leaves then placed to petridish containing 20 ml of autoclaved water agar (Agar 20 g with 1000 ml distilled water) in a laminar flow and incubated at 25±1°C near ultra-violate light for 10 days. After 10 days the growing mycelia on water agar petridish were transferred to potato dextrose agar media (200g of peeled potatoes, 20 g of dextrose, and 20 g of agar and 1000 ml of distilled water). At

10 days the fungus grew well and sporulated then freshly prepare slide was observed under compound microscope and digital microscope for the identification of the pathogen using relevant literature. The colonies of grown fungus were purified and pure culture were maintained by sub culturing at an interval every 15 days and preserved at low temperature (4°C) in refrigerator for future purpose. The observations were computed with the standard measurements and the fungal isolates were identified following by [31] (Figure 1).

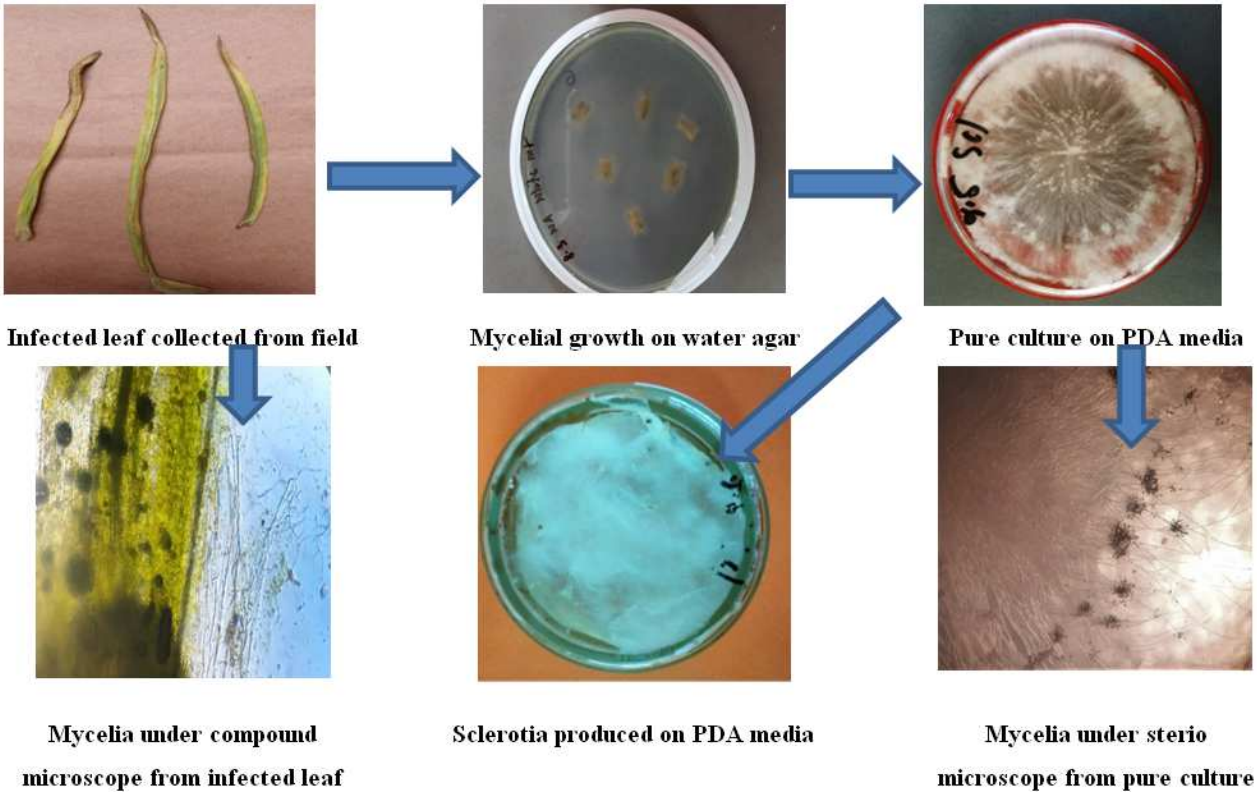

Figure 1. *Flow chart of isolation, identification and pure culture of Sclerotium cepivorum.*

2.5. Designation of Cultured Isolates

The cultured isolates were designated based on variety and location [32]. For example BAU_1I_1 represents that this isolate was cultured from BAU Rashun-1 variety.

2.6. Cultural Variability of Sclerotium Cepivorum

In cultural variation the colony diameter was recorded on the 2nd, 7th and 10th days after incubation. The data on radial growth was analyzed statistically [33]. Growth per day was calculated by the followed formula:

$$\text{mm/day}=(\text{growth observed on a day - growth on previous observation})/2.$$

2.7. Morphological Variability of Sclerotium Cepivorum

Ten days old cultures of *S. cepivorum* isolates were studied for morphological variations. In terms of conidia color, shape, size, colony character and surface structure were observed on PDA medium.

2.8. Statistical Analysis

The obtained data of different characters were subjected to statistically analyses to see the significant difference among mean using the MSTAT-C program. Conversions of the data were required when necessary. The mean values were then calculated and compared using Duncan's Multiple Range test. The least significant differences (LSD) at 5% level of probability were used to separate the means within the parameters [34].

3. Results

3.1. Evaluation of Selected Garlic Varieties Against White Rot Diseases at Field Condition

White rot is generally introduced to garlic plants through contaminated plant materials or soil. Once a small area of the field is contaminated it gradually spread to healthy plants and soil. Initially the stunted plants of garlic were considered as the first sign of white rot. Yellow striped leaves were spotted on the top of the garlic plants and eventually stopped producing leaves. The infected leaves were decayed at the base, turn yellow, wilt, and toppled over older leaves. Roots of the plants were rotted, and the top of the plant can be pulled out of the ground easily. Fluffy white mycelium was found on the remaining roots and bulb. As the infected bulbs dries and shrinks the outer scales crack it became watery (Figure 2).

A. B. C. D.

Figure 2. *Symptoms of white rot disease A. Symptoms on plants; B. Symptoms at early stage; C. Severe symptoms at late stage; D. Symptoms on harvested plant.*

3.2. Prevalence of % Disease Incidence and Disease Severity of White Rot Disease Among Selected Garlic Varieties

Though white rot of garlic prevailed on garlic plant after 45 days of sowing, data were collected concerning disease incidence and severity after 60 DAS. Percent disease incidence and severity on different varieties of garlic showed significant variation at 60 DAS and 90 DAS are shown in Table 1. The disease incidence varied from 5.00% to 33.33%. Besides, the disease severity varied from 36.00% to 89.33%.

At 60 DAS, BARI Rashun-3 scored the maximum disease incidence (29.44%). The highest disease severity (63.33%) was found on BARI Rashun-3 variety that was statistically similar to BARI Rashun-4 (58.67%) variety, respectively. On the contrary, Local Indian variety gave the lowest disease incidence (5.00%) which was statistically alike with BAU Rashun-2 (6.11%). The lowest disease severity was noted 36.00% on Local Indian variety.

At 90 DAS, the highest disease incidence (33.33%) and disease severity (89.33%) was observed on BARI Rashun-3 variety. BARI Rashun-4 gave the second highest disease incidence (21.11%) and disease severity (77.33%). On the other hand, Local Indian variety showed the lowest disease incidence (5.00%) and disease severity (60.00%) which was statistically similar to BAU Rashun-2 disease incidence of 6.11% and disease severity of 64.00%.

Table 1. *Prevalence of % disease incidence and severity of white rot disease among selected garlic varieties.*

Variety	(%) Disease Incidence		(%) Disease severity	
	60 DAS	90 DAS	60 DAS	90 DAS
BAU Rashun-1	7.22 de	7.22 d-f	44.00 bc	63.33 c
BAU Rashun-2	6.11 e	6.11 ef	47.33 bc	64.00 c
BARI Rashun-1	9.44 cd	9.44 cd	46.67 bc	69.33 bc
BARI Rashun-2	12.22 c	12.22 c	46.67 bc	70.67 bc
BARI Rashun-3	29.44 a	33.33 a	63.33 a	89.33 a
BARI Rashun-4	17.78 b	21.11 b	58.67 ab	77.33 b
Local Deshi	7.22 de	8.33 de	44.00 bc	66.00 bc
Local Indian	5.00 e	5.00 f	36.00 c	60.00 c
CV	15.37	14.19	18.65	9.65

CV=Coefficient of variance; in a column mean values having similar letter (s) are statistically similar and those having dissimilar letter (s) differ significantly as per 0.01% level of significance.

3.3. Cultural Studies of Sclerotium Cepivorum

Sclerotium cepivorum is a fast growing pathogen in PDA media. The reviews on colony shape and growth of the pathogens were noted for 10 consecutive days (Table 2).

Colony growth of the pathogen appeared after 2 days of inoculation. Maximum increase of colony diameter (5.20 mm) was noted on BAU_2 I_2 and LD I_7, respectively. Conversely, the minimum radial mycelial growth was observed on $BARI_1$ I_3 (4.10 mm). After 7 days of inoculation, $BARI_3$ I_5 scored 7.65 mm maximum colony diameter. The minimum increment of colony diameter (6.00 mm) was found in $BARI_4$ I_6. As the pathogen is fast growing, at 10 DAI the maximum pathogen covered the whole petridish of 9.00 mm except $BARI_1$ I_3 (7.65 mm) and $BARI_2$ I_4 (7.00 mm).

Table 2. *Radial mycelial growth of Sclerotium cepivorum.*

Isolates	Radial mycelial growth (mm)		
	2 DAI	7 DAI	10 DAI
BAU_1 I_1	4.50	7.50	9.00
BAU_2 I_2	5.20	7.45	9.00
$BARI_1$ I_3	4.10	6.50	7.65
$BARI_2$ I_4	5.00	7.00	7.00
$BARI_3$ I_5	4.50	7.65	9.00
$BARI_4$ I_6	5.00	6.00	9.00
LD I_7	5.20	7.45	9.00
LIND I_8	4.50	7.50	9.00

In the column BAU_1 I_1=BAU_1 $Isolate_1$; BAU_2 I_2=BAU_2 $Isolate_2$; $BARI_1$ I_3=$BARI_1$ $Isolate_3$; $BARI_2$ I_4=$BARI_2$ $Isolate_4$; $BARI_3$ I_5=$BARI_3$ $Isolate_5$; $BARI_4$ I_6=$BARI_4$ $Isolate_6$; LD I_7=Local Deshi $Isolate_7$ and LIND I_8=Local Indian $Isolate_8$

3.4. Morphological Studies of Sclerotium Cepivorum

The pure culture of pathogen *S. cepivorum* was observed regularly under the microscope for morphological characteristics viz. color, shape and surface texture (Table 3, Figure 3).

Almost all of the isolates had cottony fluffy growth on potato dextrose agar. The colony color varied from milky white to cottony watery white. Smooth, cotton like sometimes striped surface texture was found. After 10 days of incubation all the isolates except $BARI_1$ I_3 and $BARI_2$ I_4

had suppressed growth on PDA media as it is a fast growing pathogen. The shape of the colony was regular, circular. At the full growth of *S. cepivorum* dark brown color cotton like sclerotia was found.

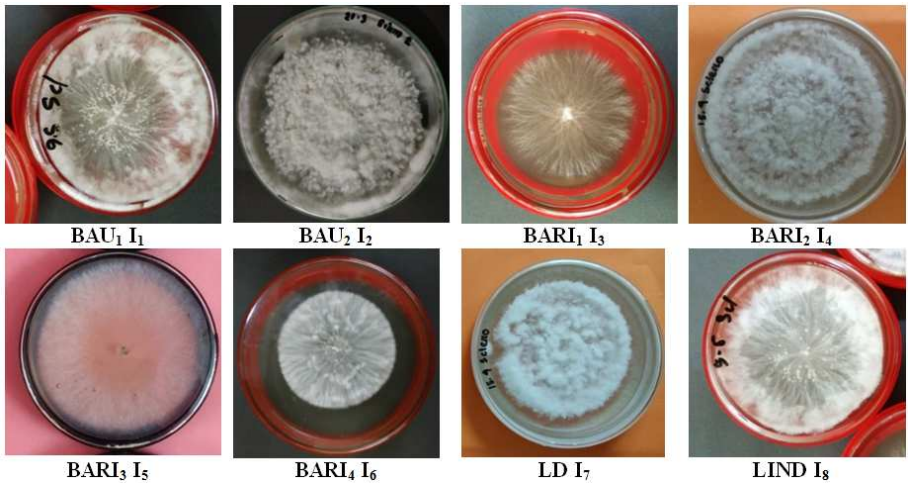

Figure 3. *Mycelial growth of Sclerotium cepivorum on PDA media at 10 days after incubation.*

Table 3. *Colony characteristics of Sclerotium cepivorum on PDA media.*

Isolates	Colony characteristics		
	Color	Surface texture	Shape
BAU_1 I_1	Milky white	Fluffy	Regular
BAU_2 I_2	Milky white	Fluffy	Irregular
$BARI_1$ I_3	Watery white	Cottony	Regular
$BARI_2$ I_4	Cottony white	Cottony	Regular
$BARI_3$ I_5	Watery white	Cottony	Regular
$BARI_4$ I_6	Milky white	Fluffy	Regular
LD I_7	Cottony white	Cottony	Regular
LIND I_8	Milky white	Fluffy	Irregular

In the column BAU_1 I_1=BAU_1 $Isolate_1$; BAU_2 I_2=BAU_2 $Isolate_2$; $BARI_1$ I_3=$BARI_1$ $Isolate_3$; $BARI_2$ I_4=$BARI_2$ $Isolate_4$; $BARI_3$ I_5=$BARI_3$ $Isolate_5$; $BARI_4$ I_6=$BARI_4$ $Isolate_6$; LD I_7=Local Deshi $Isolate_7$ and LIND I_8=Local Indian $Isolate_8$

4. Discussions

White rot showed stunted plant growth, followed by yellowing and death of the outer leaves. In severe cases the bulb completely rotten. Similar symptoms were found in Painter, Woodhall and Jenson where initially, infected plants showed yellowing and wilting of the leaves, starting with the older leaves [35]. Tamire, Cemeda, Sakuja and Seid stated that constant growing of garlic leads to congregate of the white rot sclerotia in the soil which exacerbates the occurrence of white rot [36]. Once the bulb is infected, the plant promptly loses vigor, the leaves turning yellow and wilt, and fluffy, white mycelia fill the bulb and then abundant sclerotia were spotted to increase future infections [37, 38]. The most easily noticeable symptom is the yellowing and dieback of the leaves beginning at the tips and progressing downward accompanied by death of the infected leaves [39].

BARI Rashun-3 scored the maximum disease incidence (29.44% and 33.33%) and severity (63.33% and 89.33%) at 60 and 90 DAS. Minimum disease incidence (5.00%) and severity (36.00% and 60.00%) founded on Local Indian variety at 60 and 90 DAS. It was similar to Crowe, Hall, Greathed and Baghott where the incidence of both onion and garlic white rot disease (10- 100%) [40]. The lowest disease incidence of white rot and highest yield in BARI Piaz-3, Indian big and Indian small among nine onion cultivars [41]. BARI Piaz-1 showed lower performance in respect of all parameters. A survey was conducted in different districts of Ethiopia where 97% of the fields of North Shewa of Ethiopia were infested with white rot. The highest mean disease incidence (77%) and severity (57%) were recorded from the garlic field of Menz Mama Midir as compared to the other districts [42].

Most of the isolates of *S. cepivorum* had cottony fluffy growth on potato dextrose agar. The colony color varied from milky white to cottony watery white. After 10 days of inoculation all the isolates had suppressed growth on PDA media as it is a fast growing pathogen. The result of the study was similar to [43] where the colony color of *S. vesicarium* varied from greenish brown to dirty white, deep grey to whitish, light grey to whitish, raised to flat and texture varying from cottony, fluffy to velvety. Arzanlou, Khodaei and Babai-Ahari reported that the colonies of *S. vesicarium* on PCA medium were grey to brownish grey, flat, attaining a diameter of 50 mm after 7 days with sparse aerial mycelium [44].

5. Conclusions

Garlic is considered as the second important spice crop next to onion in Bangladesh and grown under a wide range of favorable condition. Due to pathogenic and environmental factor garlic production is decreasing day by day. The present study was done to determine % disease incidence and severity owing to white rot and identification of the pathogen for morphological characterization. The study revealed that among eight selected garlic varieties BARI Rashun-3 variety showed the highest disease incidence (33.33%) and severity (89.33%) whereas, lowest disease incidence (5%) and severity (60%) was found in Local Indian variety. After 10

days of incubation in PDA media the growth of the pathogen suppressed the whole petridish. As the pathogen remains viable in the soil more than two decades and introduced by agronomic practices, so farmer should take some precautions. They should maintain a long term rotation schedule, should not follow allium crops with other crops. For the reduction of pathogen colonization they should use row spacing with reduced plant density.

Conflict of Interest

All authors declare that they have no conflict of interest.

Compliance with ethical standards the present manuscript does not contain any studies with human participants or animals performed by the authors.

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