Nepal J Biotechnol. 2022Jul;10 (1): 13-24 Research article DOI: https://doi.org/10.54796/njb.v10i1.226

Nepal Journal of Biotechnology
Publisher: Biotechnology Society of Nepal ISSN (Online): 2467-9313
Journal Homepage: https://nepjb.com/index.php/NJB ISSN (Print): 2091-1130

Occurrence of Purple Blotch Disease Associated with Selected Garlic Varieties and its Management Through Bio-Agent, Botanicals and Fungicides

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Received: 5 Jul 2021; Revised: 21 Jul 2022; Accepted: 26 Jul 2022; Published online: 30 Jul 2022

Abstract

Purple blotch of garlic caused by *Alternaria porri* is recognized as a prominent diseases posing threat to garlic cultivation throughout the world including Bangladesh. The experiments were conducted to determine the prevalence of purple blotch disease on garlic varieties in field condition, to test the pathogenicity of isolated causal organism and to find out the suitable management options of the disease. 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 in prevalence study and nine management option comprising a bio-agent *Trichoderma harzianum* (T_1), five botanicals viz. *Lantana camara* (T_2), *Spilanthes paniculata* (T_3), *Ocimum sanctum* (T_4), *Raphanas raphanistrum* (T_5) *and Azadirachta indica* (T_6), two fungicides Mancozeb 80% WP (T_7) and Sulcox 50% WP (T_8) and, an untreated control (T_9) were explored in the experiments. BARI Rashun-3 showed the highest disease incidence (40.00%) and severity (92.00%) of purple blotch disease. Isolation, identification of pathogen and pathogenicity test was carried out as well. In case of management, all botanicals and bio-agent were tested significantly beneficial in lessening the disease incidence and severity of purple blotch disease. The results revealed that *Lantana camara* (T_2) was found most effective for minimizing the disease incidence (26.67, 26.67 and 33.33%) at 30, 45 and 60 DAS, respectively while maximum disease incidence was recorded in control (T_9) (86.67, 96.67 and 100.00%). T_2 also reduced disease severity at 30 DAS (11.00%) whereas, at 45 DAS (18.67%) and 60 DAS (19.33%) T_1 performed well against the disease.

Keywords: Purple blotch; *Alternaria porri*; *in-vivo*; bio-agent; plant extracts and fungicides.

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Introduction

Garlic (*Allium sativum*) belongs to Alliaceae family is considered as the most demanded and universal spices within the world especially in Bangladesh. The position of garlic is second among all the important spices in the world [1]. Garlic helps in controlling hypertension, diabetes, cancer, ulcer, rheumatism, germs, fungal and bacterial diseases etc. [2]. According to Asfand *et al.* (2019)[3] the pungent taste of garlic is reduced by the field fresh exfoliated garlic which consist just about 0.8% fiber, 63% water, 7% protein, 28% carbohydrate, 0.2% fat and an excellent amount of sulphur compounds.

From the report of FAOSTAT, (2021)[4] the United Nations Food and Agriculture Organization reported that from 1,634,634 hectares production with 30,708,243 tonnes of garlic globally each year. Total production of Garlic in the year of 2019 in Bangladesh was 466, 389 tonnes from 71734 ha land. Though garlic production is enhancing gradually, but due to expanding population rate in the low income country Bangladesh, the domestic need cannot be fulfilled. To meet up the demand Bangladesh imports enormous amounts of garlic from abroad every year [5].

Though garlic has many importance, the yield is below average in many parts around the world. Various factors viz. diseases, insects, soil, climatic condition and lack of technical knowledge etc. affect the quality of garlic bulb and the yield greatly. [6]. Soil borne diseases are major in garlic. Among the fungal diseases, purple blotch caused by *Alternaria porri* (Ellis) Cif., is a major constraint that leads to considerable loss in yield and quality of garlic [7]. The disease is considered as a crucial disease all over the world including Bangladesh [8]. Epidemic may cause total failure of the crop in favorable conditions. Gupta and Srivastava (1993) [9] carried out a study in Maharashtra during Kharif where extreme loss was noted owing to purple blotch disease. In Punjab, Haryana and Maharashtra purple blotch spotted as serious disease and caused 20-60% loss [10, 11, 12]. The purple blotch disease acting as more dreadful for seed crops in contrast to bulb crops that caused sometimes 100% loss on productivity of onion seed [13, 14, 15]. Hence, in existing situation convenient

management strategies of purple blotch of garlic has become a turning topic. To control the plant pathogens, farmers around the world need the chemical pesticides with a view to sustain the standard and dismissal of agricultural products [16]. Sharma *et al*. (2012)[17] approximated in her study that because of pests cause 37% of crop loss and 12% crop loss is due to pathogens. On the contrast, issues of environmental pollution and various health complications arose because of the immoderate and the inappropriate use of pesticides over the past decades around the world. Patent resistant organisms can be developed by the extreme use of chemical pesticides [18]. However, now a days strict regulation are applied on the implementation of chemical fungicides because of their carcinogenic effects, problems of residual toxicity, environmental pollution and development of fungicide-resistant strains [19, 20]. Kumar & Palakshappa, (2008) [21] stated that, biological control of plant pathogens through antagonistic microorganisms is proved as an effective, not harmful to the environment and a suitable strategy other than an optimistic alternative of chemical uses. *Trichoderma sp*. is a biological control agent and botanicals have been found to be very effective for several soil borne plant pathogenic fungi. Plant extract possess an anti-fungal activity in opposition to a wide range of plant pathogenic fungi. These are less phytotoxic, biodegradable and host metabolism stimulatory. Various experiments were undertaken over the past many years to control purple blotch disease through bio-agents, botanicals and fungicides [12, 15, 21, 22, 23, 24, 25]. The current research work was aimed to assess the occurrence of purple blotch disease incidence and severity of selected garlic varieties, to isolate and identify purple blotch disease and pathogenicity test of *Alternaria porri,* and its management using bio-agent, plant extracts and fungicides.

Materials and methods

The experiments were conducted 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) during the Rabi season of 2018-2019 and 2019-2020 with three replications consists of 24 units plots in Randomized complete block design (RCBD). Recommended doses of fertilizer (Cowdung @10 tons/ha, Triple Super Phosphate (TSP) @417kg/ha, Muriate of Potash (MP) @165kg/ha, Urea @320kg/ha, Gypsum 100kg/ha, Zinc oxide @5kg/ha and Boric acid @5kg/ha) were applied during field preparation and after sowing of garlic clove and, 2 packets of Sevin were applied to control from the attack of ant [26].

Source of garlic seeds

Eight different fresh and disease-free garlic variety seed were collected from three different places. BAU Rashun-1 (V_1) and BAU Rashun-2 (V_2) varieties were collected 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.

Disease incidence

Garlic varieties were assessed on the basis of symptoms appeared on the above ground plants and recorded. For calculation of 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 was used: [27]

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

Disease severity

Disease severity of purple blotch was assessed using 0-5 scale [28], as follows by randomly selected 10 plants from each plot and final data were calculated for PDI (percent disease index) estimation.

Grade	Symptoms description
0	Free from infection
1	Small sized lesion towards the tip, covering less than 10% leaf area
2	Several dark purplish brown patches covering less than 20% leaf area
3	Several patches with paler outer zone, covering up to 40% leaf area
4	Long streaks covering up to 75% leaf area or breaking of leaves/stems from the center
5	Complete drying of the leaves/stems or breaking of the leaves/stems from the base

The percent disease index (PDI) was computed according to the formula given by (Wheeler, 1969 and Islam *et al*., 2003) [29, 30].

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

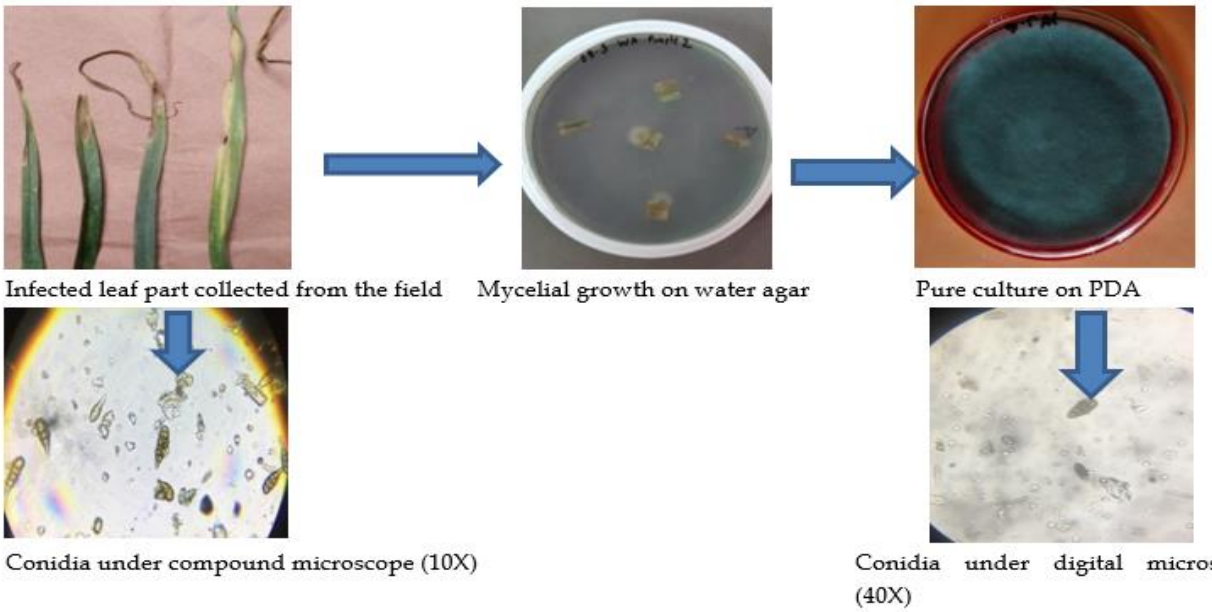


Plate 1: Flow chart of Isolation, Identification and pure culture of *Alternaria porri*

Yield per hectare

Yields of harvested garlic bulbs were computed using electric balance after solar drying of bulbs for 10 days. The yields was expressed as Kg/hectare.

Isolation and identification of *Alternaria porri*

Diseased leaf samples were collected from field, put into brown paper envelope and, taken to the central laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for isolation. Collected diseased leaves were cut into pieces regarding 1 to 1.5 mm with the help of sterilized scalpel, cleaned with sterilized distilled water and disinfected using 0.1 percent $MgCl_2$ solution (30 to 60 seconds). Then sterilized cut pieces were washed three times right away with double sterilized distilled water frequently to remove the traces of mercuric chloride and dried with a towel on sterilized filter paper, then placed to petri plates 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 for 10 days. After 10 days the growing mycelia on water agar petri plates 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 14 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. After identification of *Alternaria porri* the pure culture was maintained by sub culturing at an interval every 15 days and preserved at low temperature (4ºC) in refrigerator for future purpose. The observations were equated with the standard measurements following by Ellis (1971) [31] for the identification of the pathogens (**Plate 1**).

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.

Cultural variability of *Alternaria porri*

Colony diameter was recorded on the 2nd, 7th and 14th days after incubation. The data on radial growth was analyzed statistically [33]. Growth per day was calculated by the followed formula:

mm/ day = (growth observed on a day - growth on previous observation)/2.

Morphological variability of *Alternaria porri*

Fourteen days old cultures of *A. porri* isolates were studied for morphological variations viz. conidia color, shape, size, colony character and surface structure.

Pathogenicity test of *Alternaria porri*

To prove the association of isolated organism with the disease pathogenicity test was done using Koch's Postulates conducted on the Agri found onion red varieties [34, 35]. For testing the virulence level of *Alternaria porri* isolates, BARI Rashun-3 variety was selected. The plants were raised in sterilized plastic pot under greenhouse condition. Soil was sterilized for three consecutive days in an autoclave at 20 lbs per sq. inch pressure for one and half an hour. The plastic pots were cleaned entirely with water, rinsed with two per cent formalin and before using it was dried in the sunlight. The fumigated pots were filled with this sterilized soil

and covered with disinfected polythene sheet to block aerial contamination. After that air dried sandy loam soil and cow dung were mixed thoroughly at the ratio of 4:1 and then filled in earthen pots (20 cm diameter). No chemical fertilizers were used in the pot soil. The conidial suspension ($5x10^5$ spores mL^{-1}) was mixed in prepared distilled water from 10 days old culture of *A. porri* isolates. The garlic plants of 30 days old were inoculated with these spore suspensions after garlic leaves were injured by sterile toothpick. Water was sprayed consequently to the plants both before 24 hour and after inoculation the plants were covered with moist polythene bag to keep up high relative humidity (%RH) and also to inhibit natural contamination with other fungal conidia or spores. The inoculation was done on cool evening hours. The inoculated plants were maintained in greenhouse condition. On the 17 days after inoculation the severe symptoms were observed and compared with original symptoms.

Collection of data on leaf infection

After 5 days of inoculation on garlic leaves the size of lesions was recorded on 5th, 7th, 9th, 11th, 13th, 15th, and 17th days after inoculation. Size of lesions increased per day was calculated by the formula:

Leaf infection per day =

$$\frac{\text{Leaf infection observed on a day} - \text{Leaf infection on previous observation}}{2}$$

Management of Purple blotch of garlic caused by *Alternaria porri* through *Trichoderma*, botanicals and fungicides

Experimental design

The experiment was conducted in a Randomized Complete Block Design (RCBD) with three replication and nine treatments with the objective to attain management of Purple blotch of garlic caused by *Alternaria porri.* It was conducted in Rabi season 2019-2020 at Central Farm, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh.

Variety and Treatments

From previous evaluation, most susceptible variety BARI Rashun-3 was used for the management strategies of purple blotch disease. One bio-agent, five botanicals, two fungicides and a control used as treatments (**Table 1**).

Preparation of plant extract

Fresh and healthy leaves of all five test plants were collected from the surrounding field of university for the preparation of plant extract. For the removal of dust material adhering to surfaces the collected leaves were first washed under running tap water and then in distilled water. One hundred grams (100 g) leaves from each sample were then mixed with sterile water (100 ml) at 1:1 (w/w) with the help of mortar and pestle. After through grinding the extract was filtered through muslin cloth and then through Whatman filters paper no1. Then the extract was passed through sieve filter to eliminate contamination. After that the extract is used as standard plant extract solution of 100% concentration of 1:1 ratio. Prepared plant extract was treated at 60°C for 15 minutes for demolition of other microorganism contamination. Then after the initial appearance of disease at 30 days after sowing, the foliar spray of botanicals was applied given for 3 times at 15 days interval. The foliar spray was given using hand sprayer at afternoon for better result [36].

Table 1. Treatments used for management of Purple blotch of garlic

Treatment Name	Common Name	Scientific Name / Chemical name	Plant part/media used
T_1	Trichoderma	*Trichoderma harzianum*	Liquid solution
T_2	Lantana	*Lantana camara*	Leaf
T_3	Shormoni	*Spilanthes paniculata*	Leaf
T_4	Tulsi	*Ocimum sanctum*	Leaf
T_5	Bon mula	*Raphanas raphanistrum*	Leaf
T_6	Neem	*Azadirachta indica*	Leaf
T_7	Mancozeb 80% WP	Ethylene (bis) di thio carbamate	Powder
T_8	Sulcox 50% WP	Copper oxychloride	Powder
T_9	Control	-	-

Preparation of bio-agent and fungicides

The bio-agent *Trichoderma harzianum* liquid solution was sprayed 3 times at 15 days interval after the initial appearance of disease at 30 DAS [36].

Fungicidal solutions were prepared following the recommended doses of selected fungicides. The fungicides were mixed thoroughly using required quantity with sterilized water. It was required 2 gm/liter of Mancozeb 80% WP and 3 gm/liter of Sulcox 50% WP for preparation of solution for recommended concentration. The solutions of the fungicides were sprayed 3 times at 15 days interval at afternoon by hand sprayer [37]. A control treatment was maintained in each block where spraying was done with normal water only.

A. B. C. D.

Plate 2. Symptoms of Purple blotch of Garlic
A. Symptoms on leaf tip; B. Symptoms at early stage;
C. Symptoms at mid growth stage; D. Symptoms at late stage

Table 2. Prevalence of % disease incidence and disease severity of purple blotch disease among selected garlic varieties

Variety	(%) Disease Incidence		(%) Disease Severity	
	60 DAS	90 DAS	60 DAS	90 DAS
BAU Rashun-1	1.80 c	1.80 d	36.00 d	68.00 c
BAU Rashun-2	1.47 c	1.47 d	36.00 d	68.00 c
BARI Rashun-1	8.33 b	8.33 c	49.33 b-d	76.00 bc
BARI Rashun-2	8.88 b	15.00 b	56.00 bc	89.33 ab
BARI Rashun-3	34.44 a	40.00 a	70.67 a	92.00 a
BARI Rashun-4	6.67 b	13.89 b	62.67 ab	82.67 ab
Local Deshi	7.78 b	7.78 c	51.33 bc	76.00 bc
Local Indian	6.66 b	7.22 c	44.00 cd	64.00 c
CV	14.80	18.89	15.39	9.46

Parameters observed

Data were noted on plant height (cm), number of leaves, disease incidence, and disease severity. Observations on *Alternaria porri* disease intensity were recorded on randomly selected six plants from the diseased infected leaves. Screening was assessed using 0-5 rating scale [28] based on leaf area covered by the pustules. Measurement of PDI described before.

Statistical Analysis

The data of different characters were statistically analyzed which were obtained from the experiment to observe the significant difference among the treatment by using the MSTAT-C program. Conversions of the data were required when necessary. The mean values of treatments were calculated and analyzed using Duncan's Multiple Range test. DMRT test were executed to determine the level of significant differences and to separate the means within the parameters at 5% level of probability [38].

Results

Evaluation of selected garlic varieties against purple blotch diseases at field condition

Small, whitish and sunken like lesions were marked on leaves and stalks initially as the symptoms of purple blotch disease. Subsequently watersoaked lesions developed and transferred to brown. While the disease advanced, these lesions expanded and became zonate and turned into purplish color. The border of the lesions turned to purplish red encircling by yellowish brown or pale color margin. Upwards and downwards extension were founded in these lesions. Infected leaves turned yellow and wilted at advanced stages (**Plate 2**).

Prevalence of % disease incidence and severity of purple blotch disease among selected garlic varieties

Significant variation was found at different days after planting in % disease incidence and severity. The results are presented in **Table 2**. Disease incidence and severity varied depending on cultivars and climatic condition. The disease incidence varied from 1.47 to 34.44% and 1.47 to 40.00% at 60 DAS and 90 DAS, respectively. Whereas, the disease severity varied from 36.00 to 70.67% and 64.00 to 92.00% at 60 DAS and 90 DAS, respectively.

At 60 DAS, the highest disease incidence (34.44%) and disease severity (70.67%) was noted on BARI Rashun-3, respectively. Statistically similar disease severity (62.67%) was reported on BARI Rashun-4. On the contrary, the lowest disease incidence (1.47%) was observed on BAU Rashun-2 which was statistically alike with BAU Rashun-1 (1.80%). The lowest disease severity (36.00%) was found in BAU Rashun-1 and BAU Rashun-2 variety that was statistically similar to BARI Rashun-1 (49.33%) and on Local Indian variety (44.00%), respectively.

At 90 DAS, the highest disease incidence (40.00%) and disease severity (92.00%) was recorded on BARI Rashun-3 variety. BARI Rashun-2 showed statistically similar disease severity (89.33%) with BARI Rashun-3 along with BARI Rashun-4 (82.67%). Conversely, the lowest disease incidence (1.47%) was observed BAU Rashun-2 followed by BAU Rashun-1(1.80%), Local Indian (7.22%), Local Deshi (7.78%) and BARI Rashun-2 (8.88%). Local Indian was found as less infected variety with lowest disease severity of 64.00% which was statistically similar to BAU Rashun-1 and BAU Rashun-2(68.00%) respectively.

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.

Correlation of purple blotch percent disease severity with yield (t/ha)

The yield of the garlic plant affected due to disease severity. Correlation analysis was done to find out the assessment of yield loss owing to disease severity. To determine the effect of disease severity on yield of selected garlic varieties correlation of coefficient was considered at 0.01% level of probability. From this correlation, it was estimated that the fresh and dry weight of yield showed negative correlation with disease severity of purple blotch of garlic and became significant at 0.01% level of probability. These values clearly expressed that less disease severity provide higher yield and vice versa (**Figure 1**).

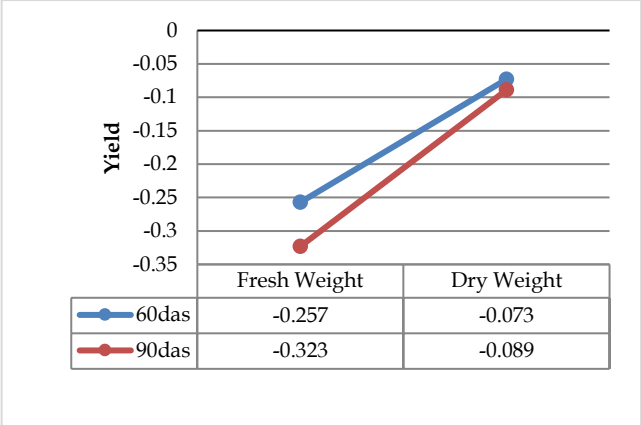


Figure 1. Correlation between % disease severity of purple blotch with yield (t/ha)

Isolation, identification and pathogenicity of *Alternaria porri*

Cultural studies of *Alternaria porri*

Isolated pathogens (*Alternaria porri*) from infected leaves of garlic transferred to water agar thereafter cultured in petri plates on potato dextrose agar (PDA). Cultured pathogen incubated at 25±1°C, afterwards sub cultured for future use. The colony appearance and growth of the pathogens were monitored and noted for 14 successive days (**Table 3**).

Table 3. Radial mycelial growth of *Alternaria porri* on PDA media

Isolates	Radial mycelial growth (mm)		
	2 DAI	7 DAI	14 DAI
BAU_1 I_1	2.80	7.65	9.00
BAU_2 I_2	4.00	6.50	9.00
$BARI_1$ I_3	3.05	7.00	9.00
$BARI_2$ I_4	2.90	7.50	9.00
$BARI_3$ I_5	4.10	7.65	9.00
$BARI_3$ I_6	2.60	6.45	9.00
$BARI_4$ I_7	4.10	6.50	9.00
$BARI_4$ I_8	2.65	7.65	9.00
LD I_9	3.10	7.45	9.00
LIND I_{10}	4.00	7.65	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; $BARI_3$ I_6= $BARI_3$ Isolate 6; $BARI_4$ I_7= $BARI_4$ Isolate 7; $BARI_4$ I_8= $BARI_4$ Isolates 8; LD I_9= Local Deshi Isolate 9 and LIND I_{10}= Local Indian Isolate 10

Radial mycelial growth of 10 isolate of *A. porri* from eight different varieties of garlic varied significantly on PDA media. *Alternaria porri* is a fast growing pathogen. Colony growth of the pathogen appeared after 2 days of incubation; maximum increase (4.10 mm) of colony diameter was recorded in $BARI_3$ I_5 and $BARI_4$ I_7 isolates along with isolates BAU_2 I_2 and LIND I_{10} (4.00 mm), $BARI_1$ I_3 (3.05 mm), LD I_9 (3.10 mm). The minimum increment (2.60 mm) of colony diameter was found in $BARI_3$ I_6 followed by isolates $BARI_4$ I_8 (2.65 mm), BAU_1 I_1 (2.80 mm) and $BARI_2$ I_4 (2.90 mm).

After 7 days of incubation, the maximum increment (7.65 mm) of colony diameter was recorded in BAU_1 I_1, $BARI_3$ I_5, $BARI_4$ I_8 and LIND I_{10} respectively followed by $BARI_2$ I_4 (7.50mm), LD I_9 (7.45 mm) and $BARI_1$ I_3 (7.00mm). On the other hand, the minimum increase (6.45 mm) of colony diameter was recorded in $BARI_3$ I_6.

After 14 days of incubation all the colonies covered whole petridish which was 9.00mm.

Morphological studies of *Alternaria porri*

Regular inspection was done to the pure culture of pathogen *Alternaria porri* under the microscope to determine morphological characteristics of the pathogen viz. color, shape and surface texture. The results of morphological studies of *A. porri* are shown in **Table 4**, **Plate 3** and **Plate 4**.

Almost all the isolates showed fluffy growth appearance on potato dextrose agar. The colony color varied from olivaceous green in BAU_2 I_2 to greyish white in $BARI_1$ I_3, ashy black in BAU_1 I_1, $BARI_2$ I_4 and LIND I_{10}, off-white in $BARI_4$ I_8 and black in rest of the isolates. Most of the surface texture of the isolates was smooth, cottony black center with whitish to greyish periphery.

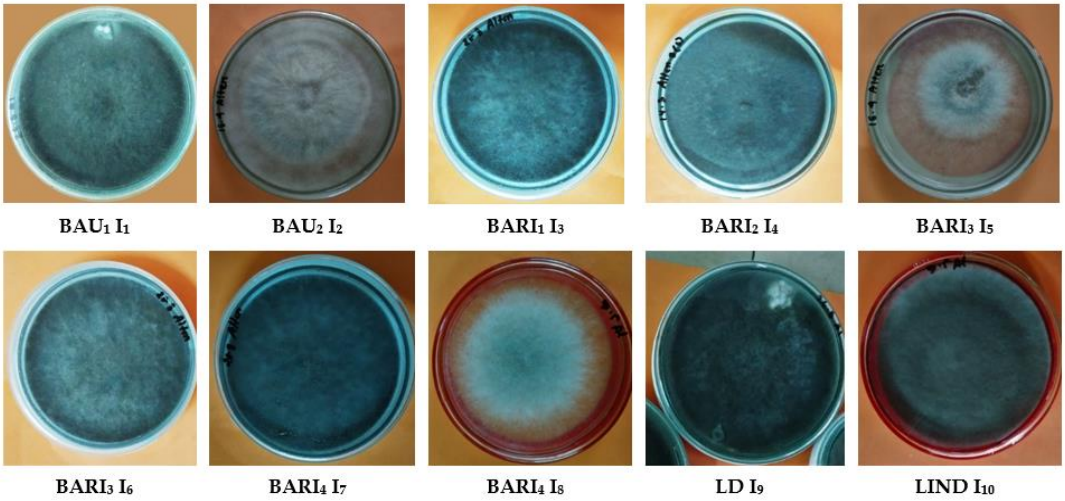


Plate 3. Mycelial growth of *Alternaria porri* on PDA media at 14 days after incubation

Table 4 Colony characteristics of *Alternaria porri* on PDA media

Isolates	Colony characteristics			
	Color	Surface texture		Shape
BAU_1 I_1	Ashy black	Velvety smooth		Regular
BAU_2 I_2	Olivaceous green	Fluffy	Regular	
$BARI_1$ I_3	Greyish white	Fluffy	Regular	
$BARI_2$ I_4	Ashy black	Velvety smooth	Irregular	
$BARI_3$ I_5	Olivaceous green	Cottony	Regular	
$BARI_3$ I_6	Smoky ash	Fluffy	Regular	
$BARI_4$ I_7	Black	Fluffy	Irregular	
$BARI_4$ I_8	Off white	Fluffy	Regular	
LD I_9	Dark	Velvety smooth	Regular	
LIND I_{10}	Ashy Black	Velvety smooth	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; $BARI_3$ I_6= $BARI_3$ Isolate 6; $BARI_4$ I_7= $BARI_4$ Isolate 7; $BARI_4$ I_8= $BARI_4$ Isolates 8; LD I_9= Local Deshi Isolate 9 and LIND I_{10}= Local Indian Isolate 10

After 14 days of inoculation all the isolates had suppressed growth on PDA media. From the microscopic study of *Alternaria porri* it was found that at first the mycelium of the fungus was hyaline then eventually turned to pale brown through olivaceous brown, smoky ash to black blended black tinge. The hypha of the conidia was septate. Colony shape of the isolates was regular with concentric ring sometimes irregular.

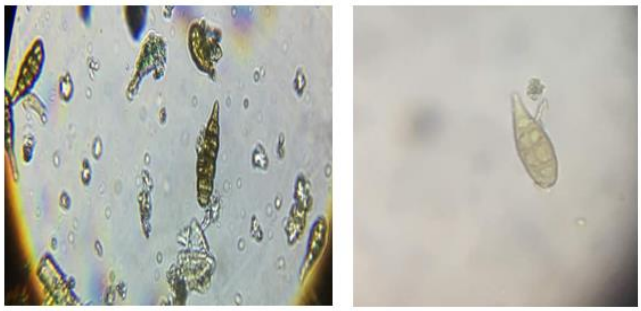
Plate 5. Pathogenicity test of *Alternaria porri* in selected garlic plant

Pathogenicity test of *Alternaria porri*

Symptoms developed on inoculated plants was recorded from time to time. After 5 days of inoculation, tiny, water soaked, sunken, and whitish lesions on the inoculated leaves were visible. While the disease progressed, the lesions expanded, changed to elliptical to oblong, zonate and transformed reddish brown to purple encircled by pale yellow halo enlarging upwards and downwards. After 15 days of inoculation, chlorosis of the leaves was observed on the plants. The inoculated plants were dried completely after 21 days of inoculation. The symptoms were almost identical to those under field experiment. Conidia of *A. porri* were found under compound microscope from the sectioned diseased leaves (**Plate 5**).

Efficacy of selected treatment on percent disease incidence and severity of purple blotch of garlic in treated condition

In case of % disease incidence, all botanicals and *Trichoderma* were found significantly effective in reducing disease incidence as compared to control and fungicides (**Table 5**).



A. B.

Plate 4. Microscopic view of *Alternaria porri*, A. 10X; B. 40X

Table 5. Efficacy of selected treatment on percent disease incidence and severity of purple blotch of garlic

Treatment	(%) Disease incidence			(%) Disease severity		
	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
T_1	33.33 cd	43.33 cd	43.33 cd	13.33 c-e	18.67 e	19.33 e
T_2	26.67 d	33.33 d	33.33 d	11.00 e	19.33 de	20.67 e
T_3	53.33 b	60.00 b	60.00 bc	11.33 de	22.00 c-e	26.00 de
T_4	46.67 bc	46.67 b-d	53.33 bc	14.67 b-e	23.33 c-e	26.00 de
T_5	50.00 b	53.33 bc	56.67 bc	15.33 b-d	26.00 cd	46.67 c
T_6	56.67 b	56.67 bc	60.00 bc	16.00 bc	26.67 c	30.67 d
T_7	53.33 b	56.67 bc	70.00 b	18.00 b	46.00 b	55.33 b
T_8	43.33 bc	50.00 bc	56.67 bc	11.33 de	44.00 b	44.00 c
T_9	86.67 a	96.67 a	100.00 a	28.67 a	81.00 a	100.00 a
CV	16.67	16.50	17.22	15.50	11.91	11.17

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. Here, T_1=*Trichoderma harzianum*; T_2=*Lantana camara*; T_3=*Spilanthes paniculata*; T_4=*Ocimum sanctum*; T_5=*Raphanas raphanistrum*; T_6=*Azadirachta indica*; T_7=Mancozeb 80% WP; T_8=Sulcox 50% WP and T_9=Control

Table 6. Mean performance of different treatments on growth and yield parameters against purple blotch disease of garlic

Treatments	Plant height (cm)			No. of leaf per plant			Fresh weight (gm/m²)	Dry weight (gm/m²)
	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS		
T_1	17.58 c	24.77 bc	28.80 b-d	3.67 a	4.33 cd	5.33 a-c	69.33 c	48.33 c
T_2	21.55 ab	25.60 bc	29.08 b-d	4.00 a	5.00 a-c	5.67 a-c	64.33 cd	42.00 cd
T_3	18.48 bc	23.53 c	28.12 cd	4.00 a	4.00 d	4.33 c	62.00 d	39.00 de
T_4	21.55 ab	29.07 a	32.67 a	4.00 a	5.00 a-c	6.33 a	87.00 b	66.33 b
T_5	18.70 bc	26.07 b	29.16 a-d	4.00 a	5.33 ab	6.33 a	99.33 a	75.00 a
T_6	21.55 a	27.20 ab	32.02 ab	4.00 a	5.67 a	6.67 a	91.00 b	65.67 b
T_7	16.98 c	27.17 ab	30.58 a-c	3.67 a	4.67 b-d	6.00 ab	52.67 e	33.67 e
T_8	16.72 c	25.24 bc	28.12 cd	3.67 a	5.00 a-c	6.33 a	52.00 e	33.67 e
T_9	17.97 c	23.20 c	25.77 d	4.00 a	4.00 d	4.67 bc	41.67 f	24.33 f
CV	7.46	5.66	6.91	7.42	8.18	15.59	5.96	9.09

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. Here, T1=Trichoderma harzianum; T2=Lantana camara; T3=Spilanthes paniculata; T4=Ocimum sanctum; T5=Raphanas raphanistrum; T6=Azadirachta indica; T7=Mancozeb 80% WP; T8=Sulcox 50% WP and T9=Control

The results revealed that *Lantana camera* (Lantana leaf) extracts designated as T_2 was found most effective in minimizing the disease incidence (26.67%, 26.67% and 33.33%) at all 30, 45 and 60 DAS, respectively. The second effective treatment was T_1 (33.33% and 43.33%) which was statistically alike with T_8 (43.33%, 50% and 56.67%) followed by T_5 (50%, 53.33% and 56.67%) respectively, whereas the maximum disease incidence was recorded in control T_9 (86.67%, 96.67% and 100.00%) at 30, 45 and 60 DAS.

In case of % disease severity, *Lantana camara* (T_2) was found most effective in reducing disease severity at 30 DAS (11.00%) whereas, at 45 DAS and 60 DAS (18.67% and 19.33%) T_1 (*Trichoderma harzianum*) was found most effective. At 30 DAS the second effective treatment was 11.33% in T_3 and T_8 in reducing the disease severity. On the other hand, at 45 and 60 DAS T_2 gave the second lowest severity of (19.33% and 20.67%). All the treatments showed the statistical similar result in reducing disease incidence and severity over control.

Mean performance of different treatment on growth and yield parameters against purple blotch disease of garlic

The results of mean performance of yield parameter due to treatment are presented in the **Table 6**. The data revealed that all the botanical plant extracts, bio-agent and fungicides given significantly better results in comparison to control. The best plant height was noted on T_4 treatment (21.55 cm, 29.07 cm and 32.67cm) at 30, 45 and 60 DAS, respectively in contrast to control. Maximum no. of leaf per plant was found on T_2, T_3, T_4, T_5, T_6 and T_9 (4.00) and minimum no. of leaf per plant was found on other remaining treatment (3.67). Maximum fresh weight and dry weight was found on T_5 (99.33 and 75.00gm/m²) statistically similar with T_6 (91.00 and 65.67gm/m²) in contrary, T_9 gave poor fresh weight and dry weight of 41.67 and 24.33gm/m².

Discussions

Preliminary symptoms of purple blotch disease were visible on the leaves in the shape of tiny, whitish,

sunken like lesions. At the final stage, water soaked large zonate lesions of purplish red color enclosed by yellowish pale brown border was found. The symptoms and advancement of the disease were similar and have been stated by completely different researchers who recorded that white flecks like symptoms appeared at initial stage on older leaves, that enlarged and developed into elliptical to rectangular sunken zonate purple lesions with a yellow to pale brown margin under favorable environmental conditions [39, 40, 41, 42].

The highest disease incidence (34.44% and 40.00%) and severity (70.67% and 92.00%) was found on BARI Rashun-3 variety at 60 DAS and 90 DAS, respectively. On the contrary, the lowest disease incidence (1.47% and 36.00%) was recorded on BAU Rashun-2 variety. The study was almost similar to Patil (1999) [43] throughout survey of purple blotch incidence of garlic he reported, maximum incidence of disease in Dharwad and Gokak taluks during kharif 1998 and rabi 1998-99. An Indian Study carried out in the horticulture garden of Raichur, where the highest disease severity (49.63%) was noted and least (10.00%) was in Neermanvi [44]. Yadav (2013) [45] studied on the onion purple leaf blotch (PLB) disease in Navsari district of Gujrat over two Rabi seasons where the severity of the disease ranging from 11.29 to 63.73%. Relative results were mentioned in [46] wherever, the highest per cent disease index (46.00) were found at the fields of Sangreshkoppa village in Belgaum district and at Hulkund village in Belgaum district the least per cent disease index (3.00) of purple blotch disease was recorded. Angadi *et al.* (2018)[47] also attained purple blotch disease from his survey.

Negative correlation with yield at 0.01% level was found. These values clearly expressed that less infection of disease severity provide higher yield and the more the disease is severe the more the degradation of yield occurred. Similar correlation was done by Jannatun *et al.* (2020) [48] wherever leaf height (cm) showed negative correlation with the entire yield parameters considered and at 0.01% levels of probability it become significant. Number of leaves showed positive correlation with total yield defining characters except clove diameter (-.859).

Almost all of the *A. porri* isolates showed fluffy growth on potato dextrose agar. The colony color varied from olivaceous green in BAU_2 I_2 to greyish white in $BARI_1$ I_3, Ashy black in BAU_1 I_1, $BARI_2$ I_4 and LIND I_{10}, off-white in $BARI_4$ I_8 and black in rest of the isolates. After 14 days of incubation all the isolates had suppressed growth on PDA media. The causal agent, *A. porri* was isolated and pure culture was cultured on PDA media. The isolated pathogen was identified as *A. porri* following morphological features given by Neergaard (1938) [49]. In accordance with Chethana (2010), Chowdhury (2013) and Yadav *et al.* (2017) [25, 50, 51] potato dextrose agar was the most acceptable culture media for mycelial growth and sporulation of *Alternaria porri*. The cultural characteristics of different isolates of *A. porri* were examined by Shahnaz *et al.* (2013) [52] where she recorded that almost all of the isolates had fluffy growth on PDA with colony color varied from pinkish white through dull orange to olivaceous and black with distinct to diffuse patterns of zonation. Mohsin *et al.* (2016) [53] used 27 isolates of *Alternaria porri* which were isolated from diseased leaf samples collected from completely different onion growing regions of Bangladesh and later characterized for cultural, morphological and pathogenic variabilities where colony color ranged between light to dark olivaceous and grayish white with irregular, regular with concentric ring and regular without concentric ring shape. Isolates impregnated media with color ranged between grey to brown on the reverse of the plates.

All botanicals and *Trichoderma harzianum* were found significantly effective in reducing disease incidence and severity compare to untreated control and fungicides. The result revealed that *Lantana camara* designated as T_2 was found most effective in minimizing the disease incidence (26.67%, 26.67% and 33.33%) at 30 DAS, 45 DAS and 60 DAS. However, the maximum disease incidence was recorded in control T_9 (86.67%, 96.67% and 100.00%). Again, *Lantana camera* (T_2) was found most effective in reducing disease severity at 30 DAS (11.00%) contrarily, at 45 DAS and 60 DAS (18.67% and 19.33%) T_1 (*Trichoderma harzianum*) was found most effective. At 45 DAS and 60 DAS T_2 gave the second lowest severity of (19.33% and 20.67%). Datar (1994) [54] evaluated six plant extracts under field condition and noticed that maximum depletion of purple blotch was attained with leaf extract of. *Polyalthia longifolia*. A field trial were assessed by Prasad and Barnwal (2004) [55] on Stemphylium blight of onion (cv. N-53) during rabi, 1998-1999 and 1999-2000 crop season in Bihar where, disease intensity was lowest (38.1% and 38.2%) with 20% leaf extracts of *Azadirachta indica*. The obtained results correspond with [36, 56]. Consistent with Uddin *et al.* (2006) [57] after 10 days of sowing disease incidence (19.95 %, 13.63 %) and severity (38.87 %, 34.59 %) were reduced due to the bulb treatment with either Dithane M-45 (0.45 %) or Rovral 50 WP (0.2 %) followed

by foliar spraying with the same, and increased seed yield by 64.82 % and 42.18 % respectively. Similar results were obtained by [23, 37, 58, 59, 60, 61, 62, 63] where different treatments had inhibitory effect on fungus based on phytochemical present in plants.

Conclusions

The present investigations showed purple blotch highest disease incidence (40.00%) and severity (92.00%) was found on BARI Rashun-3 variety at 90 DAS, respectively. On the contrary, the lowest disease incidence (36.00%) was recorded on BAU Rashun-2 variety. Negative correlation was found between disease severity and yield (t/ha) against all identified diseases. In laboratory *Alternaria porri* was isolated from infected leaves. After 14 days of incubation mycelial growth covered the whole petridish by *Alternaria porri*. Cultural and morphological variability exits in purple blotch (*Alternaria porri*). All botanicals and *Trichoderma* were found significantly effective in reducing disease incidence and severity compare to control. *Lantana camara* (T_2) was found most effective in minimizing the disease incidence (26.67%, 26.67% and 33.33%) at 30 DAS, 45 DAS and 60 DAS. Again, *Lantana camara* (T_2) was found most effective in reducing disease severity (11.00%) at 30 DAS and *Trichoderma harzianum* (T_1) (18.67% and 19.33%) at 45 DAS and 60 DAS against purple blotch disease in compare to control and fungicides.

Acknowledgements

We would like to express cordial gratitude to Prof. Dr. Abdur Rahim, Horticulture Department, Bangladesh Agricultural University, Mymensingh and Late Arpon Haider, Scientific officer, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur for providing the different garlic varieties and good cooperation. Special thanks to all the teacher and staffs of Plant Pathology department, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh for helping throughout the research work.

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.

Funding

The research work did not receive any funding from any institution or organization.

Author contributions

Umme Habiba Akter conducted the whole research and wrote the manuscript; Jannatun Nahar Prinky and Mst. Rehena Khatun collected the purple blotch samples from field and helped in analyzing the data; Fatema Begum designed and supervised the research work and helped to correct the manuscript; M. R. Islam co-supervised the research work, read the manuscript contributed to the conceptualization, and methodology of the study.

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