Comperative performance of some selected onion cultivers against "*Stemphylium vesicarium*" causing white blotch disease under field condition

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CERTIFICATE

This is to certify that the thesis entitled "*Comperative performance of some selected onion cultivers against "Stemphylium vesicarium" causing white blotch disease under field condition* " submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of *MASTER OF SCIENCE IN PLANT PATHOLOGY,* embodies the result of a piece of bona fide research work carried out by *PANNA KANTI DAS. 08-03234,* under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma elsewhere.

I further certify that any help or sources of information availed during the course of this inquire have been duly acknowledged and the contents and style of the thesis have been approved and recommended for submission.

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Comperative performance of some selected onion cultivers against "*Stemphylium vesicarium*" causing white blotch disease under field condition

BY

PANNA KANTI DAS

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Dedicated To

My

Respectable

Parents

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ABSTRACT

An experiment was conducted in the Department of Plant Pathology and the field of Sher-e-Bangla Agricultural University (SAU) farm, Dhaka to determined the performance of some selected cultivers of onion against stemphylium blight disease (*Stemphylium vesicarium*) during the period from October, 2009 to April, 2010. Nine onion cultivars viz. BARI Peas-1, BARI Peas-2,BARI Peas-3,Thakurgaon local, Faridpur local, Manikgonj local, Taherpuri Indian big and Indian small were evaluated against the disease.. The performance were measured on different parameters like disease incidence, disease severity and yield performance. At seedling stage in net house, no disease incidence were rrecorded in case of BARI Peas-3,Indian big and Indian small, while local cultivars of Faridpur local, Manikgonj local and Thakurgaon local showed disease incidence of 14.33%,13.33% and 13.33% respectively. The lowest disease incidence was found in BARI Peas-3, Indian small and Indian big irrespective of different days after transplanting (DAP). The highest disease incidence at 30 DAP, 45 DAP, 60 DAP, 75 DAP, and 90 DAP, were observed in case of local cultivars Taherpuri, Fridpur local, Thakurgaon local and Manikgonj local. The highest yield (12.67 t/ha) was recorded in BARI Peas-3 followed by Indian small (10.33 t/ha) and Indian big (9.84 t/ha).The lowest yield was found in Taherpuri (4.34 t/ha) followed by Faridpur local (4.84 t/ha). In case of disease reaction, BARI Peas-3 (9.00%) was performed best followed by Indian small (11.00%), Indian big (11.33%) , and BARI Peas-2 (18.00%) regarding stemphylium blight disease. The local cultivars Taherpuri (37.33%), Faridpur local (37.00%), Thakurgaon local (34.33%), Manikgonj local (34.00%) and BARI Peas-1 (26.00%) showed poor performance. BARI Peas-3 performed best in respect of all the parameters evaluated against stemphylium blight of onion.

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ABBREVIATIONS USED

AEZ	=	Agro-Ecological Zone
@	=	At the rate
ANOVA	=	Analysis of variance
Anon.	=	Anonymous
B	=	Boron
BARI	=	Bangladesh Agricultural Research Institute
BAU	=	Bangladesh Agricultural University
BBS	=	Bangladesh Bureau of Statistics
cm	=	Centimeter
CV	=	Co-efficient of variance
DAP	=	Days after planting
DMRT	=	Duncan's Multiple Range Test
eg.	=	Example
FAO	=	Food and Agricultural Organization
ha	=	Hectare
LAD	=	Leaf Area Diseased
LSD	=	Least Significant Difference
PDI	=	Percent Disease Index
RCBD	=	Randomized Complete Block Design
RH	=	Relative Humidity
SAU	=	Sher-e-Bangla Agricultural University
T	=	Treatment
t / ha	=	Ton per hectare
TSP	=	Triple Super Phosphate
wt.	=	Weight
w/v	=	weight per volume
Zn	=	Zinc
$ZnSO_4$	=	Zinc Sulfate
^0C	=	Degree Centigrade
%	=	Percent

Chapter 1
Introduction

CHAPTER 1

INTRODUCTION

Onion *(Allium cepa L.)* is an important spice as well as vegetable crop, commercially grown in many countries of the world including Bangladesh. On worldwide basis, onion ranked as one of the five most important fresh market vegetable crops (Cramer, 2000). This is the member of the family *Alliaceae.* Onions are used both as food and seasoning of food. The immature and mature bulbs are eaten raw or they may be cooked and eaten as a vegetable (Messiaen, 1994). Onion, like garlic, contains the lachrymatory agent, a strong antibiotic, with fungicidal, bacterial and nematicidal properties (Purseglove, 1972).

In Bangladesh its commercial cultivation is concentrated in the greater districts of Faridpur, Comilla, Jessore, Pabna, Rajshahi, Dinajpur, Mymensingh, Dhaka and Rangpur (BBS, 2006). In terms of global vegetable production nearly 28 million tons onion per annum, next to tomatoes and cabbages bears importance (FAO, 1991). In Bangladesh, the production of onion is nearly 889260 metric tons from 309309 acre of land (BBS, 2008). In 2008, the highest production of onion was obtained in Korea Rep (67.25 MT/ha) followed by USA (53.91 MT/ha), Spain (52.06 MT/ha), Japan (47.55 MT/ha) and in Bangladesh the production was only 7.10 MT/ha (FAO 2008), which was quite low than other onion growing countries of the world.

In Bangladesh, the local varieties namely Faridpuri and Taherpuri are commonly grown. The high yielding varieties, such as- BARI Peas-1, BARI Peas-2, and BARI Peas-3 also are now famous for cultivation in Bangladesh. The demand of bulb onion as well as the onion seeds is increasing every

year in Bangladesh and the price of the onion bulb and true seeds remain fairly high in each season.

As per the world literatures, Onion crop is affected by about 66 diseases including 38 fungal, 10 bacterial, 6 nemic, 3 viral, 1 mycoplasmal, 1 parasitic plant and 7 miscellaneous diseases and disorders (Khatun, 2007). In case of seed production of onion in Bangladesh, several major diseases become widespread and serious enough to limit production.

Among the major diseases, stemphylium blight *(Stemphylium vesicarium)*, purple blotch *(Alternaria porri)*, downy mildew *(Peronospora destructor)*, grey mold *(Botrytis sp.)* and basal/pink rot (*Fusarium sp.)* etc are the most destructive diseases that damage the crop and sometimes reduced seed yield up to 100%. (Haque, 2008). Among those diseases stemphyllium blight commonly known as white blotch, caused by *Stemphylium vesicarium*, is noted as an important disease throughout the world including Bangladesh (Bose and Som 1986; and Castellanos-Linares *et al.* 1988).. Stemphylium blight has become more widespread in Bangladesh in the onion growing area during the recent years. Disease cycle and epidemiology of Stemphylium leaf blight are similar to purple botch. (Haque, 2008)

Stemphylium leaf blight of onion caused by *Stemphylium vesicarium* was first reported in Egypt (Hassan, *et at.*, 2006). Stemphylium blight has been recorded on onion and garlic from many parts of the world viz , Europe, Africa, North and west America (Ellis, 1971). The disease has now become serious in northern parts of Bangladesh (Khatun, 2007). In India about 90% losses in seed yield were recorded due to the *Stemphylium blight* (Anonymous, 1982).

The first symptoms of disease appear on the radical leaves at 3-4 leaf stage. The disease symptoms are developed in the leaf as small, yellow to orange flecks or streaks, which soon develop into elongated, spindle shaped to ovate elongate, diffusate spots surrounded by characteristic margin. These spots turn gray at the centre, later brown to dark olive brown with the development of conidiophores and conidia of the pathogen (Miller, M.E. 1983). The spots frequently coalesce into extended patches, blighting the leaves and gradually cover entire foliage. The infection is usually confined to the leaves and do not extend down to the scales of the bulb. Similar symptoms are developed on the inflorescence stalk (peduncles) of the onion. (Gupta and Pandey, 1986).

Damage of foliage and breaking of floral stalks due to Stemphylium blight resulting failure of seed production of onion are common. Onion production in Bangladesh is gradually decreasing due to the disease (BBS, 2001 and Khatun, 2007). For its less production, Bangladesh are being depended to the neighboring countries like India, Barma, Pakistan for importing onion bulb and onion seed to meet up the consumption and cultivation demand.

Under tropical conditions, the disease is a limiting factor for yield of onion. Onion seed production is severely affected because the disease bulb and seed comes breaking of floral stalks (Munoz, 1984). The cultivation area of onion is increasing but its rate of production per unit area in Bangladesh is gradually decreasing due to disease problem (BBS, 2006). So Bangladesh has to import a large quantity of onion every year to fulfill the national demand at the cost of foreign currency.

In India few works have been made on varietals screening. Onion cultivar Pusa Red, N-53 (Niphed53), dark red has higher degree of adaptability in India (Singh, D. 1997). But no reports are available in the literature about the resistant varieties of onion against stemphylium blight disease in the country.

Considering the above facts, the present piece of research is proposed to screen out the Performance of onion against "*Stemphylium vesicarium*" causing white blotch disease.

Chapter 2

Review of Literature

CHAPTER 2

REVIEW OF LITERATURE

Stemphylium blight caused by *Stemphylium vesicarium*, is a common disease of onion in Bangladesh. The disease is considered as serious one as it has profound effect in reducing the yield of onion. Now it is an acute problem in the country for the onion growers. Management of the disease through resistant varieties are being explored in many countries of the world. Literatures in relation to Stemphylium blight of onion are presented below:

2.1. Epidemiology

Verwoperd & Du Plessis (1931) reported that the pathogen *Stemphylium* spp are widespread in Asia and Europe and has been recorded previously on onion plants in South Africa.

Simmons (1969) conducted an experiment and reported that the telemorph of *Stemphylium vesicarium* was as *Pleospora allii*.

Wu (1979) surveyed the seed-born fungi of vegetables. Results of the survey on onion showed that *Alternaria porii* and *Stemphylium botryosum* (*Pleospora herbgrum*) reduced germination of onion seeds.

Tomaz & Lima (1988) stated that *Stemphylium vesicarium* can cause severe damage especially to the onion seed crop and losses of 80- 85% on onion by affecting leaves and seed stalk. This was the first report for Egypt.

Khare and Nema (1982) also reported that the temperature ranged between 22^0 C to 25^0 C was not only suitable for growth and sporulation of *Stemphylium vesicarium* and *Alternaria porri* but also optimum for spore

germination as well as for infection in onion. They also argued that spore germination on leaves decreased with the increase of nitrogen doses to the host. They also reported that temperature, humidity and nutrients seemed to play important roles for ensuing infection of *Stemphylium vesicarium* and *Alternaria porri* in onion. Cent percent (100%) spore germination occurred in vitro within 4 hrs at 22^0 C, while maximum germination was recorded within 6 hrs at 25^0 C on the host surface.

Gupta *et all.*, (1996) stated that Stemphylium Blight (*Stemphylium vesicarium*) and purple blotch (*Alternaria pori*) are important diseases causing considerable damage to onion crops in-India. Diseases are severe during the rainy season especially when thrips are also associated with the crop. Nuchart Joglaekha *et al.* (1982) observed that most of the conidia produced germ tubes and penetrated through wounds on leaves within 8 hrs. after inoculation. The conidia were club shaped with cross and longitudinal septa. This fungus produces spores when the temperature lies between 18-26^0C.

Raju and Metha (1982) demonstrated an experiment on certain nutritional aspects of *Stemphylium vesicarium* and *Alternaria porri* (Ellis) Ciferri on onion in vitro and summarized that potato dextrose agar, having P^H 6, was the best to culture the fungus. Temperature ranging 22-25^0C was optimum for mycelial growth and sporulation of *Stemphylium vesicarium* and *Alternaria porri.*

Larka (1999) conducted an experiment at the Choudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India, found that

numerous blotches were observed on older leaves and scapes when fortnightly dew fall was >1. 0 mm, mean maximum relative humidity> 75% and mean maximum temperature 20-30°C with> 18 hr favorable temperature (10-30 °C) duration. Exposure of leaf and/or scape to wetness for 8 hr was a pre-requisite for conidial germination with increasing disease intensity, every yield component was adversely affected; the most severe infection reduced the number of scapes/ plant, the height of scape, the number of umblets/umbel, the number of seeds/umblet, 1000-grain weight of seeds/plant and the seed yield/ plant by 28.7, 74.5, 1.7, 35.7, 95.7 and 97.3%, respectively, compared with healthy plants.

Sharma *et al.* (2002) reported that onion seed production in Punjab was reduced by 60% - 70% due to the severe downy mildew (*Peronospora destructor*) disease outbreak on seed stalks resulting in low seed recovery and poor seed health and vigor. They detected *Fusarium*, *Aiternaria*, *Stemphylium* and *Aspergilius spp.* in the onion seeds of N-53, ADR, and PRR, Punjab selection, Punjab white, Punjab naroya and Punjab 48 cultivars.

Hassan *et al.* (2006) reported that onion plants (*Allium cepa* cv. 'Giza 6') in several commercial fields in upper Egypt, exhibited symptoms of blight on the leaves and seed-stalk. Initial symptoms on leaves consisted of tip necrosis followed by small white and/or large purple spots. A fungus was consistently isolated from diseased tissue and identified as *Stemphylium vesicarium.*

Everts and Lacy (1990), examined formation of conidia by *Stemphylium vesicarium* and *Alternaria porri* under variable dew duration and controlled relative humidity (RH). Viable conidia produced on lesions after 9 hrs of dew to 38 hrs and conidia formed during 16 hrs of dew duration caused typical lesions. Conidia were formed at all RHs tested (75-100%); numbers were very low at 75-85% RH but increased with increasing RH. Conidia formed on lesions on senescent leaves when incubated in dew chamber at 25^0C and conidia formed repeatedly (up to eight cycles) on lesions to alternating low RH (35-50%) and high (100%) RH.

2.2. Symptomology

Padule and Utikar (1977) tested 32 onion cultivars under field conditions against white and purple blotch and recorded all the cultivars to be susceptible.

Thirumalachar and Mishra (1953) reported about the existence of some varietal resistance and they stated that the fungus *Alternaria porri* (purple blotch) and *Stemphylium vesicarium* (white blotch) caused severe scorching of some onion varieties at the College of Agriculture Sabour; but the indigenous red variety had remained uninfected.

Sandhu *et al.* (1982) reported that none of 102 genotypes they screened was resistant to *Stemphylium vesicarium* and *Alternaria porri.* However, they could locate 12 genotypes which showed moderate resistance reaction. The genotypes that had flat erect leaves showed moderately resistance reaction. Whereas all those with curved, drooping leaves were susceptible.

Sixty-days-old onion plants (cv. Nasik Red) were most susceptible to the white blotch (*Stemphylium vesicarium*) and purple blotch pathogen (*Alternaria porri*) (Gupta and Pathak, 1986). Plants inoculated at high RH (100%) for 120 hours resulted in maximum disease severity and shortest incubation period.

Ariosa-Terry and Herrera-Isla (1986) measured the damage of onion due to white blotch (*Stemphylium vesicarium*) and purple blotch caused by *A. porri.* The first symptoms appeared 50 days after sowing and disease intensity was the highest at 110 days. White onions were more affected than red onions.

Basallote-Urebaa *et al.*,(1999) Surveys between 1989 and 1993 in the major garlic production areas of Spain identified a new leaf spot disease, characterized by white and purple lesions followed by extensive necrosis. Isolation and pathogenicity tests with fungal isolates taken from these spots indicated that *Stemphylium vesicarium* was the causal agent. Pseudothecia of the teleomorph stage, *Pleospora* sp., were found on leaf debris from affected plants. Inoculation of garlic and onion plants with residues carrying mature pseudothecia, or with ascospore suspensions obtained from the pseudothecia, resulted in the development of white and purple leaf spots. Wetness periods longer than 24 h were required for symptom development under controlled conditions. Isolates of *S. vesicarium* from garlic, onion and asparagus caused disease in all three hosts. In garlic, cv. Blanco de Vallelado was most susceptible, while lines B4P17 and B6P1, and cvs Iberose and Golourose were less susceptible to the disease.

2.3. Morphology of *Stemphylium vesicarium*

Ellis (1971) conducted an experiment and described that the conidiophores were pale to medium brown with dark bands, smooth or minutely verruculose; conidia oblong to ovoid, densely verrucose with 1- 5 transverse and several longitudinal septa, 13-21 x 25-40 µm. Ascomata forming in culture contained hyaline, bitunicate, clavate asci with 8 ascospores that were light to medium brown, ellipsoidal, verrucose with 5-7 transverse and several longitudinal septa, usually in incomplete series, 9-17 x 17-46 µm.

2.4. Other information regarding the pathogen

Srivastava *et at.*, (1996) conducted an in vitro studies to determine the role of infected plant debris and soil in the perpetuation of disease and air borne spore of purple blotch (*Alternaria porii*) and Stemphylium blight (*Stemphylium vesicarium*) on onions in Harayana, India, in order to establish a forecasting system for effective control measures. The pathogens remained viable for 4 months on diseased plant debris, 3 months at soil in depths of 2.5, 5.0 and 7.5 cm and for 2 months at soil in depths of 10.0 and 15.0 cm. It was suggested that the inoculum load of *Alternaria pori* and *Stemphylium vesicarium* during ploughing of infected soil was higher during the winter.

Chapter 3

Materials and Methods

CHAPTER 3

MATERIALS AND METHODS

This chapter described the materials and methods that were used in carrying out the experiment. It included a description of screening of onion varieties in the net house and in field conditions. These comprised collection of germplasms from major onion growing area, isolation and identification of *Stemphylium vesicarium"* from infected onion plant ,conduction of field experiments and recording compilation and analysis data.

3.1. Experimental site

The in vitro research was conducted in the MS laboratory, at the Department of Plant Pathology and the field experiment was conducted at the farm of Sher-e-Bangla Agricultural University (SAU), Dhaka-1207, during the period from October , 2009 to April, 2010. The experimental field is located at $90^0 33'$ E longitude and $23^0 77'$ N latitude at a height of 9 meter above the sea level. (Fig.1).The land was medium high and well drained.

3.2. Climate

The experimental area was under the sub-tropical climate which characterized by the comparatively low rainfall, low humidity, low temperature, relatively short day during October to March, and high rainfall, high humidity, high temperature and long day period during April to September.

The annual precipitation and potential evapotranspiration of the site were 2152 mm and 1297 mm, respectively. The average maximum and minimum temperature was 30.34^0C and 21.21^0C, respectively with mean temperature of 25.17^0C. (Appendix- II)

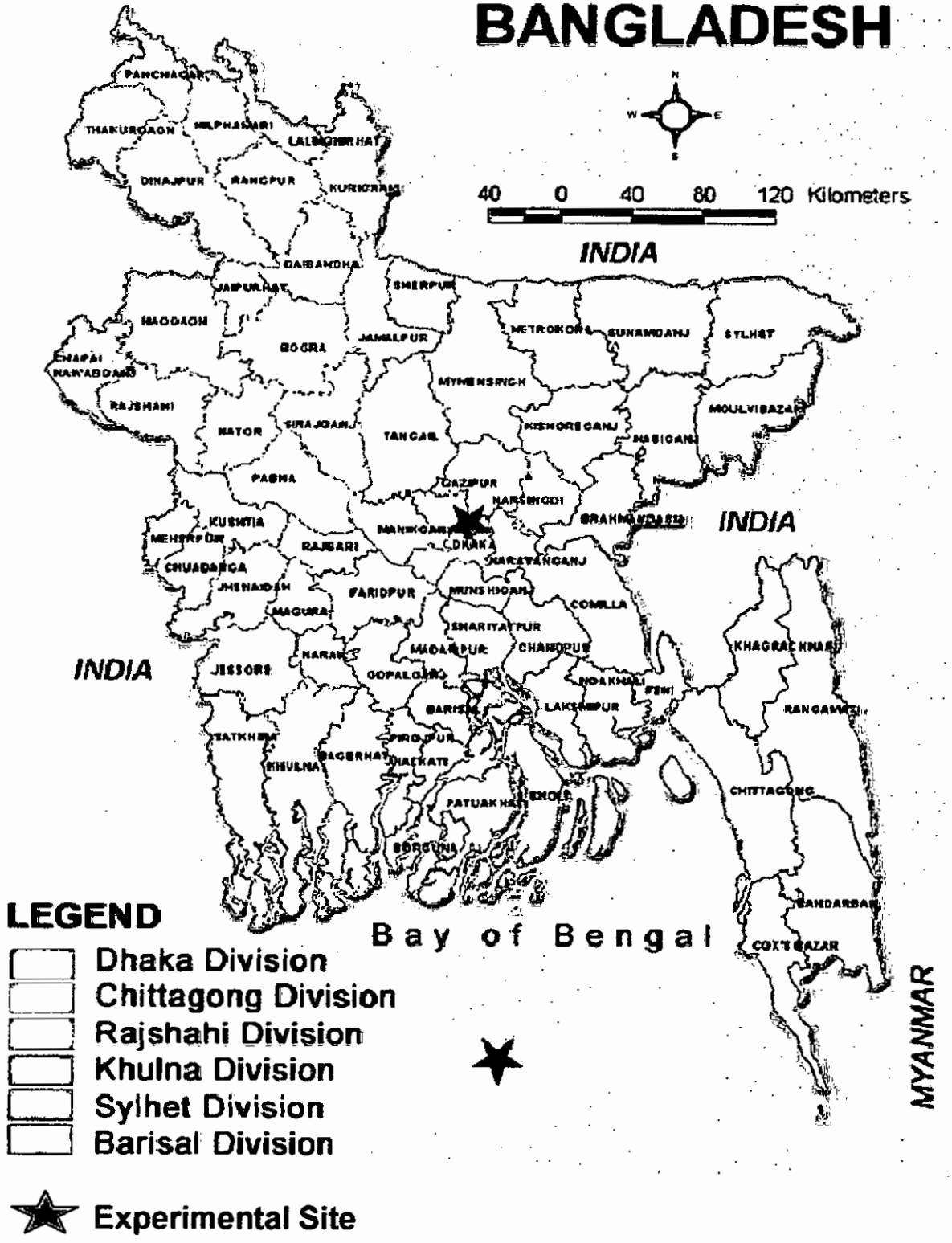


Fig.1. Map showing the experimental site under study

Temperature during the cropping period ranged between 12.2^{0}C to 31.2^{0}C. The humidity varied from 73.52% to 81.2%. The day length ranged between 10.5-11.0 hours only and there was 250-300mm rainfall during the last part of the experiment.

3.3. Soil type

The soil of the experimental site belongs to the Agro-Ecological Region of "Madhupur Tract" (AEZ No. 28). It was Deep Red Brown Terrace soil and belongs to "Nodda" cultivated series. The top soil is slightly clay loam in texture. Organic matter content was very low (0.82%) and soil pH varied from 5.47-5.63. The information about AEZ 28 is given below:

Characteristics of AEZ-28

Land type	Medium high land
General soil type	Non-Calcareous Dark gray floodplain soil
Soil series	Tejgaon
Topography	Upland
Elevation	8.45m
Location	SAU Farm, Dhaka
Field Level	Above flood level
Drainage	Fairly good
Firmness (consistency)	Compact to friable when dry

3. 4. Land preparation

The experimental field was ploughed with power tiller drawn rotovator. After ploughing the field it was left to nature for 10 days for sun and nature to work upon. Subsequent cross ploughing was done followed by laddering to make the land level. Then the soil clods were broken by a wooden

hammer and all weeds, stubbles and residues were removed from the field. Later, Cowdung @ 10 ton/ha and chemical fertilizer like Urea, Triple Super Phosphate (TSP) and Muriate of Potash (MP) was mixed with soil during final land preparation.

3. 5. Fertility status of the field soil

The soil of experimental site was analyzed in Soil Resource Development Institute (SRDI), Dhaka and found as loamy soil which contains total Nitrogen 0.061(%), Phosphorus 35022 microgram per gram of soil, Sulphur 22.60 microgram per gram of soil, Potassium 0.030 miliequivalent per 100gram soil and Calcium-2.67 miliequivalent per 100 gram soil.

Physical and chemical properties of the experimental soil

Soil properties	Value
Soil texture	clay loam
Soil pH	5.8
Organic matter (%)	1.35
Total N (%)	0.08
C : N ratio	10 : 1
Available P (ppm)	35
Exchangeable K (me/100g soil)	0.18
Available S (ppm)	40

3. 6 . Fertilizer application

The experimental field was fertilized with Nitrogen (in the form of Urea), Phosphorus (in the form of Triple Super Phosphate -TSP), Potassium (in the form of Muriate of Potash -MP), Gypsum, ZnO and Boric powder. As per the treatment whole quantity of TSP, MP, Gypsum, ZnO, Boric powder and

one fourth of Urea were applied at final plot preparation.The rest third fourth Urea was applied later in three installments on (40, 60 and 80 days after planting). Fertilizer was applied as recommended doses. Applied doses were as follows:

Name of the Fertilizer	**Fertilizer dose (kg/ha)**	**Fertilizer applied during final land preparation (kg/183.75 m^2 land)**	**Rest installments (Urea) (kg/183.75 m^2 land)**		
			1st	**2nd**	**3rd**
Urea	320	1.47	1.47	1.47	1.47
TSP	415	7.62	-	-	-
MP	168	3.08	-	-	-
Gypsum	100	1.83	-	-	-
ZnO	5	0.09	-	-	-
Boric powder	5	0.09	-	-	-

3.7. Experimental design

The experimental plots were arranged in Randomized Complete Block Design (RCBD) with three (3) replications (Appendix-I). The experiment details were given bellow:

- Total area : 183.75 m^2
- No. of plot : 27
- Plot size : (2 × 1.5) m^2
- Block to block distance : 1.0 m
- Plot to boundary distance : 1 m
- Plot to plot distance (Length wise) : 0.5 m

- Plot to plot distance (Breath wise) : .0.5 m
- Plant to plant spacing : 15 cm
- Row to row spacing : 20 cm

3.8. Treatments of experiment

Treatments used in the experiment: The local cultivars and the onion verities released by Bangladesh Agricultural Research Institute was used as the treatments. Altogether there were nine (9) different onion varieties as stated bellow.

Treatments

T_1 = BARI Peas-1

T_2 = BARI Peas-2

T_3 = BARI Peas-3

T_4 = Thakurgaon local

T_5 = Faridpuri local

T_6 = Indian big

T_7 = Manikgonj local

T_8 = Taherpuri

T_9 = Indian small

3. 9. Growing of onion

3.9.1. Raising of Seedlings

Seedlings were raised in plastic tray from the collected germplasms in the net house with proper care and management. Trays were prepared by mixing soil sand and well decomposed cowdung in the proportion of 2:1:1. The prepared soil was heaped like a square block. Formalin solution (4%) @ 200ml/cft soil was mixed with the soil heap and the soil covered by a

polythene sheet for 48 hours for sterilization. After 7 days surface sterilized trays of (45x20) cm^2 were filled up with the sterilized soil then seeds were sown in the trays and labeled by marker pen. Watering was done to maintain the soil moisture. Shade was provided to save the young and delicate seedling from heavy showering and scorching sunlight.

3.9.2. Transplantation of seedlings

Before transplantation, the top of seedling's leaves, at length of 5 to 6 cm were cut with a sharp knife, the roots were also cut at 2 cm from the base (a usual practice followed by farmers which may help decreased transpiration and faster root development). The prepared seedling was transplanted, as per design and spacing in the evening and watered on the following days up to establishment of seedlings. A good number of seedlings were transplanted at the border for later use as gap fillers.

3.10. Intercultural operation

3.10.1 Irrigation

Irrigation was normally done after each weeding. The young plants were irrigated by a watering can. Subsequence irrigation was done as per requirements.

3.10.2. Gap filling

The dead seedlings were replaced by healthy seedlings within 10 days after transplanting.

3.10.3. Weeding and mulching

Weeding and mulching were done when required to keep the crop free from weeds, for better soil aeration and conserve soil moisture. The common

weeds were *Cynodon dactylon* L. (Durba grass), *Cyperus rotundus* L. (Mutha) etc. Weeding was done carefully keeping the delicate young plants undisturbed.

3.11. Isolation and identification of pathogens from leaf tissue

The diseased leaves were cut into pieces (4 mm diameter) and surface sterilized with $HgCl_2$ (1:1000) for 30 seconds. Then the cut pieces were washed in sterile water thrice and were dried in keeping sterilized blotting paper then placed on to acidified PDA in petridish. The plates containing leaf pieces were placed at room temperature for seven days. When the fungus grew well, and sporulated, then the slide was prepared and was identified under microscope with the help of relevant literature.

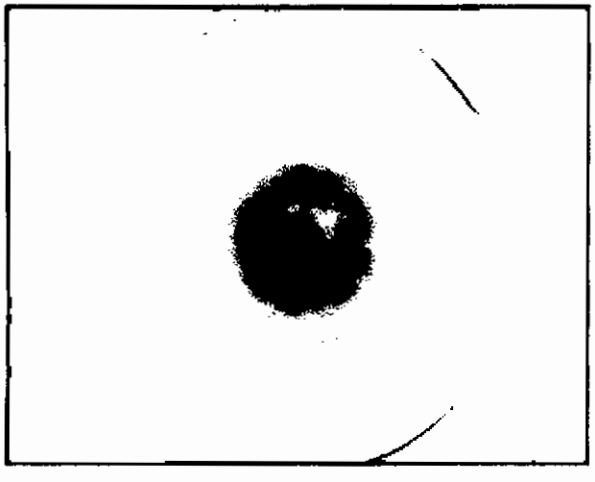


Figure 2.Pure culture of *Stemphylium vesicarium*

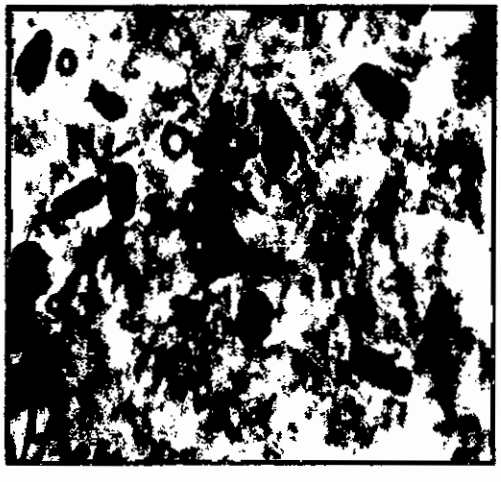


Figure 3. Conidia of *Stemphylium vesicarium*

3.12. Data collection

From forty two plants twenty one plants were selected randomly for each unit plot and tagged for data collection. Data collection was started thirty days after transplanting and continued up to 90 days with 15 days intervals.

3.12.1. No. of infected leaf/plant of different treatments

Number of leaf infected per plant were recorded and used for calculation of disease incidence. The leaf with characteristic white colored spot or blighted tip was denoted as diseased leaf.

Calculation of disease incidence of different treatments

The percent disease incidence was calculated using the following formula.

$$\% \text{ leaf infection} = \frac{\text{Number of infected leaf}}{\text{Total number of inspected leaf}} \times 100$$

3.12.2. Leaf Area Diseased (LAD)/plant in different treatment

Leaf area diseased of the twenty one plants in each plot against each treatment were measured and recorded by conversion to percentage. Mean percentage of leaf area diseased was calculated by dividing number of total observation and used for PDI (percent disease index) estimation.

3.12.3. Estimation of PDI

Evalution of leaf blotch severity: The following diseased scoring scale (0-5 scale) was used to estimate the disease severity (PDI) of white blotch disease of onion for each germplasm.(Hasan,2008)

0 = No disease symptoms (Resistant)

1 = A few spots towards the tip, covering less than 10% area. (Moderately resistant)

2 = Several spots covering less than 20% area. (Moderately resistant)

3 = Several patches with paler outer zone covering up to 40% leaf area. (Susceptible)

4 = Long streaks covering up to 75% leaf area or breaking of leaves/stem from the center. (Highly Susceptible)

5 = Complete blighting of the leaves/stem or breaking of the leaves/steam from the base. (Highly Susceptible)

Figure 4. Onion leaf showing white blotch severity.

3.13. Harvesting

Harvesting of the bulbs

Onion bulbs were harvested on 13th April, 2010, at which the plants had been showing the sign of drying out of most leaves. After harvesting the bulbs were kept in shade, dried and kept separately for weighing.

3.14. Weight of bulb per plot

Weight of onion bulbs per plot were recorded individually for each treatment.

3.15. Yield of onion per hectare

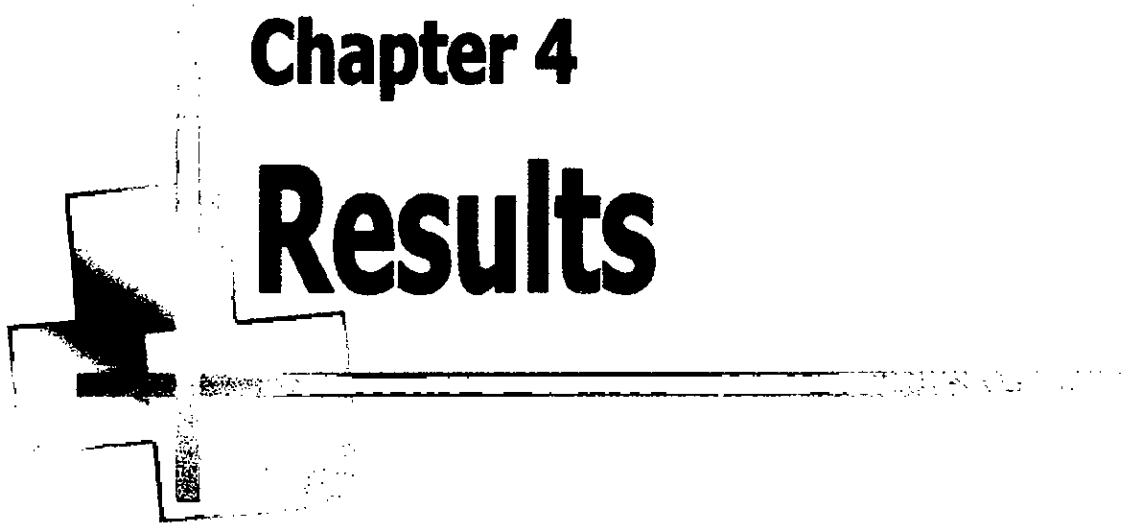
Yield of onion was calculated as ton per hectare.

3.16. Analysis of Data/Statistical Analysis

Data were analyzed statistically using MSTAT Computer Program. Data were compared using Duncan's Multiple Range Test (DMRT), (Gomez and Gomez, 1984).

3.17. Weather report

The monthly average data on temperature, rainfall and humidity during experimental period were collected from the authority of Bangladesh Metrological Department, Agargoan, Dhaka which are presented in Appendix (II).



Chapter 4
Results

CHAPTER 4

RESULTS

This chapter includes the experimental results. Different verities/cultivar viz. BARI Pias-1, BARI Pias-2, BARI Pias-3, Thakungaon local, Faridpur local, Indian big, Manikgonj local, Taherpuri and Indian small were assessed against *Stemphylium vesicarium* causing white blotch of onion under field/natural condition. Results were compiled based on disease incidence and severity of Stemphylium blight at seedling stage to maturity in net house as well as in field condition at different days after transplanting (DAP).

4.1. Incidence of Stemphylium blight on onion seedling of different cultivars at seedling stage in the net house

The incidence of stemphylium blight of onion cultivars on onion seedlings in the net house is presented in table-1. At seedling stage the different cultivars showed significant difference in respect of disease incidence among themselves. No disease incidence were recorded/ observed in case of BARI Peas-3, Indian small and Indian big. Heighest disease incidence (14.33%) was recorded in case of Faridpur local which was statistically insegnificant with Thakurgaon local, Manikgojn local and BARI Peas-1. Disease incidence of BARI Peas-2 was (5.66%) while the incidence of Taherpuri local was 10.67%.

Table 1. Incidence of Stemphylium blight on onion seedling of different cultivars at seedling stage in the net house

Treatments		Disease incidence in onion seedling (%)
T_1	BARI Peas-1	13.00 a
T_2	BARI Peas-2	5.67 bc
T_3	BARI Peas-3	0.00 c
T_4	Thakurgao local	13.33 a
T_5	Faridpuri local	14.33 a
T_6	Indian big	0.00 c
T_7	Manikgonj local	13.33 a
T_8	Taherpuri	10.67 b
T_9	Indian small	0.00 c
LSD		6.014
CV (%)		40.74

At 75 DAP, the disease incidence of Stemphylium blight of onion in the test varieties differed significantly. The lowest disease incidence was recorded in case of BARI Peas-3 (18.65%) which was statistically identical with cultivars Indian big (24.52%) and Indian small (26.11%). Statistically the highest disease incidence were noted in case of Tharpuri, Thahurgaon local Manikgonj local and Faridpur local, preceded by BARI Peas-1 and BARI Peas-2.

In 90 DAP when the crops was in mature stage the cent percent(100%) disease incidence was recorded in case of Tharpuri, Thahurgaon local, Faridpur local and Manikgonj local. The lowest disease incidence (37.3%) was recorded in case of BARI released variety BARI Peas-3 which was statistically identical with cultivars Indian big and Indian small followed by BARI Peas-2.

Table 2. Incidence of Stemphylium blight of onion cultivars recorded at different days after transplanting (DAP) growth in the fields:

Treatments	Disease incidence (%)				
	30 DAP	45 DAP	60 DAP	75 DAP	90 DAP
T_1	26.11 a	32.78 ab	46.67 cd	67.22 b	87.78 a
T_2	12.22 b	26.11 b	32.78 de	53.33 b	73.89 b
T_3	0.00c	0.00 c	12.22 f	18.65 c	37.30 c
T_4	23.33 a	38.33 ab	61.67 ab	85.00 a	100.0 a
T_5	27.30a	44.28 a	66.03 ab	83.01 a	100.0 a
T_6	0.00 c	0.00 c	19.76 ef	24.52 c	44.28 c
T_7	23.33 a	38.33 ab	53.33 bc	85.00 a	100.0 a
T_8	20.55 a	32.78 ab	73.89 a	94.44 a	100.0 a
T_9	0.00 c	5.553 c	13.09 f	26.11 c	41.11 c
CV (%)	35.76	35.09	19.18	14.75	8.98
LSD(0.05)	9.137	14.911	14.00	15.24	11.28

Treatments:

T_1 = BARI Peas-1

T_2 = BARI Peas-2

T_3 = BARI Peas-3

T_4 = Thakurgaon local

T_5 = Faridpur local

T_6 = Indian big

T_7 = Manikgonj local

T_8 = Taherpuri

T_9 = Indian small

4.3. Severity of Stemphylium blight of onion cultivars at different days after transplanting (DAP) in the fields

The severity of Stemphylium blight of onion cultivars recorded at different days after planting (DAP) are presented in Table-3. The different onion varieties showed significant variation in respect of disease severity recorded at different days after transplanting. Data were recorded with 15 days interval staring from 30 days after transplanting. At 30 DAP, the lowest disease severity was observed in case of onion variety BARI Peas-3 (2.333%) which was statistically identical with Indian small and the respective value was (3.667%). The second lowest severity was recorded in case of Indian big (5.667%) which was statistically similar with BARI Peas-2 (7.00%). The highest disease severity was recorded in case of Taherpuri (21.00%) which was statistically identical with Faridpur local (19.33%) followed by Thakurgaon local (17.33%) and Manikgonj local (17.00%).

At 45 DAP, the severity of Stemphylium blight on onion varieties varied significantly. The lowest disease severity was recorded in case of BARI released variety BARI peas-3 (3.833%) followed by Indian small (5.55%) that was statistically similar with the cultivar Indian big (7.00%). The second lowest severity (9.333%) was recorded in case of BARI Peas-2 (9.33%). The highest severity was observed in the local cultivar Taherpuri (25.33%) which was statistically identical with the local cultivar Faridpur local (23.33%). The second highest severity was noted in case of Thakurgaon local (21.00%) followed by Manikgonj local (20.00%).

At 60 DAP, the onion cultivars evaluated against Stemphylium blight also differed significantly regarding disease severity. The lowest disease severity

was recorded in BARI Peas-3 and the calculated value was (5.50%) followed by Indian small (7.667%) which was statistically identical with Indian big (8.333%). The moderate disease severity was recorded in case of BARI Peas-2 (11.67%). The highest disease severity was recorded in case of local cultivar Taherpuri (28.67%) which was statistically similar to Faridpur local (28.00%) followed by Thakurgaon local (25.67%). It was observed that the disease severity of Stemphylium blight of onion was gradually increased with the age of the crops.

At 75 DAP, the disease severity of Stemphylium blight of onion in the test varieties differed significantly. The lowest disease severity was recorded in case of BARI Peas-3 (6.833%). The second lowest severity was noted in case of Indian small (9.667%) which was statistically identical with Indian big (10.00%). Statistically the highest disease severity was noticed in case of Taherpuri (33.33%) which was statistically similar with Faridpur local (31.67%) preceded by Thakurgaon local (30.67%), Manikgonj local (30.33%) and BARI Peas-1 (21.67%).

At 90 DAP, the lowest disease severity (9.00%) was recorded in BARI Peas-3 which was statistically similar with the cultivar Indian small and Indian big. The highest disease severity was recorded in the local cultivar Taherpuri that was statistically identical with the cultivar Faridpur local followed by Thakurgaon local and Manikgonj local .

Table 3. Severity of Stemphylium blight of onion cultivars at different days after transplanting (DAP) growth in the fields:

Treatments	Disease severity (PDI-Percent Disease Index)				
	30 DAP	45 DAP	60 DAP	75 DAP	90 DAP
T_1	10.00 c	14.00 c	18.00 d	21.67 c	26.00 c
T_2	7.000 d	9.333 d	11.67 e	14.00 d	18.00 d
T_3	2.333 e	3.833 f	5.500 g	6.833 f	9.000 e
T_4	17.33 b	21.00 b	25.67 bc	30.67 b	34.33 b
T_5	19.33 a	23.33 a	28.00 ab	31.67 ab	37.00 a
T_6	5.667 d	7.000 e	8.333 f	10.00 e	11.33 e
T_7	17.00 b	20.00 b	25.00 c	30.33 b	34.00 b
T_8	21.00 a	25.33 a	28.67 a	33.33 a	37.33 a
T_9	3.667 e	5.667 ef	7.667 fg	9.667 e	11.00 e
CV (%)	9.77	9.13	8.57	7.02	6.12
LSD (0.05)	1.942	2.274	2.612	2.541	2.564

Treatments:

T_1 = BARI Peas-1

T_2 = BARI Peas-2

T_3 = BARI Peas-3

T_4 = Thakurgaon local

T_5 = Faridpur local

T_6 = Indian big

T_7 = Manikgonj local

T_8 = Taherpuri

T_9 = Indian small

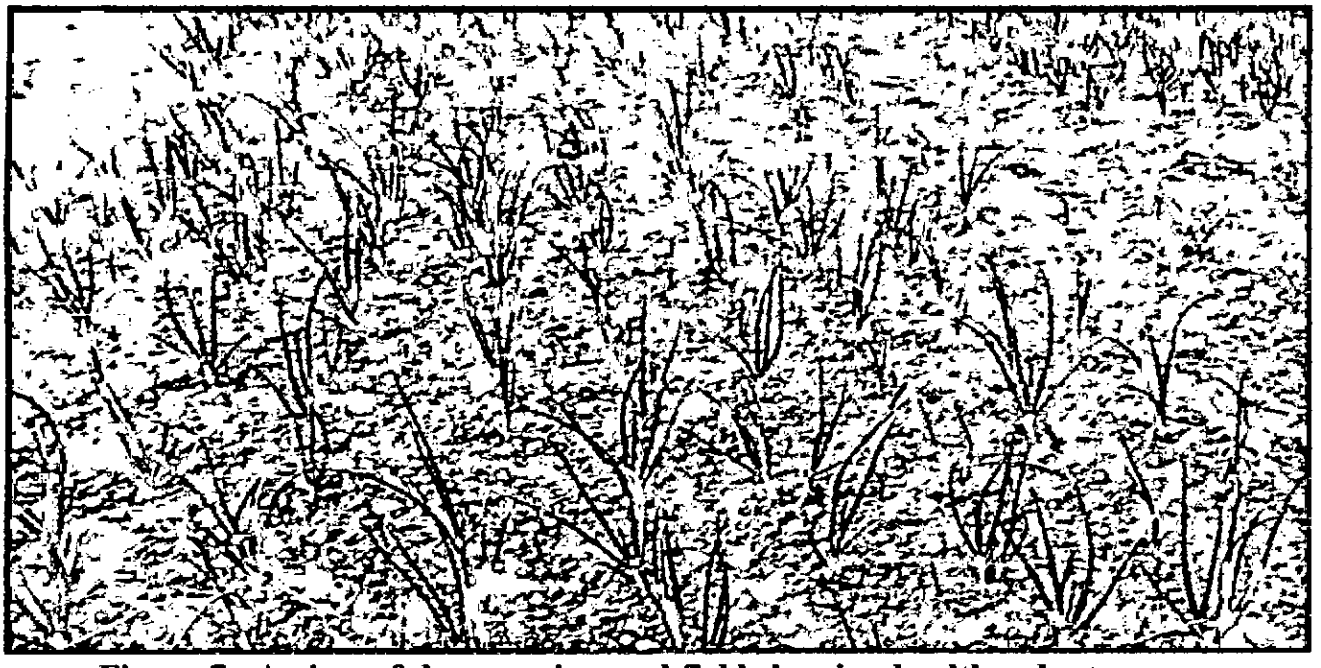


Figure 7. A view of the experimental field showing healthy plant

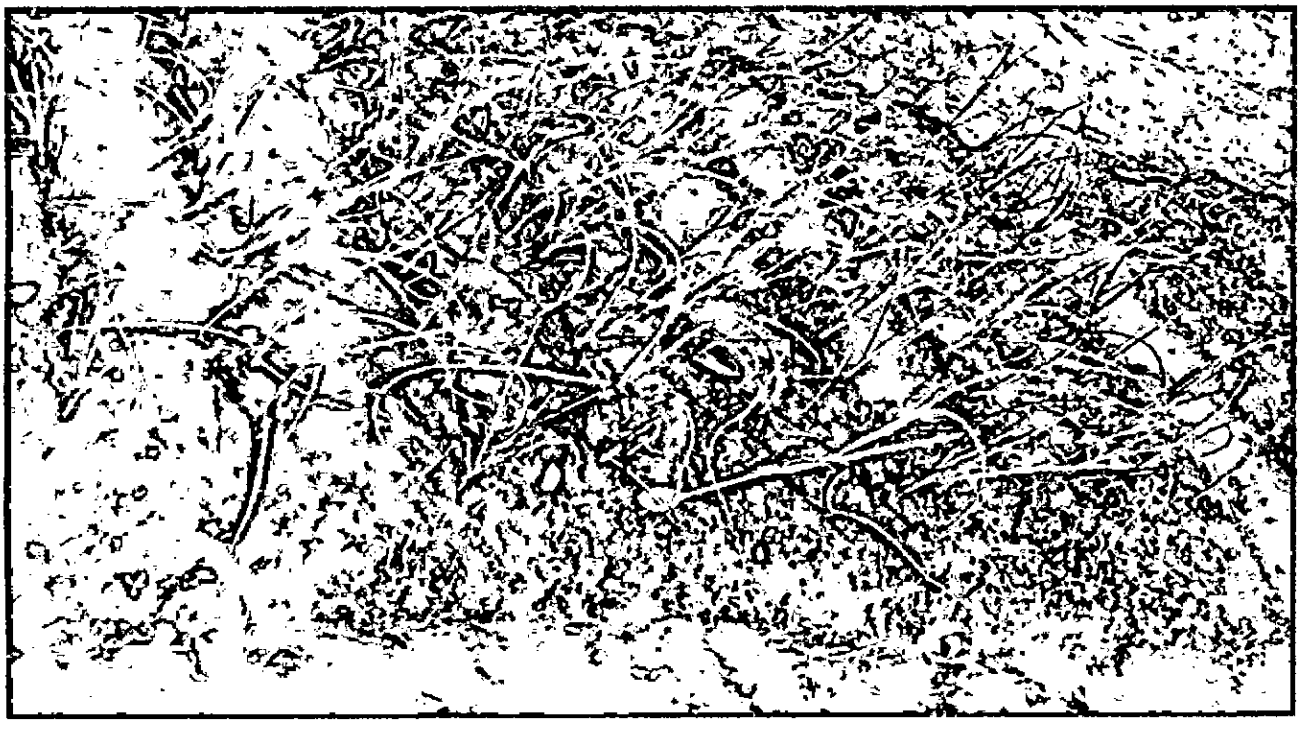


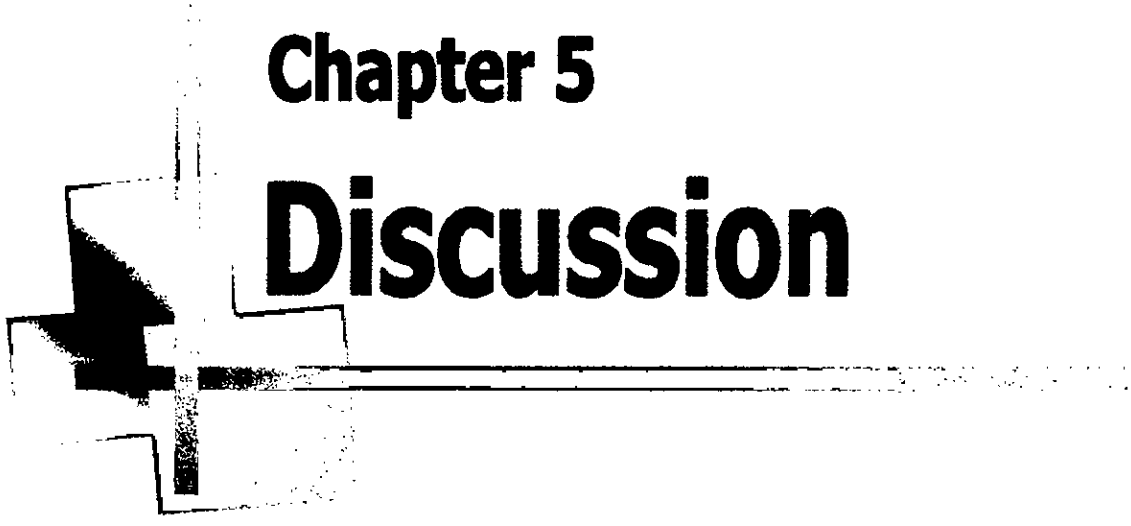
Figure 8. A view of the experimental field showing healthy plant

4.4. Yield performance of different onion cultivars against Stemphylium blight of onion

Yield performance of different test varieties against Stemphylium blight is presented in Table-4. The different varieties varied significantly in respect of yield. BARI Peas-3 showed highest yield performance (12.67 ton/ha). The second highest yield (10.33 t/ha) was obtained in case of the cultivar Indian small which was statistically identical with the cultivar Indian big (9.84 t/ha). BARI Peas-2 showed Moderate yield performance (9.00 t/ha). The lowest yield performance was obtained in case of the local cultivar Taherpuri (4.34 t/ha) which was statistically similar with the local cultivar Faridpur local (4.84 t/ha). The second lowest yield (5.67 t/ha) was noted in Takurgaon local which was statistically identical with local cultivar Manikgonj local (6.17 t/ha).

Table 4: Yield performance of different onion cultivars against Stemphylium blight of onion:

Treatments		Yield(t/ha)
T_1	BARI Peas-1	7.00d
T_2	BARI Peas-2	9.00c
T_3	BARI Peas-3	12.67a
T_4	Thakurgaon local	5.67e
T_5	Faridpur local	4.84f
T_6	Indian big	9.84b
T_7	Manikgonj local	6.17e
T_8	Taherpuri	4.34f
T_9	Indian small	10.33b
CV (%)		5.72
LSD		0.6783



Chapter 5
Discussion

CHAPTER 5

DISCUSSION

Onion (*Allium cepa*) is a popular vegetable grown for its pungent bulbs and flavorful leaves. Onion is an important spice as well as vegetable crops grown in many countries in the world including Bangladesh. The yearly onion production is 889260 metric ton from 309309 acre of land. (BBS, 2008). In Bangladesh, the productivity of onion is on the 7.1 mt/ha which is quite lower in comparison to the world average like Koreya (67.25 mt/ha), USA (53.91 mt/ha), Spain (52.06 mt/ha) and Japan (47.77 mt/ha) (FAO, 2008).White blotch complex of onion is supposed to be the major constrain for the lower yield of onion in the country. The present experiment was conducted in the net house of the department of plant pathology and in the field of SAU farm during November, 2009 to April, 2010. To find out the performance of different onion cultivars available in Bangladesh against white blotch complex (*Stemphylium vesicarium*). The cultivars used in the experiment were BARI Peas-1, BARI Peas-2, BARI Peas-3, Indian big Indian small, Taherpuri, Manikgonj local, Thakurgaon local and Faridpur local collected from HRC (Horticultural Research Center), BARI (Bangladesh Agricultural research Institute) and major onion growing areas in the countries.

5.1. Net house experiment

In the net house using the collected seeds of onion cultivars seedling were raised in a temporary constructed net house using plastic tray arranged in RCBD deign with three replications. At the age of 22-25 days old seedlings the incidence of white blotch were observed. No disease incidence was

found in case of BARI Peas-3, Indian small and Indian big. While the local varieties Faridpur, Taherpuri, Manikgonj and Thakurgaon showed disease incidence (Table-1). The highest seedling incidence was found in case of Manikgonj local followed by Taherpuri ,Thakurgaon local and Faridpur local. No pervious report on seedling incidence of onion cultivars against white blotch disease of onion is reported in the country. However it is reported that indigenous red variety of India found to be uninfected against white blotch complex of onion (*Stemphylium vesicarium*).(Sing, D.1997).

5.2. Field experiment

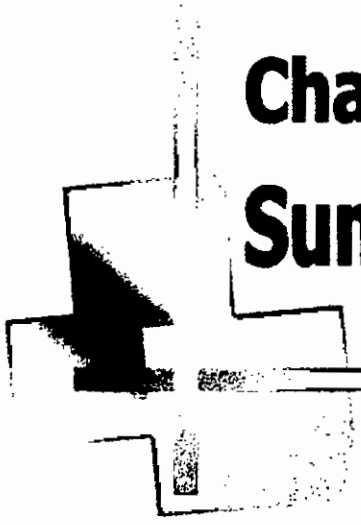
The onion seedlings raised in the net house transplanted to the field after one month of seed sowing. The incidence and severity was recorded starting from 30 days after transplanting (DAP) with 15 days intervals. In case of disease incidence it is reveals that different cultivars showed significantly different reaction against white blotch of onion. Initially at 30 DAP, no disease incidence was found in BARI released variety BARI Peas -3 and two Indian variety Indian big and Indian small while the rest of the varieties were infected by the disease. The result showed that with the increase of the age of the crops the incidence of the disease increased gradually and reached to the highest at 90 DAP in maturity. At 90 DAP, the BARI Peas-3, Indian small and Indian big were also scored significantly lowest incidence (37.30%, 41.11% and 44.28%), respectively while Thakurgaon local Faridpur local Manikgonj local and Taherpuri showed 100% disease incidence. BARI Peas -2 and BARI Peas -1 scored 73.89%, and 87.78% disease incidence, respectively. On the basis of the response of the cultivars regarding disease incidence against white blotch complex of onion it reveals that BARI released variety BARI Peas-3, Indian big and Indian small seem

to be resistant under natural conditions while the local varieties are found to be highly susceptible. Padule and Utikar (1977) tested 32 onion cultivar under natural field conditions against white and purple blotch was done and recorded all tested cultivars found to be susceptible. Thirumalachar *et al* (1953) searched varietals resistant against white blotch disease of onion reported that the indigenous red variety showed resistant reaction. No reports on the screening of local onion varieties are available in Bangladesh. In the present experiment disease severity as PDI (Percent disease index),while recorded at different days after transplanting of seedling starting from 30 DAP to 90 DAP with 15 days intervals it was noticed that a remarkable difference on disease severity was observed from 30 days after transplanting of seedling and the difference of severity appeared to be more distinct among the treatments with the progress of time and the severity counts reached to the highest stage of the crops. On the basis of disease severity or disease reactions, the cultivars were graded following the proposed severity scales (Basak 1997). As per severity scale the BARI released variety BARI Peas -3 was found to be resistant (PDI-9.00%), cultivars Indian small (PDI-11.00%), Indian big (PDI-11.33%) and BARI Peas-2 (PDI-18.00%) were graded as moderately resistant. Rest of the local cultivars including BARI Peas-1 (PDI range from 26-37%) were graded as susceptible. No research report are available in the literature on the disease reaction of onion cultivars both local and hybrid against white blotch of onion in the country.

At mature stage, the crops were harvested and bulb yield weighted was recorded separately for each test cultivar or varieties. The highest yield performance was recorded in case of variety BARI Peas-3(12.67 t/ha)

followed by Indian small (10.33 t/ha), Indian big (9.84 t/ha), BARI Peas-2 (9.00 t/ha) and BARI Peas-1 (7.00 t/ha). The yield performance of Indian big and Indian small was also identical. Significantly the lowest yield was recorded in case of Faridpur local and Taherpuri. The yield performance of Thakurgaon local and Manikgonj local were also significantly identical where the yield were 5.67 t/ha and 6.17 t/ha respectively.

From the result, it is revealed that yield performance of BARI released variety and the imported Indian varieties were higher than the indigenous local varieties. The better performances regarding the yield of BARI released varieties and two imported Indian variety Indian big and Indian small might be due to the resistant reaction of the varieties against white blotch disease. Because white blotch disease reduce the yield up to 41-44% in Bangladesh (Fakir, 2002, Hassan ,2008). The present finding agree with the report of Rahman *et al.*,1988), who reported that two widely cultivated local variety in Bangladesh like Faridpur, and Taherpuri were susceptible to white blotch complex of onion that affects the bulb seriously.



Chapter 6
Summary and Conclusion

CHAPTER 6

SUMMARY AND CONCLUSION

Onion (*Allium cepa*) is one of the most important vegetable and at the same time an important spice crop in Bangladesh. Onion suffers from many diseases of which stemphylium blight is common and devastating for itsdestructive potential bulb yield and deteriorating quality of seeds.

The present piece of research work was conducted in the Dpertment of Plant Pathology and in the field of SAU (Sher e-Bangla Agricultural University) farm allotted for the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, to screen out the resistant varieties of onion against stemphylium blight disease (*Stemphylium vesicarium*) during the period from October 2009 to April 2010. Nine onion varieties viz. BARI Peas-1, BARI Peas- 2, BARI Peas- 3,Thakurgaon local Faridpur local, Manikgonj local, Taherpuri, Indian big and Indian small were evaluated in the experiment. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications.

The onion varieties differed significantly among themselves in respect of incidence at seedling stage. No disease incidence was observed in case of BARI Peas-3, Indian small and Indian big. The highest disease incidence (14.33%) in seedling was recorded in case of Faridpur local which was statistically indifferent with Thakurgaon local, Manikgonjn local and BARI Peas-1.

In the field condition, the disease incidence and disease severity were recorded in different days after transplanting (DAP) with 15 days intervals

starting from 30DAP.The disease incidence and severity of different onion cultivars against stemphylium blight (*Stemphylium vesicarium*) differed significantly at different days after transplanting (DAP).The lowest disease incidence at 30 DAP, 45 DAP, 60 DAP, and 90 DAP respectively were 0.0%, 0.0%, 12.22%, 18.65% and 37.30% in case of BARI Peas-3 followed by Indian small and Indian big with some extent. The highest disease incidence at 30 DAP, 45 DAP, 60 DAP, 75 DAP, and 90 DAP, were observed in case of local cultivars Taherpuri, Fridpur local, Thakurgaon local and Manikgonj local.

In case of disease severity the lowest PDI (Percent disease index) was recorded in case of BARI Peas-3 irrespective of different days after transplanting (DAP) followed by Indian small and Indian big. The local cultivars Taherpuri, Manikgonj local Thakurgaon local and Manikgonjn local showed higher PDI irrespective of different DAP.

On the basis of yield performance the highest yield (12.67 t/ha) was found in BARI Peas-3 followed by Indian small (10.33 t/ha) that was statistically identical with Indian big (9.84 t/ha).The lowest yield performance was found in Taherpuri (4.34 t/ha) followed by Faridpur local (4.84 t/ha).

On the basis of disease reactions in critical stage at 90 DAP BARI Peas-3 (9.00%) was graded as resistant against stemphyliun blight. Cultivars Indian big (11.33%), Indian small (11.00%) and BARI released variety BARI Peas-2 (18.00%) categorized into moderately resistant. The local cultivars Taherpuri (37.33%), Faridpur local (37.00%), Thakurgaon local (34.33%), Manikgonj local (34.00%) and BARI Peas-1 (26.00%) were graded as susceptible. (Hasan, 2008).

Considering the performance of onion cultivars under natural field condition it may be concluded that BARI Peas-3 was resistant against stemphylium blight disease of onion Indian small, Indian big and BARI Peas-2 were moderately resistant and the local cultivars Taherpuri, Faridpur local Thakurgaon local and Manikgonj local were susceptible to stemphylium blight of onion (*Stemphylium vesicarium*). However, screening program need to carryout for consecutive years in different agro ecological zones to justify the present findings.

Chapter 7
Literature Cited

CHAPTER 7
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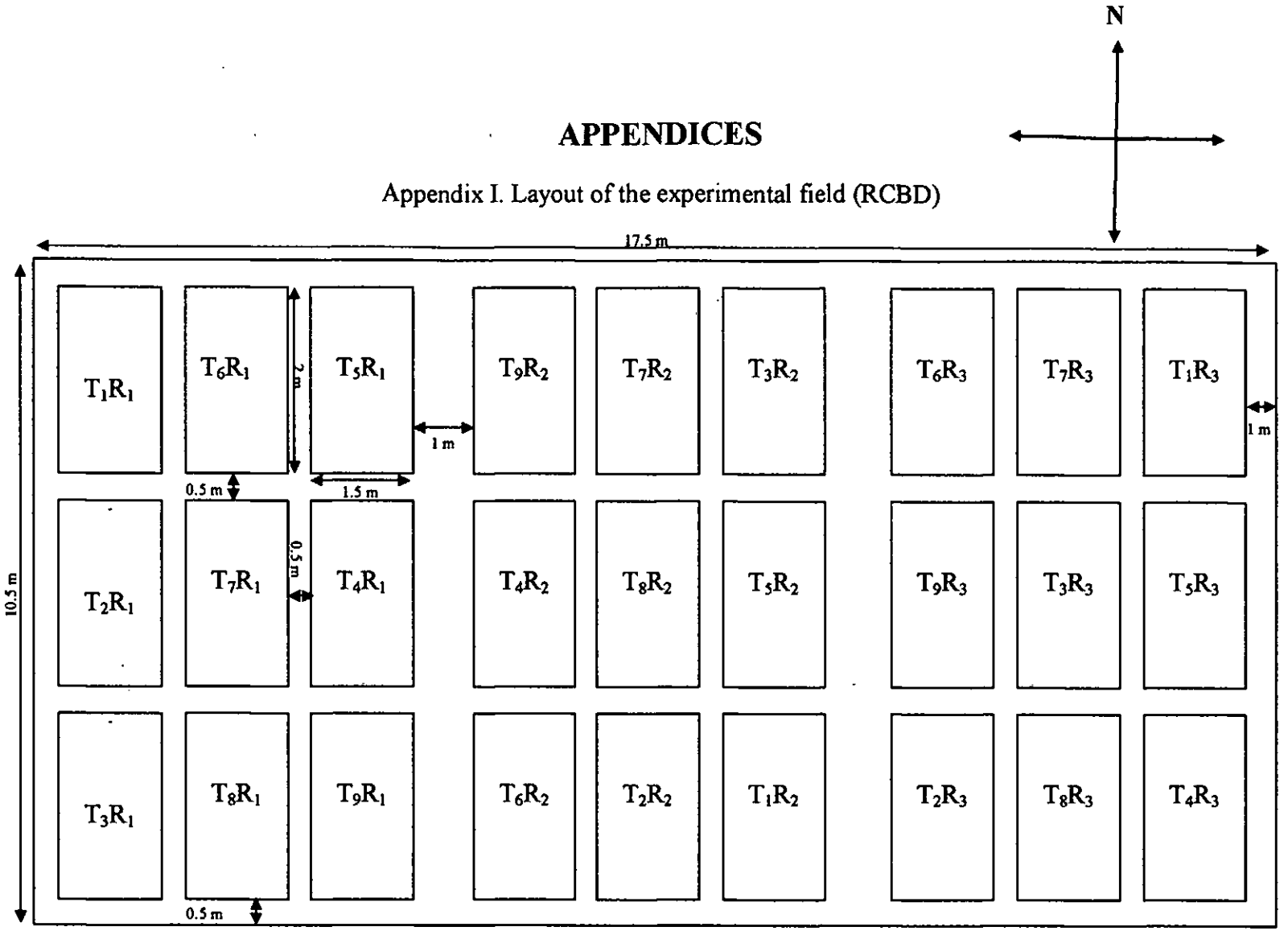
Appendices

APPENDICES

Appendix I. Layout of the experimental field (RCBD)



Total area= 17.5 x 10.5=183.75 m^2

Fig. 2. Layout of the field experiment showing treatment assigned

APPENDICES

Appendix II. Monthly average temperature, relative humidity and total rainfall of the experimental site during the period from November, 2009 to april, 2010.

Month	Air temperature (0C)			RH (%)	Total rainfall (mm)
	Maximum	Minimum	Mean		
November, 2009	29.0	19.8	24.40	73.90	3.0
December, 2009	27.0	15.7	21.35	62.79	0.0
January, 2010	24.9	13.2	19.05	67.5	3.0
February, 2010	28.1	17.8	22.95	61.5	4.0
March, 2010	32.5	22.6	27.55	66.6	155.0
April, 2010	33.74	23.87	28.81	69.41	250

Source: Bangladesh Metrological Department (Climate division), Agargoan, Dhaka-1207.

Appendix III. Analysis of variance of the data on the incidence of stemphylium blight at seedling stage

Source of variance	Degree of freedom	Mean square
Replication	2	5.59
Treatments	8	122.34
Error	16	10.13

Significant at 0.05 level of probality.

Appendix IV. Analysis of variance of the data on the incidence of stemphylium blight after different days of transplanting

Source of variance	Degree of freedom	Mean square				
		30 DAP	45 DAP	60 DAP	75 DAP	90 DAP
Replication	2	17.14	30.28	137.53	15.97	10.76
Treatments	8	422.49	7417.72	1662.35	2692.10	2313.03
Error	16	27.86	1157.78	65.39	77.53	46.63

Significant at 0.05 level of probality.

Appendix V. Analysis of variance of the data on the severity of stemphylium blight after different days of transplanting

Source of variance	Degree of freedom	Mean square				
		30 DAP	45 DAP	60 DAP	75 DAP	90 DAP
Replication	2	4.593	3.86	4.694	3.34	3.44
Treatments	8	156.67	203.54	268.79	354.73	430.33
Error	16	1.259	1.726	2.278	2.155	2.194

Significant at 0.05 level of probality.

Appendix VI. Analysis of variance of the data on the yield of different treatments

Source of variance	Degree of freedom	Mean square
Replication	2	0.009
Treatments	8	24.13
Error	16	0.197

Significant at 0.05 level of probality.