SCREENING OF SELECTED GARLIC VARIETIES AGAINST FUSARIUM ROT DISEASE CAUSED BY *Fusarium proliferatum*

JANNATUN NAHAR PRINKY

DEPARTMENT OF PLANT PATHOLOGY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY,
DHAKA-1207

DECEMBER, 2018

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BY

JANNATUN NAHAR PRINKY

REGISTRATION NO.: 17-08261

A Thesis

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 (MS)

IN

PLANT PATHOLOGY

SEMESTER: JULY-DECEMBER, 2016

Approved by:

Dr. Fatema Begum	**Dr. Md. Rafiqul Islam**
Professor	Professor
Supervisor	**Co-supervisor**
Department of Plant Pathology	Department of Plant Pathology
Sher-e-Bangla Agricultural University	Sher-e-Bangla Agricultural University
Dhaka-1207	Dhaka-1207

Dr. Khadija Akhter
Professor
Chairman
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Dhaka-1207

DEPARTMENT OF PLANT PATHOLOGY

Sher-e-Bangla Agricultural University

Sher-e-Bangla Nagar, Dhaka-1207

CERTIFICATE

*This is to certify that the thesis entitled '**Screening of selected garlic varieties against Fusarium rot disease caused by Fusarium proliferatum**' submitted to the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE in PLANT PATHOLOGY**, embodies the results of a piece of bona fide research work carried out by , **JANNATUN NAHAR PRINKY**, Registration No. **17-08261** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

Dated: December, 2019
Dhaka, Bangladesh

Dr. Fatema Begum
Professor
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Dhaka-1207

Supervisor

DEDICATED TO

MY

BELOVED PARENTS, MY

HUSBAND AND MY

GRANDMOTHER

ACKNOWLEDGEMENTS

All the praises and gratitude are due to the omniscient, omnipresent and omnipotent ***Almighty Allah****, who has kindly enabled the author to complete her research work and complete this thesis successfully for increasing knowledge and wisdom.*

The author sincerely desires to express her deepest sense of gratitude, respect, profound appreciation and indebtedness to her research Supervisor, ***Dr. Fatema Begum****, Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for her kind and scholastic guidance, untiring effort, valuable suggestions, inspiration, co-operation, constructive criticisms and for providing necessary facilities and conductive atmosphere to accomplish the research work and the preparation of the manuscript of this thesis.*

The author expresses heartfelt gratitude and indebtedness to her Co-supervisor, ***Dr. Rafiqul Islam*** *Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for his cooperation, criticisms on the manuscript and helpful suggestions for the successful completion of the research work.*

I also express my cordial thanks to respected teacher ***Dr. Khadija Akhter****, Professor and Chairman and to all respected teacher, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for their valuable teaching, encouragement and co-operation during the entire study period.*

The author wishes to extend her special thanks to ***Umme Habiba Akter*** *and* ***Nasrin Sultana Neela*** *for her great support and assistance during experimentation. Special thanks to all other friends for their support and encouragement to complete this study.*

The author is deeply indebted to her respectful father, mother, sisters for their moral support, encouragement and unquantifiable love with cordial understanding.

Finally, the author also expends her thanks to all the staff of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for their help and co-operation during the research work.

The Author

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ABSTRACT

A field experiments was carried out to study the screening of selected garlic varieties in field and isolation, identification and pathogenicity test of Fusarium rot caused by *Fusarium proliferatum* was also done. The experiment was conducted in central farm and plant pathology lab, Sher-e-Bangla Agriculture university, Dhaka-1207 with RCBD (Randomized Complete Block Design) with three replications. The entire experimental plot was divided into three blocks, each of which then divided into 24-unit plots. A total eight 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 was selected to conduct the study. In the following study the highest pre emergence mortality was observed in BAU Rashun-1 garlic variety with 31.35% germination failure and the lowest were found in BARI Rashu-1 with 14.19%. The maximum result was found for the post emergence mortality in BARI Rashun-3 with 13.22% mortality rate after germination and minimum result were found in Local Indian variety 6.15%. Leaf height showed negative correlation with the entire yield parameters considered and become significant at 0.01% level of probability and number of leaves showed positive correlation with all the yield defining characters except clove diameter (-.859). For disease incidence BARI Rashun-4 showed the highest susceptibility to Fusarium rot and maximum disease severity of garlic variety was observed in BARI Rashun-4. Maximum radial mycelial growth of *Fusarium proliferatum* was observed in BAR3I (9mm). The minimum radial mycelial growth was BAR2I (5.2mm). Pathogenicity test was done with only one susceptible variety BARI Rashun-4 in *in-vivo* condition by inoculating *Fusarium proliferatum* as soil inoculation. 27 pots were considered as test pot, 29.62% pre emergence mortality and 49.37% dead seedling was observed in *in- vivo* condition.

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ABBREVIATION AND ACRONYMS

AEZ	=	Agro-Ecological Zone
BARI	=	Bangladesh Agricultural Research Institute
BBS	=	Bangladesh Bureau of Statistics
FAO	=	Food and Agricultural Organization
N	=	Nitrogen
et al.	=	And others
TSP	=	Triple Super Phosphate
MOP	=	Muriate of Potash
RCBD	=	Randomized Complete Block Design
DAT	=	Days after Transplanting
ha^{-1}	=	Per hectare
g	=	gram (s)
kg	=	Kilogram
SAU	=	Sher-e-Bangla Agricultural University
SRDI	=	Soil Resources and Development Institute
wt	=	Weight
LSD	=	Least Significant Difference
^0C	=	Degree Celsius
NS	=	Not significant
Max	=	Maximum
Min	=	Minimum
%	=	Percent
NPK	=	Nitrogen, Phosphorus and Potassium
CV%	=	Percentage of Co-efficient of Variance
BAR3I	=	BARI Rashun-3 isolate
BAR2I	=	BARI Rashun-2 isolate

CHAPTER 2

REVIEW OF LITERATURE

In Bangladesh and in many countries of the world garlic is an important and one of the most valuable spice crops. The crop received much attention to the researcher of different countries including Bangladesh. But a few investigations have been taken on the screening of fusarium rot of different garlic varieties in vivo. There is a little or no combined research work evaluate the major fungal diseases of different garlic varieties at field conditions in Bangladesh and study the pathogenicity test of fusarium disease of garlic by inoculation of *Fusarium proliferatum*. The literature and research results related to the present study are reviewed in this chapter.

2.1 Morpho-physiology of garlic

A garlic bulb is generally four to eight centimeters in diameter, white to pinkish or purple, and is composed of numerous (8-25) discrete cloves. The foliage comprises a central stem 25-100 cm tall, with flat or keeled (but not tubular) leaves 30-60 cm long and 2-3 cm broad. The flowers are produced in a small cluster at the top of the stem, often together with several bulblets, and surrounded by a papery basal spathe; each flower is white, pink or purple, with six petals 3-5 millimeters long. The flowers are commonly abortive and rarely produce any seeds (Wikipedia, 2018).

Garlic belongs to the family Liliaceae. Common garlic is classified as *Allium sativum*, British wild garlic as *Allium oleraceum*, and American wild garlic as *Allium candense*. The field garlic of Europe and the Americas is classified as *Allium vineale*. False garlic is classified as Nothoscordum bivalve (MEOE, 2001). Garlic is a perennial that can grow two feet high or more. The most important part of this plant for medicinal purposes is the compound bulb. Each bulb is made up of 4 to 20 cloves, and each clove weighs about 1 gram. The parts

of the plant used medicinally include fresh bulbs, dried bulbs, and oil extracted from the garlic. (UMM, 2004). The Bulb, 12 inches to 18 inches tall (30-45 cm), 9 inches to 12 inches in spread (22.5-30 cm).

The roots are trimmed and the stems snipped or braided. Depending on where they are grown, the size, shape, colour, and flavour will differ. Colours can range from white to red to purple or pink (Innvista, 2005).

OSU, (2005), a garlic bulb develops from the bud primordia (2 or 3) of the cloves that are planted. Each bud primordia forms between two and six growing points, each of which develops a lateral bud which later develop into a clove. Temperatures during growth determine the rate of leaf growth, clove, and flower stalk development. Clove formation in non-bolting types differs slightly in that lateral-bud primordia (which form the cloves), form in the axil of the youngest 6-8 foliage leaves, beginning with the oldest one. At maturity, these develop into cloves. The growing point may then either form a clove and go dormant, or form an incomplete leaf that degenerates.

Sexual propagation in garlic is expected to facilitate the exchange of genetic traits from one genotype to another and to improve garlic cultivars through classical breeding (Kamenetsky *et al.*, 2004). Garlic does not produce true seed but it is propagated by planting cloves. Each bulb usually contains a dozen or more cloves and planted separately. Select only larger outer cloves of the best garlic bulbs for planting because larger cloves yield larger size and mature bulbs at harvest.

Hector *et al.*, (2012) revealed that garlic is propagated asexually, but shows a high morphological diversity among cultivars. These cultivars have a wide range of adaptation to different environments. Like onion, garlic plants have thin tape shaped leaves about 30 cm long. Roots reach up to 50 cm depth or little more. Heads or bulbs are white skinned, divided into sections called cloves. Each head

could have from 6 to 12 cloves, which are covered with a white or reddish papery layer or "skin".

Similarly, Kamenetsky *et al.*, (2001) revealed that sexual propagation in garlic is expected to facilitate the exchange of genetic traits from one genotype to another and to improve garlic cultivars through classical breeding. Garlic does not produce true seed but it is propagated by planting cloves. Each bulb usually contains a dozen or more cloves and planted separately. Select only larger outer cloves of the best garlic bulbs for planting because larger cloves yield larger size and mature bulbs at harvest.

According to Figliuolo *et al.* (2001); Ipek *et al.* (2003) garlic belongs to the genus Allium family Alliaceae, which includes important vegetable crop such as onion (*Allium cepa*), leek (*A. ameloprisum*) and shallots (*A. asacloncum*). Garlic is a diploid species (2n = 2x = 16) of obligated apomixis and propagated vegetatively.

Garlic's strap like leaves are 1-2 feet long, surrounding a central flower stalk or scape, which develops a globular cluster of tiny white blossoms (The Rodales Herb book,1987).

The leaves are flat, linear, gray-green, and longitudinally folded, with a keel on the lower surface. Six to twelve of them grow, widely spaced, along the central stalk of the plant. The bases of non-topsetting types form a semi-stiff pseudostem, which remains upright until bulb maturity, when it bends over near ground level (Garlic and friends, 1996).

2.2 Nutritional value of garlic

Gadel-Hak *et al.* (2015) studied six garlic genotypes with different skin colors (white and purple skin), and they detected significant differences in vitamin C and total fractionated oil contents (higher for purple color genotypes), as well as in total phenolic compounds and flavonoids content (higher for white color genotypes).

According to Mohammadi *et al.* (2014), there was a relationship between geographical origin and genetic diversity for various Iranian garlic landraces, whereas differences in germplasm were mostly due to genotype and transfer of plant material between the various growing areas.

Moreover, Singh *et al.* (2014), analyzed the morphological variability of 47 Indian garlic collections and they suggested the existence of only two major phylogenetic groups, which could be attributed to the vegetative nature of propagation.

Bhandari *et al.* (2014), evaluated 19 garlic genotypes from South Korea, and although they reported amounts of sucrose (up to 3.43%), fructose and glucose were detected in higher amounts (1.05% and 0.54% comparing to 0.41 and 0.27, for fructose and glucose respectively), which could be attributed to different genotypes and growing conditions.

According to Rekowska and Skupie ´n (2009), sugar content can be affected by cultivation practices such as plant density, and type of ground cover. Imen *et al.* (2013), have reported that sulfur fertilization can negatively affect reducing the carbohydrate content of rosy garlic (*Allium roseum* L.) bulbs, expressed as glucose equivalents.

Rekowska (2009) There is also a high content of non-volatile compounds with well-known medicinal and therapeutic properties, such as amides, nitrogen oxides, phenolic compounds, especially flavonoids, proteins, saponins and sapogenins, as well as antioxidants, minerals (especially P, K and Se) and vitamins (especially vitamin C and vitamins of B complex).

Significant variation according to the genotype in sugar composition and total sugar content has been previously reported by Pardo *et al.* (2007) who evaluated 14 garlic cultivars cultivated in Spain. In most cases of that study sucrose was the main sugar followed by fructose and glucose; however, there was a single case where fructose content was higher than that of sucrose (Chinese cultivar ChinoSprint).

Kimbaris *et al.* (2006) reported garlic is considered a rich source of volatile compounds, which are responsible for the distinct flavor and the bioactive properties of dry bulbs.

Gonzalez *et al.* (2009) have detected significant variability in terms of organosulfur compounds, pungency, total soluble solids and antiplatelet activity, not only between garlic clones belonging in different eco-physiological groups, but also between clones of the same group.

According to Koch and Lawson (1996), dry garlic bulbs mainly consist of water (62–68%) and carbohydrates (26–30%), while proteins are detected in relatively less amounts (1.5–2.1%). Moreover, protein contents of 4–6% are also very common in various cultivars, considering the high dry matter content of the bulbs, while ash content ranges between 0.6% and 1.0%, and energy content is around 140 kcal 100 g–1 f.w. Brewster (2008).

Kaufmann *et al.*, 1999 reported garlic bulbs are rich sources of carbohydrates and proteins. Analysis of garlic indicates that it contains 61– 64% moisture, 31% carbohydrate, 5–6% protein and only 0.2% fat. Significant levels of phosphorous (3.9– 4.6 mg/g), potassium (1.0–1.2 mg/g) and calcium (0.5– 0.9 mg/g) are present.

2.3 Production of garlic

According to FAO, 2016 garlic is cultivated in most countries both in the tropic and temperate zones. In Asia, it is commercially grown in China, Indonesia, Pakistan, Republic of Korea, Thailand, and India. World trade in garlic is dominated by the developing countries and their share of trade has been growing at the expense of that of the developed countries during the past ten years.

China is the leading producer, accounting for 66 percent of world output. China is the leading producer of garlic accounting for 20.0 million tons followed by India with 1.25 million tons per year. The other three top producing

countries of garlic include; South Korea, Egypt, and Russia producing 0.35, 0.26 and 0.26 million tons, respectively (Wikipedia, 2017).

2.4 Economic value of garlic

Spyridon *et al.* (2018) describe garlic as one of the most important herbs found in Nigeria. It is used for flavoring food. But over the years, it has also been used as a medicine to prevent or treat various types of diseases. The following are the economic importance of garlic: Cash crop: USA is said to be the world's largest import market of fresh garlic, followed by Indonesia, France, Germany, Australia and Brazil. Nigeria has a great potential in the export of garlic, if only we can cultivate it in larger quantities using improved methods and advanced cultural practices. Therefore, serving as a source of foreign exchange. Medicinal purposes: garlic is used as herbal medicine for many conditions related to the blood and heart. It also has antifungal and antibacterial properties. Flavoring in food: garlic is a common flavoring used in cooking. It serves as a food additive which prevents food poisoning. Raw material: garlic is used as raw material in pharmaceutical industries. It is used to produce supplements which have enteric coatings. Low capital requirement: in the presence of a good farm site, garlic production does not require a huge start-up capital. It is less affected by destructive pests and diseases unlike the other vegetables. It does not require sophisticated storage facility and can be stored for a very long period of time (up to 12 months) after harvest.

2.5 Fusarium rot of garlic

Armengol *et al.* (2005) reported that *Fusarium proliferatum* is a world-wide occurring fungal pathogen of several agriculturally important host plants. In particular, *F. proliferatum* has been reported as a pathogen of maize (Logrieco *et al.*1995), rice (Desjardins *et al.*1997), asparagus (Elmer 1990), date palm (Abdalla *et al.*2000) and ornamental palms.

F. proliferatum produces a number of toxins apart from fumonisins, such as moniliformin (marasas *et al.*, 1984), beauvericin (Plattner and Nelson, 1994;

logrieco *et al.*, 1998), fusaric acid (FA), (Bacon *et al.*, 1996) and fusaroproliferin (ritieni *et al.*, 1995).

Jurado *et al.* (2010) are reputed *F. verticillioides* and *F. proliferatum* to be the main sources of fumonisins in food and feed products.

Jurado *et al.* (2010) explained variability in fumonisin production by different isolates of *F. proliferatum* has been demonstrated, with the results indicating that FUM1 is the key gene for fumonisin biosynthesis in this species.

Ibrahim and Stevan (2014) mentioned garlic are typically propagated via seed cloves, but planting of aerial bulbils (borne in umbels at the apex of stalks known as scapes) is an alternative means of propagation for cultivars that produce them. We wished to know if the infection rate in bulbils would diff er from the infection rate in cloves, when both bulbils and cloves originated from plants whose bulbs were known to be infected by *F. proliferatum*.

stepien *et al.* (2011) reported that as fresh garlic is consumed worldwide, the production of mycotoxins in cloves infected with *F. proliferatum* requires serious consideration.

Recently, BEA was shown by Paciolla *et al.* (2004) to possess phytotoxic activity as it caused a severe reduction in tomato protoplast viability.

Lacy and Roberts (1982) explained it is important to assess the biological species for isolates identified as *Liseola* section members, in order to characterize the phyto-pathological and toxigenic risks that could affect the host plants. Wilting of young and adult plants of onion (*Allium cepa*) is caused by a complex of species of the genus Fusarium among which *F. oxysporum f. sp. cepae* is considered the most important onion parasite worldwide, causing rot of the basal plate of the onion bulb.

Previous cases of *F. proliferatum* isolated from onion and garlic in storage were reported in USA, showing bulb rot of onions caused by this fungus (Dugan *et al.*2003; du-Toit and Inglis 2003).

In Europe, *F. proliferatum* was isolated from onion seeds (Mannerucci *et al.*1987) and it was reported for the first time as an agent of bulb rot of garlic in Hungary during winter storage (Simey 1990). However, these reports were only based on morphological identification of *F. proliferatum* and pathogenic assays were not always performed, in order to complete Koch's postulates.

Conventional PCR approaches for specific detection of *F. proliferatum* have been applied (Mule *et al.*, 2004; Jurado *et al.*, 2006a), and recent work demonstrated that *F. proliferatum* isolated from garlic in Poland Produced fumonisins.

A real-time PCr assay developed for diagnosis and quantitation of FUM1 gene expression by Jurado *et al.*, 2010 can be used to determine the potential ability of Spanish isolates of this species from garlic to produce fumonisins.

In Egypt, *F. proliferatum* had a high incidence in the last years according to a report by Galal *et al.* (2002) who described a new disease called garlic bulb rot caused by its syn. *F. moniliforme* Sheldon.

In 2011, *F. proliferatum* (T. Matsushima) Nirenberg was identified as the causal agent of clove rot during storage (Moharam *et al.*, 2013).

F. proliferatum is an increasing problem for all kinds of onion-type crops in addition to garlic (Havey, 1995; Mahmoody, 1998; Dugan *et al.*, 2007; Stankovic *et al.*, 2007; Palmero *et al.*, 2010; Ravi and Prasand, 2012; Yisa *et al.*, 2013).

Tonti *et al.* (2012) explained *Fusarium proliferatum* (Matsush) Nirenberg is a worldwide occurring pathogen of several important crops.

According to Stankovic *et al.*2007, it is possible that the pathogen may contaminate garlic seed cloves and infect garlic plants in the field during growth and later causes CR of SB.

Previously, it has been reported on onion in the north *F. proliferatum* western USA (Mohan *et al.*, 1997), This is the first report of a *Fusarium*. Seefelder *et al.* (2002) have reported *F. proliferatum* and fumonisins in garlic bulbs in Germany.

The fungus was also reported from onion in Idaho (Mohan *et al.*, 1997), garlic in Germany (Seefelder *et al.*, 2002), and onion in Washington State (du Toit *et al.*, 2003).

Fusarium proliferatum was documented by Shin and Kim, (2001) as a pathogen of ornamental Allium in Korea.

Subsequently, *F. proliferatum* has been documented in Allium species in a growing body of literature worldwide (e.g., Alberti *et al.*, 2018; Alizadeh *et al.*, 2010; Bayraktar and Dolar, 2011; Dissanayake *et al.*, 2009; Fuentes *et al.*, 2013; Haapalainen *et al.*, 2016; Moharam *et al.*, 2013; Palmero *et al.*, 2010; Quesada-Ocampo *et al.*, 2014; Ravi *et al.*, 2014; Salvalaggio and Ridao, 2013; Sankar and Babu, 2012; Stankovic *et al.*, 2007; Tonti *et al.*, 2012).

Dugan *et al.* (2007) explained Chemical-based disease management has not been consistently cost effective, largely because the pathogen often grows deeply within inner clove scales and even systemic fungicides cannot effectively penetrate.

Recent research by Patón *et al.* (2017) on chemical-based management holds greater promise, but there are indications of fungicide resistance and of failure to control rots in storage.

Although *F. proliferatum* is not known to roduce chlamydospores, sclerotia are produced by some isolates (Leslie and Summerell, 2006), and sclerotia are also produced by *F. oxysporium f. sp. cepae*, pathogenic on garlic. Such fusaria persist in soil at least four years (Klokočar-Šmit *et al.*, 2008).

Dugan *et al.*, 2007; Patón *et al.*, 2017 experimented treatment of pathogen-free bulbils with fungicide dips, and/or in-furrow fungicides, might effectively diminish this infection window.

When combined with planting of relatively pathogen-free bul-bils, such practices might represent a cost-effective means to manage *F. proliferatum.* Planting into soil free or nearly so of *F. proliferatum* or other pathogens is important.

Detection of *F. proliferatum* by culture-based techniques is time consuming and expensive. An alternative using near-infrared spectroscopy (Tamburini *et al.*, 2016) holds promise, but is compromised in reliability unless bulbs are broken into cloves with skins removed.

2.6 Disease incidence and severity of Fusarium rot

F. proliferatum is the main causal agent of garlic bulb rot during storage. This pathogen has been isolated from water-soaked tan colored lesions on stored cloves in different countries including Spain (Palmero *et al.*2010), United States (Dugan *et al.*2003), Serbia (Stankovic *et al.*2007), Mexico (Ochoa-Fuentes *et al.*2013), India (Sankar and Babu 2012), Italy (Tonti *et al.*2012), Argentina (Salvalaggio and Ridao 2013) and Egypt (Moharam *et al.*2013). Seefelder *et al.*(2002) reported mycotoxins in garlic bulbs in Germany.

Depletion of bulbs and clove rot are serious problems affecting this crop in which different fungal species are involved, including *F. oxysporum, F. proliferatum, F. culmorum, A. niger, A. ochraceus* and *P. purpurogenum* (Kararah and El-Tobshy, 1979; Moharam *et al.*, 2013).

According to Dugan *et al.* (2007) currently, garlic clove rot caused by *F. proliferatum* has become a limiting factor to garlic production in different growing areas of Egypt and other countries, such as China, the largest garlic producer in the world.

Cloves rot (CR) of stored bulbs (SB) caused by *F. proliferatum* was recorded first in Germany (Seefelder *et al.*2002), later was found in many countries around

the world (Dugan *et al.*2003; Stankovic *et al.*2007; Palmero *et al.*2010; Stepien *et al.*2011; Sankar & Babu 2012; Tonti *et al.*2012; Xu-shuang *et al.*2012).

Recently, *Fusarium proliferatum* developing internal rot, white mycelium, bulbs and reddish stems has been found to be the cause of garlic bulb rot, in Spain, Serbia, USA and Germany (De Cara *et al.*, 2010; Stankovic *et al.*, 2007; Dugan *et al.*, 2003; Seefelder *et al.*, 2002).

Considering the report of Nelson *et al.*, 1983; Nirenberg & O'Donnell, 1998 the importance of the disease in garlic growing regions, this work aimed to confirm the presence of *F. proliferatum* in Aguascalientes, In September 2001, bulbs of garlic (cv. Italian Red) rotted in a drying shed at the Plant Introduction farm in Pullman, Washington. Softened cloves displayed water-soaked, tan lesions, often with white mycelium near the bulb axis. Similar symptoms were noted in the same cultivar commercially grown in Idaho, 10 km from Pullman, and purchased at a retail outlet in July 2002. In each case, surface-disinfested tissue plated on agar produced a *Fusarium Allium sativum sp.* with catenate microconidia borne on polyphialides. Isolates were identified as *Fusarium proliferatum.*

Dugan *et al.* (2003) explained *Fusarium proliferatum*, as a fungal pathogen of garlic (*Allium sativum*), was first detected and confirmed as a pathogen in garlic in North America in 2001.

Moreover, *Fusarium proliferatum* is a toxigenic species, producing a broad range of toxins, such as fumonisin B (FB1; Leslie *et al.*1996), moniliformin (MON; Marasas *et al.*1984) beauvericin (BEA; Logrieco *et al.*1995), fusaric acid (FA; Bacon *et al.*1996), and fusaproliferin (FUP; Ritieni *et al.*1995).

According to Lamprecht *et al.* (1994) some of these toxins have a well-known phytotoxic activity; these include FA, which is implicated in the pathogenesis of tomato-wilt symptoms (Gaeumann 1957); MON, which is toxic towards tobacco plants (Cole *et al.*1973), and FB, which has shown to be phytotoxic to maize and tomato.

2.7 Isolation and Identification of Fusarium rot

The concept of species is defined by the criteria through which they can be recognized and differentiated from others (B. A. Summerell *et al.*, 2003). In Fusarium there are three different concepts that can be used to define species; the biological, the phylogenetic and the morphological (J. F. Leslie *et al.*, 2001). H. W. Wollenweber and O. A. Reinking (1935) grouped Fusarium species into section according to morphological characteristics such as shape of macroconidia, presence or absence of microconidia, shape of microconidia, presence or absence of chlamydospores and their location (terminal or intercalary).

2.8 Pathogenecity test of *Fusarium Proliferatum*

Since most nurseries use containers for different seedling crops, I used pot in net house for the pathogenicity test. Most nurseries superficially washed containers with high-pressure water (sometimes steam) to remove residual organic debris (growing media, seedling roots) remaining after seedling extraction. This type of "cleaning" was unsatisfactory because relatively high levels of fungal inoculum remained, particularly *F. proliferatum*, on the inner walls of both styroblock and plastic cell containers (James et al. 1988). Although chlamydospore production is lacking in *F. proliferatum* (Nelson et al. 1983), the fungus is capable to survive within containers for several months, apparently colonizing organic debris remaining after "cleaning" (James et al, 1995). Swamping containers in hot water delivers most potential disease inoculum including *F. proliferatum*, nonviable (James et al. 1988; James and Woollen 1989). In spite of treating containers with warm water, they still obstructed *F. proliferatum* on roots of container seedlings by the end of the growth cycle, even those without typical disease symptoms (James et al. 1995). In general, the fungus produces long chains of microconidia that displace of drying to be carried by air currents. It is also possible that some inoculum may be brought into greenhouses from outside sources. Because *F. proliferatum* commonly infects apparently healthy seedlings, they hypothesized

that some isolates might be more pathogenic than others. Same as the situation that occurs with *F. oxysporum* (Gordon and Okamoto 1992; James et al. 1989b).

2.9 Management of Fusarium rot of garlic

Zhao *et al.* (2013) Reported that rot caused by Fusarium spp. has been controlled with synthetic fungicides, the use of biocontrol agents and plant extracts can be an efficient alternative for the control of various species of Fusarium.

It is necessary to explore other control measures for the pathogen. One alternative to the use of fungicides in the field is to use tissue culture to obtain germplasm that is free of fungi and viruses; this technique is currently routinely used by many garlic producers in Spain. Bioassays on field soil demonstrated that soil cropped to garlic was infected with *F. proliferatum* (Dugan, 2007; Palmero *et al.* 2011).

Another alternative of control is thermotherapy suggested by Vares *et al.* (2009) (hot water treatment), which has been used against fungal, nematode and mite pests in garlic. The temperature of treatment varies widely depending on the cultivar.

Previous studies have concluded that the application of fungicides (fludioxonil, benomyl and thiophanate methyl) do not control *F. proliferatum* (Dugan *et al.* 2007), especially under high disease levels conditions.

Healthy cloves coming from tissue cultures may become infected if they are planted in soil in which the pathogen is present. It is known that the fungus can survive prolonged freezing temperatures, although it does not produce chlamydospores (Leslie and Summerell, 2006; Nelson *et al.* 1983), and it may even remain viable in wheat seeds stored at −18°C after 30 years of storage (unpublished data). When cloves are not visibly symptomatic, it is very difficult to select against them for the following cycle. Because the scales of the cloves cover emerging symptoms, diseased bulbs cannot be completely removed, and the disinfection of planting stock was not always satisfactory.

2.10 Fusarium rot disease in Bangladesh

F.proliferatum have been reported as a pathogen of maize (Nguyen *et al.* 2016) garlic (Dugan *et al.* 2003), tomato (Gao *et al.* 2016), pineapple (Stepien *et al.* 2011), onion (Carrieri *et al.* 2013), The identification of genus and species of Fusarium spp. Was carried out through phenotypic characteristics and different molecular markers (Booth, 1971) many species of Fusarium contain orthologous regions making the identification unreliable through the use of only the ITS region. Some coding regions of nuclear genes have elongation factor 1-a (TEF-1-a) and tubulin (ß-Tub), that allow for identification of Fusarium species because they contain only a single copy in its genus, and it has high polymorphism even in species relatively close genetically.

CHAPTER 3

MATERIALS AND METHODS

In this study Screening of selected garlic varieties against Fusarium rot disease in field condition and isolation, identification was done in lab and finally pathogenicity test was done in pot. Materials used in this study and methodology followed are included in this chapter.

3. Experimental details

3.1: Screening of selected garlic varieties against fusarium rot disease at field condition

3.1.1 Experimental site

The experiment was conducted in Central Farm and Plant Pathology Lab, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh. Geographically the experimental area was located at 23°41′N latitude and 90°22′E longitudes at the elevation of 8.6 m above the sea level (Appendix I).

3.1.2 Climate and weather

The climate of the experimental site was under the subtropical climate, characterized by three distinct seasons, winter season from November to February and the pre-monsoon or hot season from March to April and the monsoon period from May to October. Details of the meteorological data during the period of the experiment were collected from the Bangladesh Meteorological Department, Agargoan, Dhaka (Appendix II).

3.1.3 Characteristics of soil

Soil of the experimental field was silty loam in texture. The soil of the experimental area belongs to the Modhupur Tract under the AEZ No. 28. Soil of the experimental plot was collected from a depth of 0-30 cm before conducting the experiment and analyzed in the Soil Resources Development Institute (SRDI), Soil Testing Laboratory, Khamarbari, Dhaka (Appendix III).

3.1.4 Experimental duration

The experiment was conducted from November 26, 2018 to November 19, 2019.

3.1.5 Source of garlic seed

Fresh and disease-free garlic seeds were collected from three different places. Eight selected garlic varieties and their sources are present in table 1.

Table 1. Sources of collected garlic varieties

Variety	Sources
V1=BAU Rashun-1	Bangladesh Agricultural University, Horticulture Department, Mymensingh
V2=BAU Rashun-2	Bangladesh Agricultural University, Horticulture Department, Mymensingh
V3=BARI Rashun-1	Bangladesh Agricultural Research Institute, Joydebpur, Gazipur
V4=BARI Rashun-2	Bangladesh Agricultural Research Institute, Joydebpur, Gazipur
V5=BARI Rashun-3	Bangladesh Agricultural Research Institute, Joydebpur, Gazipur
V6=BARI Rashun-4	Bangladesh Agricultural Research Institute, Joydebpur, Gazipur
V7=Local Deshi	Siddik Bazar, Dhaka
V8=local Indian	Siddik Bazar, Dhaka

3.1.6 Experimental design and layout

The experiments were conducted by using RCBD (Randomized Complete Block Design). A total of 8 varieties were applied with 3 replications for each treatment. The entire experimental plot was divided into three blocks, each of which then divided into 24-unit plots. The size of the unit plot was 2.40 m^2. Two adjacent unit plots and blocks were separated by 0.25 m, plant to plant distance 15 cm and line to line distance of 20cm.Thetreatments (8 Varieties) were distributed randomly

among the unit plots of each block. Total field size = 3m × 22m (198 m^2) (Appendix IV).

3.1.7 Land preparation

The selected experimental plot was first opened in the month of 25 November' 2018 by a tractor with disc plough. It was then thoroughly prepared by ploughing and cross ploughing with power tiller followed by laddering. The comers of the plots were trimmed by spade. The clods were broken into friable soil and the surface was until the desired tilth was obtained. All the weeds, their rhizomes and stubbles were collected and removed from the plots (Fig.1). Irrigation and drainage channels were prepared around the plots.

Figure1: Field overview of different varieties of garlic

3.1.8 Manures and fertilizers

The following doses of manure and fertilizers were applied to the plots for bulb production (BARI, 2015).

Table 2. Optimum does of Manure and fertilizer Doses/ha:

SL	**Manure/Fertilizer**	**Doses/ha**
1	Urea	4 kg/ha
2	TSP	4 kg/ha
3	MoP	3 kg/ha
4	Gypsum	2 kg/ha
5	Sevin	2 Packet
6	Cow dung	100 kg/ha

Entire amount of cow dung, urea, TSP, MP were applied in the unit plot during final land preparation of 7 days before clove sowing and manure and fertilizer was thoroughly mixed with soil.

3.1.9 Clove sowing

The clove for planting was selected from large bulb of garlic. The garlic cloves were separated from each mother bulb for sowing in the field and were planted in each unit plot as per treatment maintaining a spacing of 15 cm × 0.5 cm. The cloves were dibbed at about 4 cm depth of soil.

3.1.10 Intercultural operations

3.1.10.1 Weeding

Weeding was done regularly in all replicated plots to keep them free from weeds. Hand weeding was done when necessary.

3.1.10.2. Irrigation

Irrigation was given after sowing of seeds to bring proper moisture condition of the soil to ensure uniform germination of the cloves. The irrigation was done frequently after 10 DAS and when necessary. A good drainage system was

maintained for immediate release of rainwater from the experimental plot during the growing period.

3.1.10.3. Tagging

A label attached to the garlic plant or stuck in the soil near the base of the plant that gives information about the variety of the following garlic plant.

3.1.10.4 Harvesting

The harvest of garlic was done following sowing date after attaining full maturity, showing the sign of drying out of most of the leaves and softening of the neck of the bulb.

3.1.11 Parameters studied

The following parameters were considered in the study:

1. % Germination
2. % Pre-emergence mortality
3. % Post-emergence mortality
4. % Total mortality
5. Disease incidence (%)
6. Disease severity (%)
7. No. of leaf
8. Leaf length (cm)
9. Bulb length (cm)
10. Bulb diameter (cm)
11. Bulb neck diameter (mm)
12. Clove length (mm)
13. Clove diameter (mm)
14. No. of clove $bulb^{-1}$
15. Bulb dry weight $plant^{-1}$ (gm)
16. Average bulb weight $plant^{-1}$(gm)
17. Dry matter percentage
18. Total Yield (Kg/ha)

19. Yield loss (Kg/ha)

3.2 Procedure of data collection

Data were collected on the following parameters. Ten plants were selected randomly from each unit plot for the collection of data.

3.2.1 Pre emergence mortality (%)

Pre-emergence mortality was defined as those seedlings having the symptoms described for fusarium rot disease. The pre-emergence mortality rate was obtained by the following formula: (Zagade, 2013)

$$\% \text{ Pre emergence mortality} = \frac{\textbf{number of cloves planted}}{\textbf{total seedlings emerged}} \times \mathbf{100}$$

Emerged seedlings were considered as those with cotyledons above the substrate surface. All dead seedlings were collected, surface disinfected with sodium hypochlorite (5% v/v) for 3 min, and then transferred to Petri dishes with PDA medium to determine infection by *Fusarium proliferatum.*

3.2.2 Post emergence mortality (%)

Post-emergence mortality was defined as those seedlings having the symptoms described for fusarium rot disease. The post-emergence mortality rate was obtained by the following formula (Zagade, 2013):

$$\% \text{ Post emergence mortality} = \frac{\textbf{number of dead seedlings}}{\textbf{total seedlings emerged}} \times \mathbf{100}$$

Emerged seedlings were considered as those with cotyledons above the substrate surface. All dead seedlings were collected, surface disinfected with sodium hypochlorite (5%v/v) for 3 min, and then transferred to Petri dishes with PDA medium to determine infection by *Fusarium proliferatum.*

3.2.3 Total mortality

Total mortality was defined as those seedlings having the symptoms described for fusarium rot disease. The total mortality rate was obtained by the following formula:

Total mortality = number of cloves planted – total seedlings emerged

Then total mortality was presented in percentage.

3.2.4 Disease incidence (%)

For calculation of disease incidence every plant was counted in the field and also counted the infected plants and then expressed in percentage. The disease incidence of garlic was determined by the following formula (Manandhar, 2016):

$$\text{Disease Incidence (DI)} = \frac{\textbf{Number of diseased plants}}{\textbf{Number of total plants observed}} \times \mathbf{100}$$

3.2.5 Disease severity (%)

Disease severity was rated using 10 randomly pre-tagged garlic plants in the three central rows, using standard disease scales of 1-5 Fusarium rot severity, where, 1 = 1 - 10%, 2 = 11 - 25%, 3 = 26 - 50%, 4 = 51 -75%, and 5 = 76 - 100% of the leaf surface covered with lesion (Koike et al., 2001) and average severity of the 10 plants per plot was used for statistical analysis. The scores were changed into percentage severity index (PSI) for analysis using the formula as follows **(**Wheeler, 1969):

Figure 2: Disease symptom and severity measurement scale (0-5)

For calculation of disease severity every plant was counted in the field and also counted the infected plants and then expressed in percentage. The disease severity of garlic was determined by the following formula (Manandhar, 2016):

$$\text{Disease Severity (DS)} = \frac{\textbf{Total point score}}{\textbf{Total no of plant} \times \textbf{maximum grade}} \times \mathbf{100}$$

3.2.6 Leaf length (cm)

The average length of the longest leaf, at physiological maturity was measured in cm from the ten randomly taken plants in the three central rows.

3.2.7 Leaf number per plant

The total number of healthy leaves was counted from the ten randomly taken plants from middle three central rows at physiological maturity.

3.2.8 Bulb length (cm)

Bulb length was measured form the bulbs which the bulb diameter was measured as indicated above. It was measured at the basal end point from the bottom scar of the bulb to the tip point of the bulb using slide-caliper in cm.

3.2.9 Bulb diameter (cm)

Bulb diameter was measured from randomly taken five bulbs at the widest point in the middle portion of the bulb using slide-caliper in cm.

3.2.10 Bulb neck diameter (mm)

The average thickness was measured at the middle narrow point of the bulb neck using graduated caliper in cm. It was measured from ten randomly taken plants from the middle three rows in each plot.

3.2.11 Clove length (mm)

Clove length was measured form the bulbs which the bulb diameter was measured as indicated above. It was measured at the basal end point from the bottom of the clove to the tip point of the bulb using slide-caliper in cm.

3.2.12 Clove diameter (mm)

Clove diameter was measured from randomly taken five cloves at the widest point in the middle portion of the clove using slide-caliper in cm.

3.2.13 No. of clove bulb^{-1}

The total number of cloves produced from ten randomly taken plants were counted and divided by number of bulbs.

3.2.14 Bulb dry weight plant^{-1}

The average bulb dry matter weight of ten randomly take plants for which the average mature bulb weight was measured in gram after ten days of curing bulbs and drying in oven with a forced hot air circulation at temperature of 70°C for 72 hours.

3.2.15 Average bulb weight plant^{-1}

The average mature bulb weight per plant was recorded after weighting ten bulbs produced in the three central rows and dividing by the number of plants.

Figure 3: Bulb and clove measurement

3.2.16 Dry matter (%)

Bulb dry matter weight of ten randomly take plants for which the average mature bulb weight was measured in gram after ten days of curing bulbs and drying in oven with a forced hot air circulation at temperature of 70° C for 72 hours. Dry matter % was calculated by using following formula: (KP Haydock, 1975)

$$\text{Dry matter (\%)} = \frac{\textbf{Total dry weight (g)}}{\textbf{Total fresh weight (g)}} \times \mathbf{100}$$

3.2.17 Total yield per hectare (Kg)

Total bulb yield of plants grown in three central rows was measured after bulbs were cured or exposed for ten days to sunlight. The yields obtained from plots were converted to hectare base.

3.2.18 Yield loss (%)

Yield loss was compared with expected yield and Local Indian verity and recorded in percentage.

3.3 Isolation, Identification and pathogenicity test of *Fusarium proliferatum* causing Fusarium rot in garlic

3.3.1 Isolation and identification of *Fusarium proliferatum*

The pathogen that causes fusarium rot disease in garlic was isolated using tissue culture techniques. The surface of working clean bench was sterilized with ethanol (70%). Then the infected garlic roots were taken into the clean bench and cut into small pieces (0.5-1.0 cm). The cut pieces were sterilized in HgCl (1:1000) for one and half minutes and then taken out with the help of sterile forceps and placed on sterile distilled water in order to wash the samples in three times. After washing, these Fusarium infected leaf cut pieces were placed on sterilized blotter paper in Petridis's and also placed into Petri plates containing water ager and replaced to PDA media after two (2) days and incubated at 25°C under near ultraviolet (NUV) light following ISTA rules (ISTA, 1996). Seven days after incubation the fungal culture were studied under stereoscopic and compound microscope for identification of the desired pathogens.

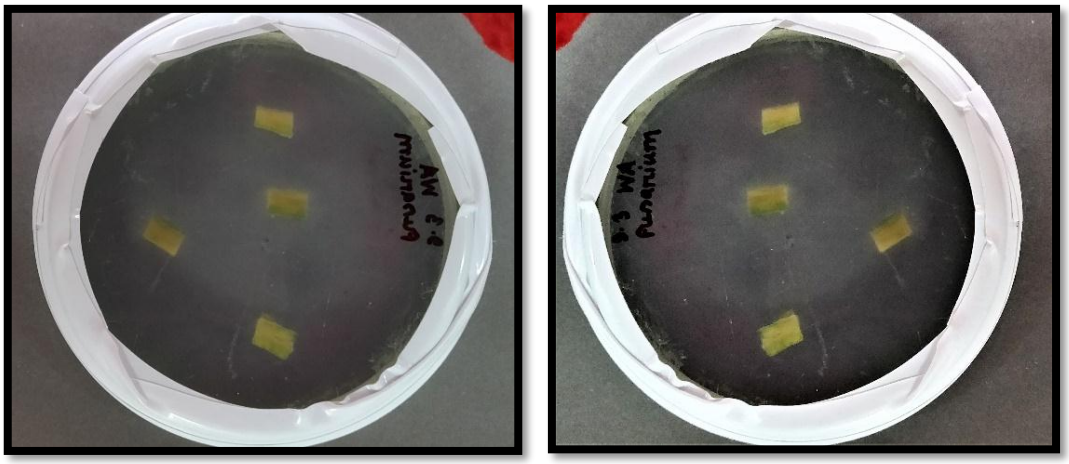


Plate 1: Fusarium rot infected specimen on water agar for isolation of *Fusarium proliferatum* by tissue planting method

3.3.2 Pure culture preparation

In order to get a huge amount of inocula of *Fusarium proliferatum* f. sp. isolates. Each isolate was sub-cultured on PDA medium and incubated at least 10 days of incubation, inocula (mycelial mat and spores) were scraped by a plastic scrapper, wrapped with aluminum foil and preserved in the room temperature. Pure culture of *Fusarium proliferatum* is shown in plate 2.

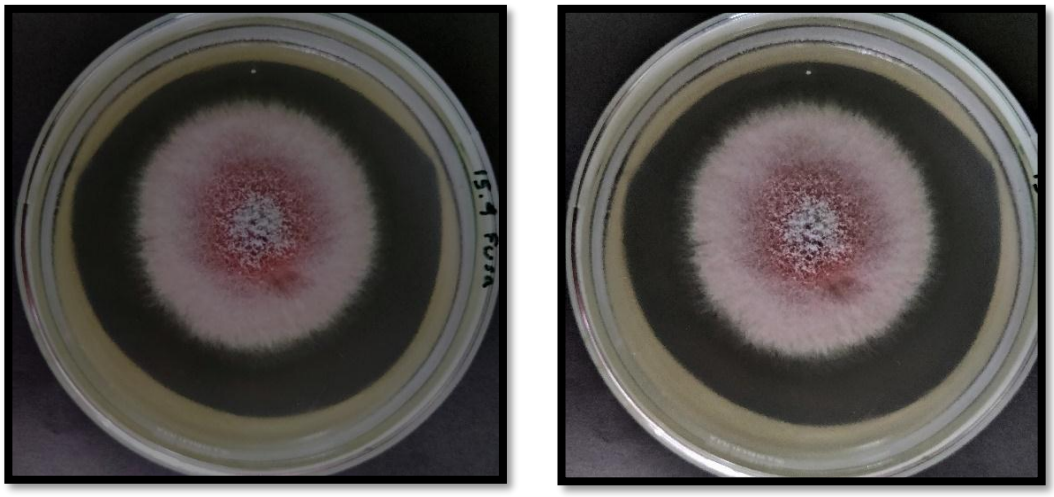


Plate 2: Pure culture of *Fusarium proliferatum* on PDA media

3.3.3 Mycelial growth of *Fusarium proliferatum*

Mycelial growth of *Fusarium proliferatum* was measured in millimeter scale at 2, 5 and 7 days after inoculation by checking the space change of the mark on the forepart of a mycelium or the diameter change of a mycelium in certain period of time, the growth of the mycelia can be observed (Juan Yang, 2011).

3.3.4 Identification of *Fusarium proliferatum*

Whole structure of *Fusarium proliferatum* was observed under stereo-microscope then conidial structure were seen under compound microscope by slide preparation. Stereo and compound microscopic view are shown in figure 4 and figure 5.

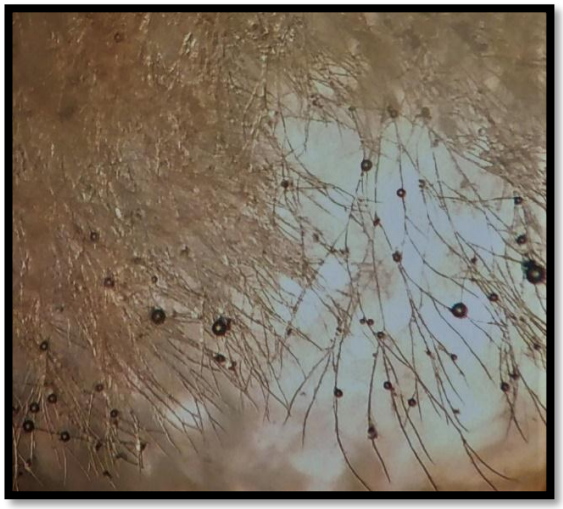


Figure 4. *Fusarium proliferatum* under stereo microscope

Figure 5.*Fusarium proliferatum* under compound microscope

3.4 Pathogenicity test

The pathogenicity test is the main criterion for the identification of fungus suspected of being the etiological agents of a plant disease. This involves reproduction of lesions following artificial infection of suitable hosts under net house conditions. Pathogenicity test was conducted by *in vitro* (figure 6) inoculation of pathogen on growth media (soil) to measure disease incidence and severity of a selected garlic variety (BARI Rashun 4).

Figure 6. Prepared pot for Pathogenicity test of *Fusarium proliferatum*

3.5 Statistical analysis

The data obtained for different characters were statistically analyzed to observe the significant difference among the treatment by using the MSTAT-C computer package program. The mean values of all the characters were calculated and analyses of variance were calculated. DMRT test were performed to determine the level of significant differences and to separate the means within the parameters at 5% level of probability (Gomez and Gomez, 1984).

CHAPTER 4

RESULTS

The results of the study regarding to screening of selected garlic varieties against fusarium rot disease caused by *Fusarium proliferatum*. Possible interpretations have been made with Figure and Table in this chapter.

Screening of selected garlic varieties against fusarium rot disease in field condition

4.1 Evaluation of selected garlic varieties on germination (%) and seedling mortality (%) in field condition

Significant variations of percent germination, pre and post emergence mortality and total mortality were found in eight varieties of garlic collected from different sources. The results of variations are shown in table 3.

4.1.1 Germination (%)

The highest number of germination (85.81%) was observed in BARI Rashun-1 (85.81%) followed by Local Indian (85.71%), Local Deshi (84.38%), BARI Rashun-3 (83.61%) respectively. On the other hand the lowest number of germination (68.98%) was found in BAU Rashun-1.

4.1.2 Pre emergence mortality (%)

Pre-emergence mortality was defined as those seedlings failed to germinate having the symptoms described for fusarium rot disease. The highest pre emergence mortality was observed in BAU Rashun-1 garlic variety with 31.35% germination failure followed by BAU Rashun-2 with 22.62%, BARI Rashun-4 with 21.11%, BARI Rashun-2 with 20.83% pre emergence mortality respectively and the lowest pre emergence mortality (14.19%) was found in BARI Rashun-1.

4.1.3 Post emergence mortality (%)

Post-emergence mortality was defined as those seedlings death after germination and failed to produce bulb having the symptoms described for fusarium rot disease. The highest post emergence mortality (13.22%) was found in BARI Rashun-3 followed by

BAU Rashun-1(13.02%), BARI Rashun-2(12.62%) respectively. And the lowest mortality rate showed in Local Indian variety with 6.15%.

4.1.4 Total emergence mortality (%)

Total mortality was measured and calculated and presented in percentage where BAU Rashun-1 variety showed the highest total mortality (44.04%) followed by BARI Rashun-4 (38.80%), BAU Rashun-2 (34.68%), BARI Rashun-2 (33.45%) respectively whereas, Local Indian variety showed the minimum total mortality of 20.44%.

Table 3: Evaluation of eight selected garlic varieties on germination (%) and seedling mortality (%) in field condition

Variety	Germination (%)	Pre emergence mortality (%)	Post emergence mortality (%)	Total mortality (%)
BAU Rashun-1	68.98 d	31.35 a	13.02 a	44.04 a
BAU Rashun-2	77.39 c	22.62 b	11.41 a	34.68 bc
BARI Rashun-1	85.81 a	14.19 c	6.470 bc	20.66 e
BARI Rashun-2	79.17 bc	20.83 b	12.62 a	33.45 bcd
BARI Rashun-3	83.61 ab	16.39 c	13.22 a	28.61 cde
BARI Rashun-4	72.23 d	21.11 b	11.03 a	38.80 ab
Local Deshi	84.38 a	15.62 c	8.640 b	24.26 de
Local Indian	85.71 a	14.29 c	6.150 c	20.44 e
LSD	4.592	3.244	2.340	8.934
CV	3.29%	9.47%	12.95%	16.66%

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.

4.2 Disease incidence (%) of Fusarium rot disease in selected garlic varieties

Significant variations of percent disease incidence and severity at 30 ,45 and 60 DAS were found in different varieties of garlic in field condition. The results of variations are shown in table 4.

4.2.1 Disease incidence of Fusarium rot

Disease incidence of fusarium rot in every plant was counted in the field also counted the infected plants and then expressed in percentage. Data was measured considering 60 plants.

At 30 DAS, BARI Rashun-4 (15.00%) showed the highest percentage of disease incidence followed by Local Deshi (12.78%), BARI Rashun-3 (10.55%) and BARI Rashun-1 (10.00%) respectively. On the other hand, the lowest disease incidence observed in local Indian variety (2.23%).

At 45 DAS, the highest disease incidence percentage observed in BARI Rashun-3 (18.22%) followed by BARI Rashun-4 (16.82%), Local Deshi (16.67%) respectively. On the contrary, Local Indian variety showed the lowest incidence same as 30DAS (2.78%).

At 60 DAS, BARI Rashun-4 (25.00%) again showed the highest disease incidence which is most likely severe than other varieties. The second highest incidence observed in Local Deshi (22.20%) followed by BARI Rashun-3 (20.33%), BAU Rashun-2 (14.45%) respectively. However Local Indian variety showed the lowest incidence at 60 DAS (3.80%).

Table 4: Disease incidence (%) of fusarium rot at 30, 45 and 60 DAS for selected garlic variety

Variety	% Disease Incidence		
	30 DAS	45 DAS	60 DAS
BAU Rashun-1	6.67 f	8.33 f	12.22 f
BAU Rashun-2	8.89 e	11.48 c	14.45 d
BARI Rashun-1	10.00 d	10.167 e	13.33 e
BARI Rashun-2	8.89 e	10.56 d	11.67 f
BARI Rashun-3	10.55 c	18.22 a	20.33 c
BARI Rashun-4	15 .00 a	16.82 b	25.00 a
Local Deshi	12.78 b	16.67 b	22.20 b
Local Indian	2.23 g	2.78 g	3.80 g
LSD	0.706	0.1767	0.7066
CV	0.04	0.86	2.66

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.

4.3 Disease severity (%) of Fusarium rot disease in selected garlic varieties

Significant variations of disease severity (%) at 30, 45 and 60 DAS were found in different varieties of garlic in field condition. The results of variations are shown in figure 7.

4.3.1 Disease severity of Fusarium rot

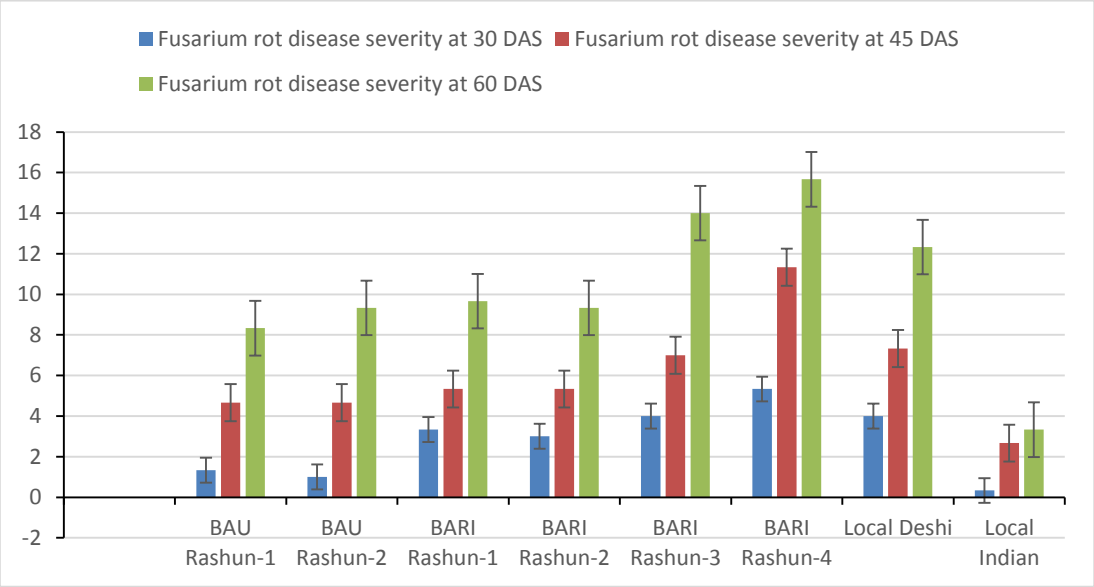
For measuring of disease severity of selected garlic variety 60 plant was considered and from disease incidence data severity was calculated.

At 30 DAS, BARI Rashun-4 showed the highest disease severity (5.33%) where BARI Rashun-3 and Local Deshi showed statistically similar and second highest percentage of disease severity (4.00%) followed by BARI Rashun-1 (3.33%) respectively. However, Local Indian variety showed the lowest percentage of disease severity (0.33%)

At 45 DAS, the highest percentage of disease severity observed in BARI Rashun-4 (11.33%) followed by Local Deshi (7.33%), BARI Rashun-3 (7.00 %) respectively. On

the other hand, the lowest percentage of disease severity observed in Local Indian variety (2.67%) .

At 60DAS, BAU Rashun-4 again statistically showed the highest percentage of disease incidence (15.67%) followed by BARI Rashun-3 (14.00%), Local Deshi (12.33%). However, local Indian showed the lowest percentage of disease severity (3.33%).



Vertical bars represent the standard errors of the treatment means at $P \leq 0.01$

Figure 7: Disease severity (%) Fusarium rot at 30, 45 and 60 DAS for selected garlic variety in field condition

4.4 Effect of fusarium rot on growth parameters of garlic varieties in field condition

Significant variations of leaf length and leaf number at 45 DAS were found in different varieties of garlic in field condition. The results of variations are shown in table 5.

4.4.1 Leaf length (cm)

The longest leaf at 45 DAS was recorded from same variety Local Indian (26.34cm) followed by BARI Rashun-3 (19.817cm), BARI Rashun-1 (19.14cm), BARI Rashun-4 (18.20cm), BAU Rashun-1 (17.74cm) but the shortest one observed from BAU Rashun-2 (17.07cm).

4.4.2 No. of leaf

No. of leaf was recorded at 45 DAS. At 45 DAS BARI Rashun-1 gave the highest no. of leaves (7cm) followed by BAU Rashun-1 (6.84cm), Local deshi (6.8cm), BARI Rashun-2 (6.57cm), BARI Rashun-3 (6.5cm), Local Indian (6.3cm), BAU Rashun-2 (6cm) and BARI Rashun-4 variety gave the lowest no. of leaves (5.14cm)

Table 5: Leaf length and no. of leaves at 30 and 45 DAS for selected garlic variety

Variety	Leaf length	No. of leaves
	45 DAS	45 DAS
BAU Rashun-1	18.05 de	6.84 b
BAU Rashun-2	17.07 e	6.070 f
BARI Rashun-1	19.14 bcd	7.00 a
BARI Rashun-2	17.74 de	6.57 cd
BARI Rashun-3	19.81 bc	6.50 d
BARI Rashun-4	18.20 cde	5.14 g
Local Deshi	20.55 b	6.80 bc
Local Indian	26.34 a	6.30 e
LSD	1.6909	0.115
CV	4.98	0.0884

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.

4.5 Effect of fusarium rot on different yield parameters in field condition

Significant variations of different yield parameters at 30 ,45 and 60 DAS were found in different varieties of garlic in field condition. The results of variations are shown in table 6.

4.5.1 Bulb length (cm)

Bulb length was measured in cm. and found statistically significant. The BARI Rashun-1 showed the longest length of 2.77 cm which is statistically similar to BAU Rashun-1 (2.43 cm) and BAU Rashun-2 with (2.16cm).

4.5.2 Bulb diameter(cm)

Higher bulb diameter indicates better yield. BARI Rashun-2 showed the largest diameter of 1.53 cm which is statistically similar to BARI Rashun-3 (1.43cm). Other verities were found almost similar as BARI Rashun-2 showed the narrowest diameter of 1.00 cm.

4.5.3 Clove length (cm)

Clove length was measured form the bulbs which the bulb diameter was measured as indicated above. It was measured at the basal end point from the bottom of the clove to the tip point of the bulb and the results showed statistically significant. The longest length was found from BARI Rashun-1 and Local deshi rashun of 2.47 cm and the shortest length from Local Indian rashun of 2.1 cm.

4.5.4 No. of clove per bulb

The total number of cloves produced in a single bulb was measured and found statically significant. The highest no. of clove producer in a single bulb was BARI Rashun-3 of 16 cloves and the lowest was from BARI Rashun-4 of 7 cloves per bulb only

Table 6: Effect of Fusarium rot on bulb length, diameter and bulb neck diameter for selected garlic variety

Variety	Bulb length (cm)	Bulb diameter (cm)	Clove length (cm)	No. of clove bulb^{-1}
BAU Rashun-1	2.433 ab	1.20 ab	2.300 a	11.333 ab
BAU Rashun-2	2.167b	1.53 a	2.233 a	13.333 ab
BARI Rashun-1	2.767 a	1.40 ab	2.467 a	11.667 ab
BARI Rashun-2	2.367 ab	1.00 ab	2.400 a	14.000 a
BARI Rashun-3	2.233 b	1.43 ab	2.233 a	16.333 a
BARI Rashun-4	2.267 b	1.23 ab	2.400 a	7.667 b
Local Deshi	2.267 b	1.13 ab	2.467 a	13.667 ab
Local Indian	2.1 b	1.33 ab	2.1 a	14.667 a
LSD	0.4593	0.4290	0.4593	6.0375
CV	11.41	19.12	11.41	27.18

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.

4.6 Effect of fusarium rot on bulb fresh weight(kg/ha), and dry weight(kg/ha), (%) dry matter and total yield weight(kg/ha) of selected garlic varieties in field condition

Significant variations were found in different varieties of garlic in field condition on different yield parameters due to the effect of Fusrium rot. The results of variations are shown in figure 8, 9 and 10.

4.6.1 Bulb fresh weight (kg/ha)

The average mature bulb fresh weight per bulb was measured. The highest fresh weight was collected from BAU Rashun-1 (579.403gm). BARI Rashun-1, BARI Rashun-2, BARI Rashun-3, BARI Rashun-4 and Local Deshi variety showed statistically similar result. The lowest one was collected from Local Indian variety (400.333gm)

4.6.2 Bulb dry weight (kg/ha)

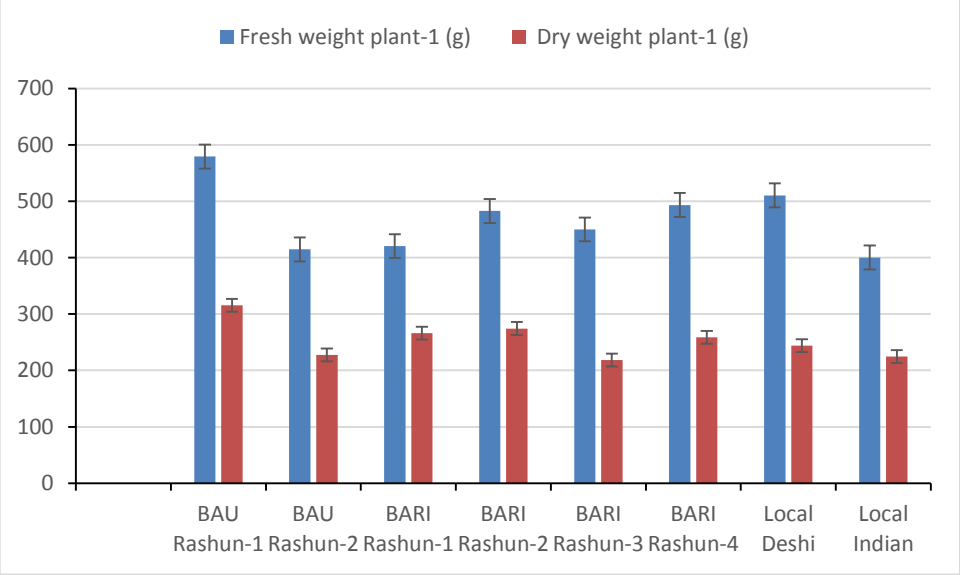
The average mature bulb dry weight per bulb measured and found statistically significant. The highest fresh weight was collected BAU Rashun-1 (315.333gm). The lowest one was collected from BARI Rashun-3 (218.667gm)

4.6.3 Dry matter percentage

Dry matter percentage of garlic is positively correlated with its nutritious value and yield (Figure 9). The dry matter percentage of selected garlic variety was measured and found statistically significant. Highest dry matter found in of Local Indian variety (70.930%) and the lowest from BARI Rashun-1 (38.353%).

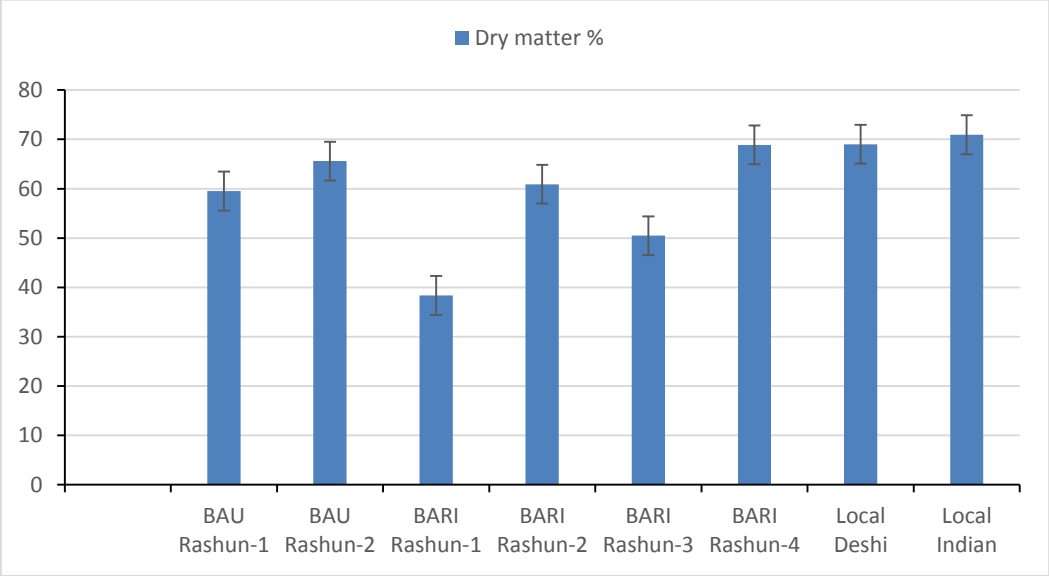
4.6.4 Total yield (Kg/ha)

Total bulb yield of plants grown in three central rows was measured after bulbs were cured or exposed for ten days to sunlight. The yields obtained from plots were converted to kilogram per hectare. The calculated data was statistically significant. Highest yield producing variety was BARI Rashun-2 of 2891.820 kg/ha production. Lowest Yield was gathered from BARI Rashun-3 of 2206.347 kg/ha.



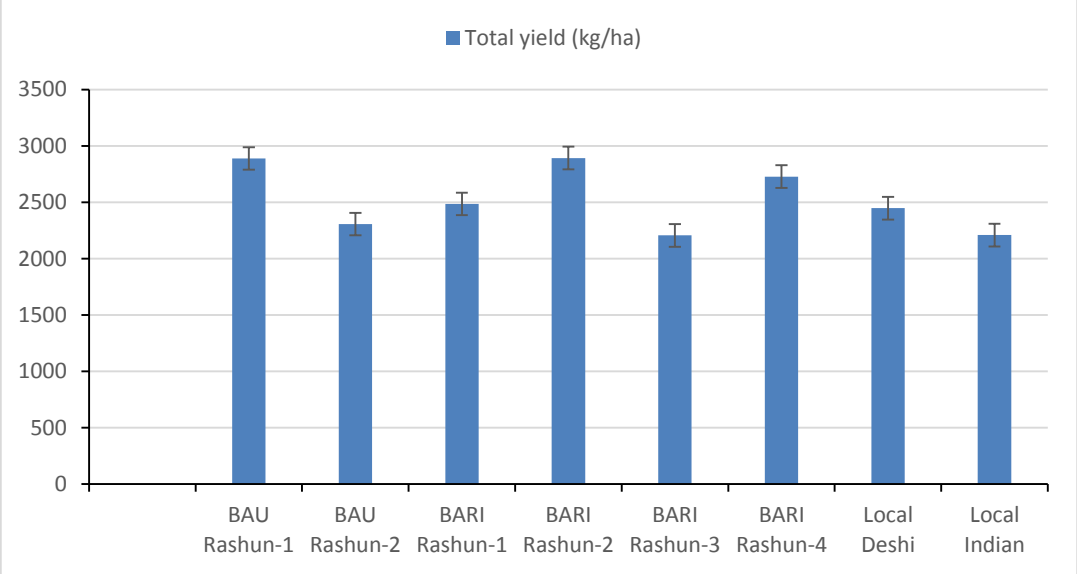
Vertical bars represent the standard errors of the treatment means at $P \leq 0.01$

Figure 8: Average bulb fresh and dry weight (g) per plant for selected garlic variety



Vertical bars represent the standard errors of the treatment means at $P \leq 0.01$

Figure 9: Dry matter percentage for selected garlic variety



Vertical bars represent the standard errors of the treatment means at $P \leq 0.01$

Figure 10: Yield performance for selected garlic variety

4.7 Effect of fusarium rot on total yield and yield loss of different garlic varieties in field condition

Significant variations of total yield and yield loss were found in different varieties of garlic in field condition due to the effect of Fusrium rot. The results of variations are shown table 7 and figure 11

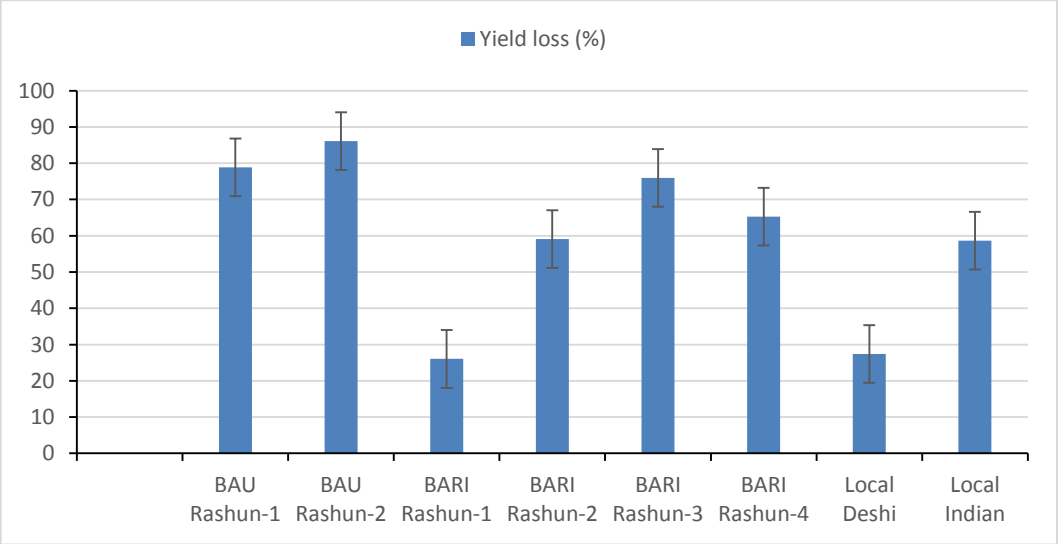
4.7.1 Determination of yield loss of eight garlic variety

Yield loss was compared with expected yield and BAU Rashun-2 (86.08%) showed the worst performance whereas from BARI Rashun-1 (26.04%) best result was obtained.

Table 7: Expected yield, total yield, yield loss (Kg/ha) and yield loss (%) for selected garlic variety

Variety	Expected yield (kg/ha.)	Total yield (kg/ha.)	Yield loss (kg/ha.)	Yield loss (%)
BAU Rashun-1	11000	3101.6800	7898.32	78.90
BAU Rashun-2	11000	2393.8400	8606.16	86.08
BARI Rashun-1	6500	2895.0533	3604.94	26.04
BARI Rashun-2	8500	2592.7200	5907.28	59.07
BARI Rashun-3	10905	3307.5000	7597.50	75.97
BARI Rashun-4	8500	1972.8400	6524.16	65.24
Local Deshi	5000	2263.2800	2736.72	27.37
Local Indian	7500	1635.48	5865.00	58.64

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.



Vertical bars represent the standard errors of the treatment means at $P \leq 0.01$

Figure 11: Yield loss for selected garlic variety

4.8 Correlation between growth parameters and yield parameters

Significant variations of correlation between growth parameters and yield parameters were found in selected varieties of garlic in field condition because of fusarium rot. The result of variations are shown in figure 12 and 13.

4.8.1 Correlation of leaf height and yield parameters

To determine the effect of growth parameters on yield of selected garlic varieties correlation of coefficient was considered at 0.01% level of probability. Here leaf height showed negative correlation with the entire yield parameters considered and become significant at 0.01% level of probability. These values clearly express less leaf height give higher yield and longer leaf cause degradation of garlic yield performance.

4.8 .2 Correlation of no. of leaf with yield parameters

The effect of growth parameters on yield of selected garlic verities correlation of coefficient was considered at 0.01% level of probability. Here no.of leaf showed positive correlation with all the yield defining characters except clove diameter (-.859). These value express better performance of yield can be considered as predeterminer of better yield but narrower cloves.

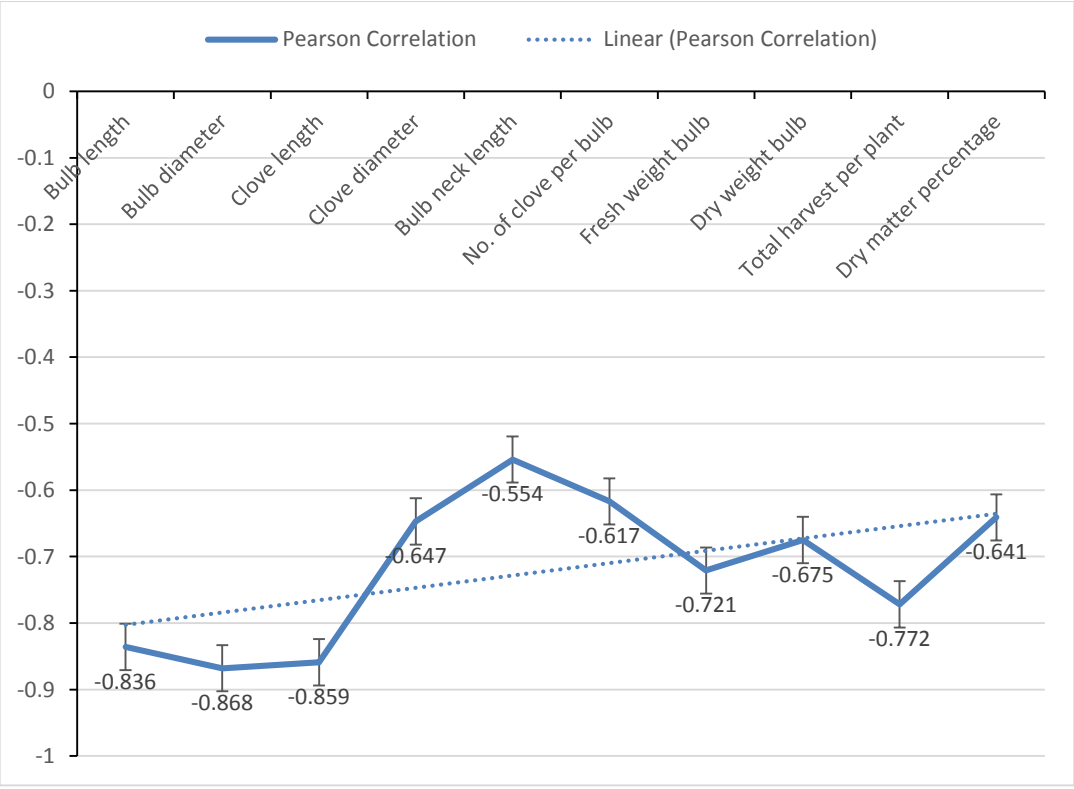


Figure 12: Correlation of leaf height and yield parameters

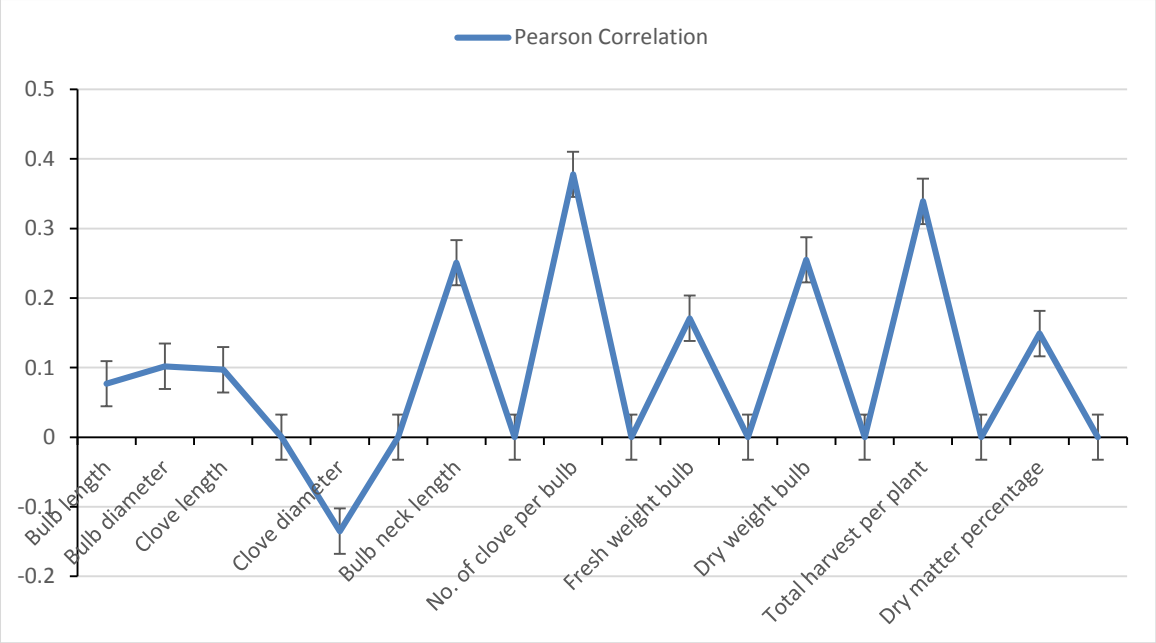
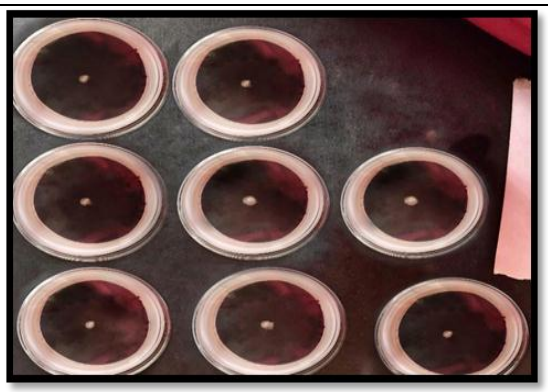


Figure 13: Correlation of no. of leaf with yield parameters

Isolation, Identification and pathogenicity test of fusarium rot caused by *Fusarium proliferatum*

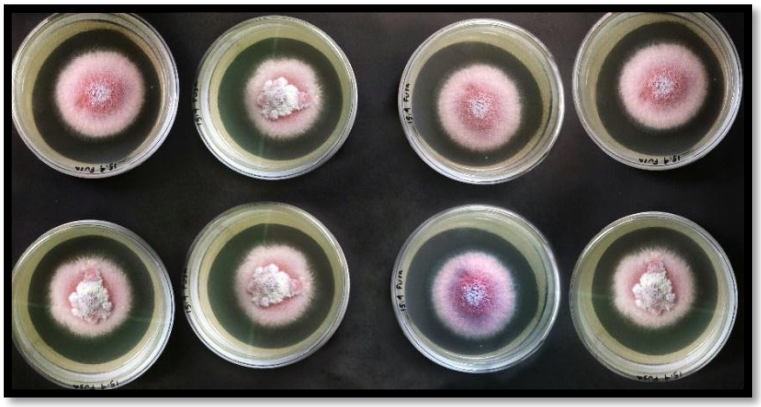
4.9 Isolation and determination of mycelial growth of Fusarium in PDA media

Eight isolates of *Fusarium proliferatum* were obtained from fusarium rot infected garlic each variety. Isolations were made from fusarium rot infected garlic leaf part on Water agar (WA) medium (Distilled water=1L, Dextrose=20g, Agar=20g) and after 3 days when mycelia was grown then it transferred into potato dextrose agar (PDA) medium (Potato=200g, Dextrose=20 g, Agar=20 g and Distilled water=1 L). In order to get a huge amount of inocula of *Fusarium proliferatum* f. sp. isolates each isolate was sub-cultured on PDA medium and incubated at least 10 days of incubation, inocula (mycelial mat and spores) were scraped by a plastic scrapper, wrapped with aluminum foil and preserved in the room temperature. Mycelial growth and cultural characters of eight isolates of *Fusarium proliferatum* at 2, 5 and 7 days on PDA media are shown in Figure 15 and table 13. After 7 days of inoculation the maximum radial mycelial growth of *Fusarium proliferatum* was observed in BARI3I (9 mm). The minimum radial mycelial growth was BARI2I (5.25 mm).



Mycelial growth after 2 days

Mycelial growth after 5 days



Mycelial growth after 7 days

Plate 3.Radial mycelial growth of *Fusarium proliferatum* on PDA media

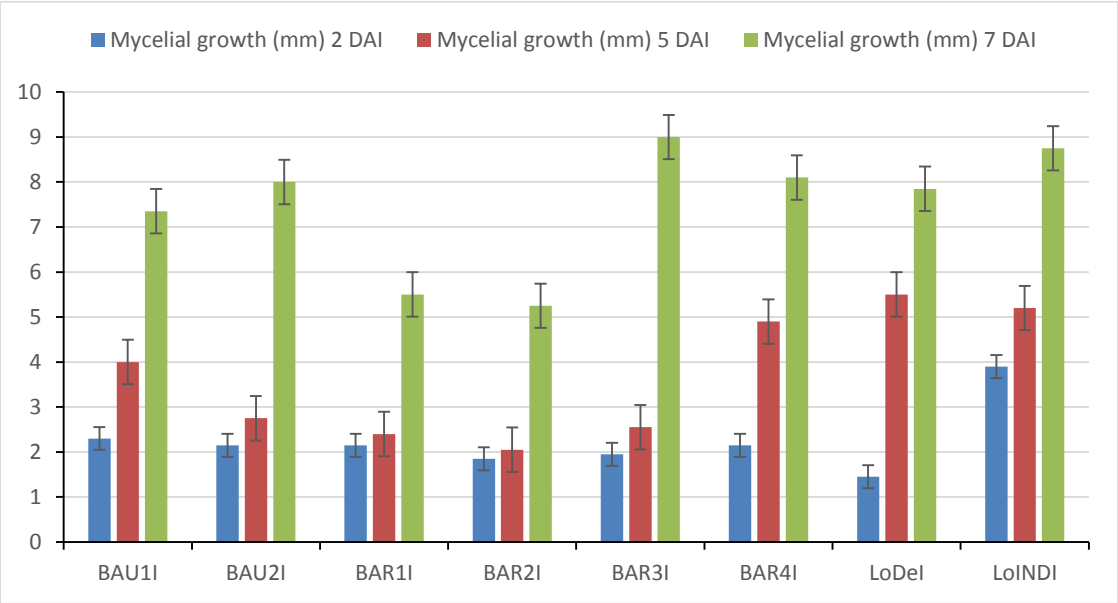


Figure 14: Mycelial growth of 10 isolates of *Fusarium proliferatum* at different days on PDA media

4.10 Colony characterization of 8 isolates of *Fusarium proliferatum* at different days on PDA media

All the isolates exhibited variation in colony characteristics in respect of color, surface texture. But the shape and mycelia growth characterization was found same at all 8 isolates. Colony colors were White centering with pink color, milky white, watery whitish and pinkish encircling white color. Surface texture were velvety smooth, puffy velvate and smooth colony centering black color. All isolates shapes were recorded regular and mycelia growth were medium in size.

Milky white colony color was found in isolates BAU2I, BAR1I AND BAR2I; pinkish color in isolate BAR4I, LoDeI and LoINDI; watery whitish in isolate BAR3I; and whitish centering pink in isolate BAU1I (Table 14, plate 3, 4 & 5).

Puffy velvet smooth surface texture was found in isolates BAU2I, BAR1I, BAR2I, BAR4I, LoDeI and LoINDI; black centering smooth colony in isolate BAR3I; and velvety smooth texture was found in isolate BAU1I (Table 8, plate 3,4 & 5).

Table 8: Colony characterization of 8 isolates of *Fusarium proliferatum* at different days on PDA media

Isolates	Colony			Mycelial Growth
	Color	Surface texture	Shape	
BAU1I	White, circle with pink center	Velvet, smooth	Regular	Medium growth
BAU2I	Milky white	Puffy, velvet	Regular	Medium growth
BAR1I	Milky white	Puffy, velvet	Regular	Medium growth
BAR2I	Milky white	Puffy, velvet	Regular	Medium growth
BAR3I	Watery whitish	Smooth colony, black center	Regular	Medium growth
BAR4I	Pinkish puffy mycelium	Puffy, velvet	Regular	Medium growth
LoDeI	Pinkish puffy mycelium	Puffy, velvet	Regular	Medium growth
LoINDI	Pinkish puffy mycelium	Puffy, velvet	Regular	Medium growth

4.11 Pathogenicity test for Fusarium rot

During screening of fusarium rot disease in garlic BARI Rashun-4 found highest disease incidence and severity which were selected for pathogenicity test. BARI Rashin-4 showed highly susceptible with *Fusrium proliferatum* which showed 29.62% germinated seedling and 49.37% deed seedling.

Table 9: Pathogenicity test for Fusaium rot disease in garlic by *Fusarium proliferatum* inoculated in soil

Variety	Total plant	Germinated seedling (%)	Dead seedling (%)
BARI Rashun-4	27	29.62%	49.37%

	
Fusarium on inoculated pot	**Symptom on garlic seeding after soil inoculation**

Figure 15. Pathogenicity test of *Fusarium proliferatum* in pot

CHAPTER 5

DISCUSSION

Garlic (*Allium sativum* L) is the most widely used cultivated allium species belonging to the family Alliaceae. It is consumed both fresh as well as in dried form as an important ingredient for flavoring various vegetarian and nonvegetarian dishes. Garlic is the second most widely used cultivated bulb crops after onion. It is being used as a spice and vegetable both by poor as well as rich in one or the other form. It is a rich source of carbohydrates (29%), proteins (6.3%), minerals (0.3%) and essential oils (0.1-0.4 %) and contains fat, vitamin C and Sulphur (Memane *et al*., 2008). Ascorbic acid content is very high in green garlic. In addition to this garlic has several medicinal values. It has antibacterial (Arora and Kaur 1999), and antiprotozoal properties (Reuter *et al*., 1996). It is beneficial to cardiovascular and immune system and has antioxidant and anticancer properties (Harris *et al*., 2001). China is the leading producer of garlic accounting for 20.0 million tons followed by India with 1.25 million tons per year. The other three top producing countries of garlic include; South Korea, Egypt, and Russia producing 0.35, 0.26 and 0.26 million tons respectively. (FAO, 2018). In Bangladesh and other Asian and Middle- East countries, it is being used in several food preparations, notably in chutneys, pickles, curry powders, curried vegetables, meat preparations, tomato ketchup and the like (Bose and Some, 1990). But the highest national yield is recorded from Armenia (40 t/ha), and the major garlic producing countries are china, South Korea, India, Spain, Egypt, United States, Thailand, Turkey and Mexico. The average yield of garlic in this country is only 2.89 t/ha (FAO, 2018). The total production of garlic is 425401 metric tons (BBS, 2019), but the requirement is 85,000 metric tons (Rahim, 1992).

The yield and quality of garlic bulb depend on several factors like diseases, Insects, soil and climate condition. The incidences of fungal diseases of garlic as fusarium rot (*Fusarium proliferatum)* and some nemic diseases are commonly reported in Bangladesh as a major reason for yield loss.

In the following study the highest number of germination percentage was observed at BARI Rashun-1 85.81% and the lowest number of germination percentage was found in BAU Rashun-1 68.98%.The highest pre emergence mortality was observed at BAU Rashun 1 garlic verity with 31.35% germination failure and the lowest for BARI Rashun-1 garlic variety at 14.19% germination failure, for the post emergence mortality for BARI Rashun-3 with 13.22% mortality rate after germination and the lowest result showed in local Indian variety with 6.15% mortality rate after germination. Maximum emergence mortality was found in BAU Rashun-1with 44.04% and minimum emergence mortality was found in local Indian variety 20.44%. The finding of the present study reflects the finding of (Ibrahim and stevan, 2014) mentioned garlic are typically propagation via seed cloves but planting of bulb is an alternative means of propagation for cultivars that produce them. We wished to know if the infection rate in bulbils would differ from the infection rate in cloves, when both bulbils and cloves originated from plants whose bulbs were known to be infected by *F. proliferatum* as a major cause of pre emergence and post emergence as well as total mortality of garlic.

For the measurement of disease incident BARI Rashun-4 at 30 DAS showed highest number of healthy plant and the lowest in local Indian verity. At 45 DAS and 60 DAS similar results were observed. As *Fusarium proliferatum* is a toxigenic fungal species, producing a broad range of toxins, such as fumonisin B (FB1; Leslie *et al.*1996), moniliformin (MON; Marasas *et al.*1984) beauvericin (BEA; Logrieco *et al.*1995), fusaric acid (FA; Bacon *et al.* 1996), and fusaproliferin (FUP; Ritieni *et al.*1995). The present study demonstrates that at 30, 45 and DAS BARI Rashun-4 give better performance which is statistically similar BARI Rashun-2. (Figure 4) Considering heavy infestation of nematode local Indian verity failed to germinate and expressed by the lowest disease severity.

Nine isolates of *Fusarium proliferatum* were obtained from fusarium rot infected garlic plants. Isolations were made from fusarium rot infected garlic plants on potato dextrose agar (PDA) media. In order to get a huge amount of inocula of *fusarium proliferatum* f. sp. isolate. Each isolate was sub-cultured on PDA

medium and incubated at least 10 days of incubation, inocula (mycelial mat and spores) were scraped by a plastic scrapper, wrapped with aluminum foil and preserved in the room temperature. Mycelial growth of 8 isolates of *Fusarium proliferatum* at 2, 5 and 7 days on PDA media was observed. At 2 days after the lowest growth was observed in LoDeI with whitish to milky white and greenish color, velvety smooth surface texture with regular shape was found. After 7 days on PDA media BARI3I showed the largest mycelial growth.

In the following study as growth parameter the longest leaf was observed at 15 DAS was from local indian verity and the shortest from BARI Rashun-1. At 30 DAS the longest leaf was found from same verity as before but the shortest one observed from BAU Rashun-2. For 30 and 45 DAS highest number of leaves was observed for BARI Rashun-3 and for 60 DAS BARI Rashun-1 gave the highest no. of leaf (7).

The present experiment indicates BARI Rashun-1 as the longest bulb length of 2.76 cm and for shortest local Indian with 2.10 cm was found. Higher bulb diameter indicates better yield. BAU Rashun-2 showed the largest diameter of 1.53 cm and the lowest diameter found in BARI Rashun-1 variety. The longest clove was obtained from BARI Rashun-1 and the shortest was collected from local Indian of 2.10 cm which is statistically similar to Local Deshi verity. The highest no. of clove producer in a single bulb was BARI Rashun-1 of 16 clove and the lowest was from BARI Rashun-4 of 9 clove per bulb only. The highest fresh weight was collected from BAU Rashun-1 which is statistically similar to BARI Rashun-3. The lowest one was collected from Local Indian verity as it was failed to produce any bulb. The highest fresh weight was collected BARI Rashun-3. Highest dry matter found in of BARI Rashun-1 (71.67%) (Figure 9) and the lowest from Local Indian verity. Highest yield producing verity was BARI Rashun-3 of 3307.5 kg/ha production. The following results supports the report of Dugan *et al.* (2007) that, garlic clove rot caused by *F. proliferatum* has become a limiting factor to garlic production in different growing areas of Egypt and other countries, such as China, the largest garlic producer in the world and stepien *et al.* (2011)

reported that as fresh garlic is consumed worldwide, the production of mycotoxins in cloves infected with *F. proliferatum* requires serious consideration.

In the following study to determine the effect of growth parameters on yield of selected garlic verities correlation of coefficient was considered at 0.01% level of probability. Here leaf height showed negative correlation with all the yield parameters considered and become significant at 0.01% level of probability. These values clearly express less leaf height give higher yield and longer leaf cause degradation of garlic yield performance. leaf height showed positive correlation with all the yield defining characters except clove diameter (-0.135). These value express better umber of yield can be considered as predeterminer of better yield but narrower cloves.

For pathogenicity test 7 days old fusarium culture and conidia was harvested from petridishes were mixed with soil and prepared 27 pot for inoculation. Pathogenicity was done with the BARI Rashun-2. Germination % and seedling mortality % was calculated to measure the susceptibility of the following disease. 29.62% seedling was germinated, and 49.37% dead seedling was observed.

CHAPTER 6

SUMMURY AND CONCLISION

Genus *Allium* is formally classified in the family Liliaceae, represented by 280 separates genera and 4000 species. However, recent taxonomic revisions have seen members of this genus placed in the family Alliaceae. It is one of the oldest vegetables and its positive effect on human has been known for thousand years. The experiments were carried out to study the screening of major fungal disease in selected garlic verities in field and isolation, identification and pathogenicity test of fusarium rot caused by *Fusarium proliferatum* was done also.

The experiment was conducted in Central Farm and Plant Pathology Lab, Sher-e-Bangla Agricultural University. The experiments were conducted using RCBD (Randomized Complete Block Design). A total of 8 varieties was selected with 3 replications for each treatment. The entire experimental plot was divided into three blocks, each of which then divided into 24-unit plots.

In the following study the highest pre emergence mortality was observed at BAU Rashun-1 garlic variety with 31.35% germination failure and the lowest were found in BARI Rashun-1 with 14.19%. The maximum result was found for the post emergence mortality for BARI Rashun-3 with 13.22% mortality rate after germination and minimum result were found in local Indian variety 6.15%. Whereas, BAU Rashun-1 showed the maximum total mortality of 40.04% and local Indian variety showed minimum total mortality of 20.44%.

For disease incident data was measured considering 60 plant and for BARI Rashun-4 showed the highest result at 30 DAS, BARI Rashun-3 showed the highest result at 45 DAS and at 60 DAS BARI Rashun-4 showed the maximum result. For the minimum incidence data local Indian variety showed the lowest result at 30 DAS, 45 DAS and 60 DAS, respectively.

For measuring of disease severity of selected garlic variety at 30 and 60 DAS maximum result observed in BARI Rashun-4 and at 45 DAS BARI Rashun-3

showed maximum result. However local Indian variety showed statistically minimum disease severity at 30 DAS, 45 DAS and 60 DAS, respectively.

To determine the radial mycelial growth 7 isolates of *Fusarium proliferatum* at 2, 5 and 7 days on PDA media after 7 days of inoculation the maximum radial mycelial growth of *Fusarium proliferatum* was observed in BARI3I (9 mm). The minimum radial mycelial growth was BARI2I (5.25 mm).

For pathogenicity test 7 days old fusarium culture and conidia was harvested from Petri dishes were mixed with soil and prepared 27 pot for inoculation. Pathogenicity was done with the BARI Rashun-2 *in vitro* condition. 27 pots were considered as test plot 29.62% pre emergence mortality and 49.37% dead seedlings were observed.

For the measurement of different growth parameters of 8 garlic variety leaf length was measured with 15 days interval at 15 DAS and 30 DAS and the longest leaf was observed at 15 DAS was from local Indian variety and the shortest from BARI Rashun-1. At 30 DAS the longest leaf was found from same variety as before but the shortest one observed from BAU Rashun-2. The highest number of leaves was observed for BARI Rashun-3 and for 60 DAS BARI Rashun-1 gave the highest no. of leaf (7).

For the measurement of different yield parameters of 8 garlic variety the BARI Rashun-1 showed the longest length of bulb 2.77 cm and the in shortest local indian with 2.10 cm was found. Higher bulb diameter indicates better yield. BARI Rashun-3 showed the largest bulb diameter of 1.43 cm and the longest clove was from BARI Rashun-1 and local deshi of 2.47 cm and the shortest from Local indian of 2.10 cm. The widest clove was obtained from BARI Rashun-1 and local deshi of 2.47 cm and the narrowest was collected from local indian of 2.1 cm. The highest no. of clove producer in a single bulb was BARI Rashun-3 of 16 clove and the lowest was from BARI Rashun-4 of 7 clove per bulb. The lowest bulb fresh weight was collected from Local Indian variety. The highest fresh weight was collected BAU Rashun-1. Highest dry matter found in local indian (70.93%) (Figure 6) and the lowest from Local Indian variety. Highest yield producing

variety was BARI Rashun-3 of 3307.5 kg/ha production. Yield loss was compared with expected yield and local indian variety showed the worst performance whereas from BARI Rashun-1 (26%) best result was obtained.

However, further research is needed to conduct in the interaction among *Fusarium proliferatum* with different garlic cultivar. To produce *Fusarium proliferatum* resistant garlic variety, advance research will be needed.

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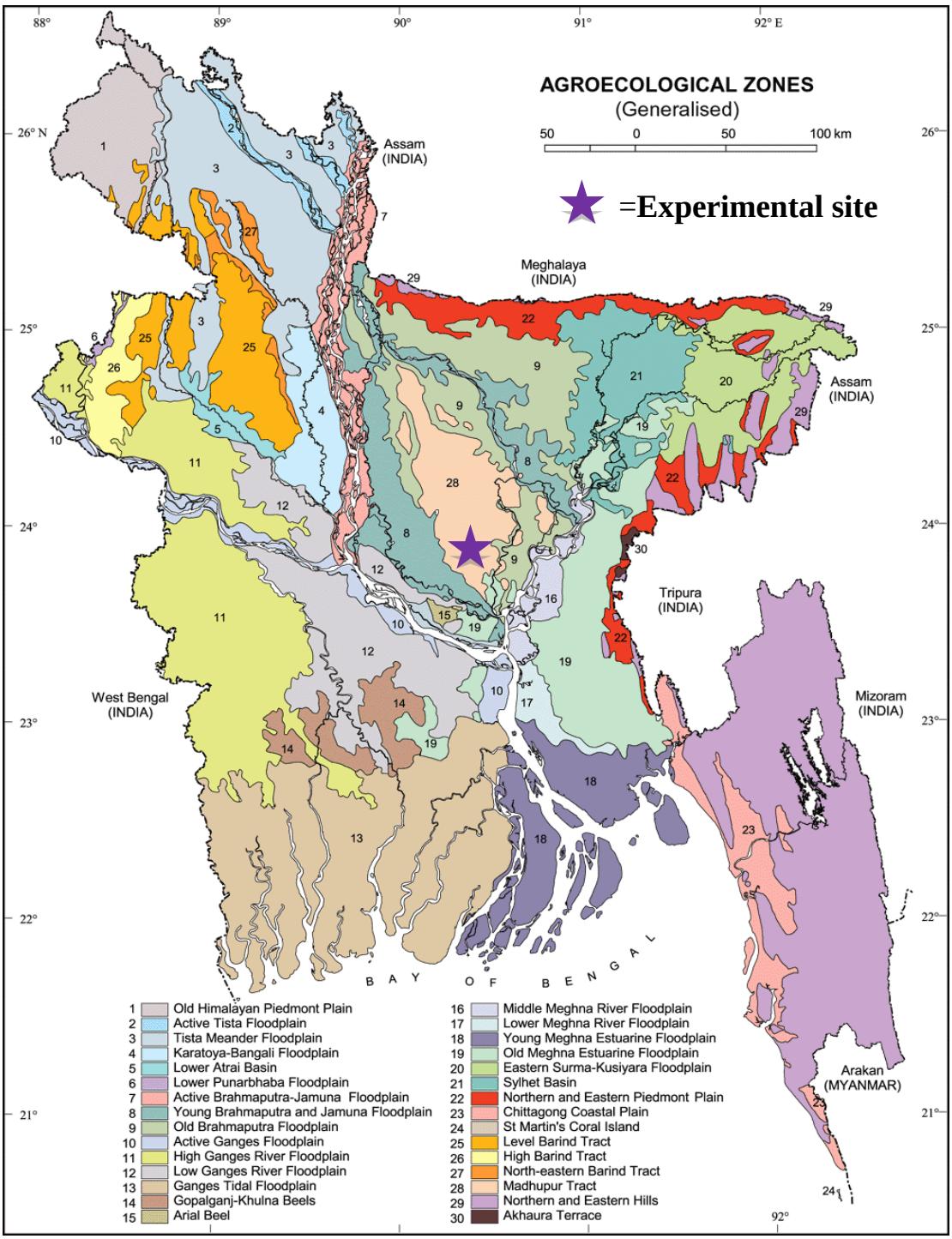
APPENDICES

Appendix I. Map showing the experimental site under study



Appendix II. Characteristics of soil of experimental field

A. Morphological characteristics of the experimental field

Morphological features	Characteristics
Location	Sher-e-Bangla Agricultural University Agronomy research field, Dhaka
AEZ	AEZ-28, Modhupur Tract
General Soil Type	Shallow Red Brown Terrace Soil
Land type	High land
Soil series	Tejgaon
Topography	Fairly leveled

B. The initial physical and chemical characteristics of soil of the experimental site (0 - 15 cm depth)

Physical characteristics	
Constituents	**Percent**
Sand	26
Silt	45
Clay	29
Textural class	Silty clay
Chemical characteristics	
Soil characters	**Value**
pH	5.6
Organic carbon (%)	0.45
Organic matter (%)	0.78
Total nitrogen (%)	0.03
Available P (ppm)	20.54
Exchangeable K (me/100 g soil)	0.10

Appendix III. Monthly meteorological information during the period from November, 2018 to April, 2019

Year	Month	Air temperature (^{0}C)		Relative humidity (%)	Total rainfall (mm)
		Maximum	Minimum		
2018	November	28.10	11.83	58.18	47
	December	25.00	9.46	69.53	00
2019	January	25.2	12.8	69	00
	February	27.3	16.9	66	39
	March	31.7	19.2	57	23
	April	33.50	25.90	64.50	119

Source : Metrological Centre, Agargaon, Dhaka (Climate Division)

Appendix IV. Analysis of variance of the data on Fusarium rot disease incident as influenced by different garlic variety

Source of variation	**df**	**Mean square of disease incident of Fusarium rot**		
		30 DAS	**45 DAS**	**60 DAS**
Replication	2	0.5417	0.1667	3.125
Variety	7	43.7083*	78.8512*	128.167*
Error	14	0.3512	0.5476	3.506
Grand mean		9.4583	11.458	14.750
CV		6.27	6.46	12.69

*Significant at 5% level of significance

^{NS} Non significant

Appendix V. Analysis of variance of the data on Fusarium rot disease severity as influenced by different garlic variety

Source of variation	df	Mean square of disease severity of Fusarium rot		
		30 DAS	45 DAS	60 DAS
Replication	2	0.29167	4.0417	0.3750
Variety	7	9.04167*	20.0417*	43.4048*
Error	14	0.29167	0.7560	0.5655
Grand mean		2.7917	6.0417	10.250
CV		19.35	14.39	7.34

*Significant at 5% level of significance

^{NS} Non significant

Appendix VI. Analysis of variance of the data on Fusarium rot pathogenicity as influenced by different garlic variety

Source of variation	df	Mean square of pathogenicity of Fusarium rot			
		%Germination	%pre-emergence mortality	%post-emergence mortality	Total mortality
Replication	2	17.120	17.120	18.6936	68.073
Variety	7	123.000 ^{NS}	123.000 ^{NS}	23.4630 ^{NS}	222.322^{NS}
Error	14	152.460	152.460	11.5625	122.945
Grand mean		79.660	20.340	10.276	30.617
CV		15.50	60.70	33.09	36.22

*Significant at 5% level of significance

^{NS} Non significant

Appendix VII. Analysis of variance of the data on growth parameters influenced by Fusarium rot of different garlic variety

Source of variation	df	Mean square of growth parameters	
		Leaf length	No. of leaf plant^{-1}
Replication	2	6.2014	2.0395
Variety	7	19.9612*	26.0251*
Error	14	1.9864	0.7993
Grand mean		15.480	19.617
CV		9.10	4.56

*Significant at 5% level of significance

^{NS} Non significant

Appendix VIII. Analysis of variance of the data on yield parameters influenced by Fusarium rot of different garlic variety

Source of variation	df	Mean square of yield parameters				
		Blub length	Bulb dia.	Clove length	Clove dia.	No. of clove buld^{-1}
Replication	2	0.04875	0.005	0.00875	0.02042	8.6667
Variety	7	0.11500 ^{NS}	0.091 ^{NS}	0.14613*	0.12262^{NS}	44.6429*
Error	14	0.08018	0.060	0.05113	0.06994	15.2857
Grand mean		2.3250	1.283	2.3625	0.9417	13.083
CV		12.18	19.12	9.57	28.08	29.88

*Significant at 5% level of significance

^{NS} Non significant

Appendix IX. Analysis of variance of the data on Fusarium rot disease incident as influenced by different garlic variety

Source of variation	df	Mean square of yield parameters			
		Bulb fresh weight	Bulb dry weight	Dry matter	Total harvest
Replication	2	8210.23	1362.25	104.32	345689.84
Variety	7	75224.39 ^{NS}	21779.63 *	2608.58 ^{NS}	1693426.83*
Error	14	97975.80	4607.75	1300.15	3143931.08
Grand mean		468.968	253.625	60.456	2520.258
CV		17.84	7.15	15.94	18.80

*Significant at 5% level of significance

^{NS} Non significant