MANAGEMENT OF PURPLE BLOTCH COMPLEX OF ONION (*Allium cepa)* FOR SEED PRODUCTION

Registration No. 13-05688

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

DECEMBER, 2020

MANAGEMENT OF PURPLE BLOTCH COMPLEX OF ONION (*Allium cepa)* FOR SEED PRODUCTION

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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
IN
PLANT PATHOLOGY

SEMESTER: JULY-DECEMBER, 2020

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CERTIFICATE

This is to certify that the thesis entitled, **"MANAGEMENT OF PURPLE BLOTCH COMPLEX OF ONION (*Allium cepa*) FOR SEED PRODUCTION"** *submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirement for the degree of* **MASTER OF SCIENCE IN PLANT PATHOLOGY** *embodies the results of a piece of bona fide research work carried out by* ***MD. MAHAMUDUL HASAN,*** *bearing Registration No.* **13-05688** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma, elsewhere in the country or abroad.*

I further certify that such help or sources of information, as have been availed of during the course of this investigation has duly been acknowledged.

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Dedicated

To

My Beloved Parents

And Teachers

ACKNOWLEDGEMENT

Firstly, I am expressing my deep sense of gratitude to the ***"Almighty Allah" (SWT),*** *the most gracious and benevolent who has made me able to perform this research work and to submit the thesis successfully for the degree of Master of Science (M.S.) in Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. My deepest respect and love form the core of my heart is for "****Prophet Muhammad" (SM)*** *who is a torch of knowledge forever and guidance for humanity.*

This is my greatest opportunity to convey my sincere respect to my supervisor ***Dr. Md. Rafiqul Islam****, Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for his scholastic guidance, continuous encouragement and support during the total tenure of my research work. I am grateful to him for introducing me to the intricacies of science. He has given me the full liberty in my work, which helped me to grow the ability of working independently and widen the sphere of my thinking. He has helped me by giving suggestions for execution of my ideas in the way of my research whenever I needed. I am really thankful to him for his competent guidance and uncompromising attitude during the thorough assessment of this manuscript.*

It is my pleasure to express my deep respect to my Co-supervisor, ***Prof. Abu Noman Faruq Ahmmed****, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for his guidance, criticism, co-operatives to conduct the research work.*

I express my indebtedness to ***Dr. Fatema Begum****, Professor and Chairman, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for his valuable advices, suggestions and continuous inspiration. I found him always helpful to provide the research facilities as a chairman of the department.*

My deepest respect goes to ***Dr. Md. Belal Hossain****, Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for his intellectual advices and academic instructions to conduct the research.*

I wishes to express my cordial respect to ***Prof. Dr. M. Salahuddin M. Chowdhury****,* ***Prof. Nazneen Sultana****,* ***Prof. Dr. F. M. Aminuzzaman, Prof. Khadija Akhter****,* ***Prof. Dr. Nazmoon naher Tonu****,* ***Associate Prof. Md. Tohidul Islam, Shukti Rani Chowdhury****,* ***Assistant Prof. Md. Ziaur Rahman Bhuiyan****,* ***Sayed Mohammad Mohsin, Hosna Ara Chowdhury Nisha****, and* ***Lecturer Salma Saker****,* ***Sanjida Hoque****,* ***Jannatul Ferdous Tanni & Nahid Akter****, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for their valuable teaching, academic advices, and encouragement during the study period.*

I am thankful to lab technician ***Rahana Akhter****,* ***Pritilata Sarder*** *and* ***Khorshed Al-Alam****, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for their cordial assistance during the research work.*

I want to acknowledge ***Dr. Md. Anwarul Islam****, Librarian, Sher-e-Bangla Agricultural University, Dhaka for providing various information, journal, books related to the research.*

I wish to extend thanks to my seniors, fellow mates and friends ***Md. Rakibul Hasan Nitol,******, Tanvir Ahmed****,* ***Kawsar Mahmud****,* ***Rubaiya Islam Sharna****,* ***Tanjila Hasan Sinthia, Shovan Krishna Das*** *for their friendly co-operation and inspiration during the study period.*

I would like to express my gratitude to ***Moksedur Rahman****, Maintenance Engineer, Central laboratory Sher-e-Bangla Agricultural University, Dhaka for his cordial help during the frequent use of central lab, Sher-e-Bangla Agricultural University.*

Finally, I recall indebtedness to my beloved parents and the family members for their boundless affection and well wishes during the whole study period.

The Author

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By

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ABSTRACT

The study was carried out in the central farm of Sher-e-Bangla Agricultural University and in the Seed Health Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka with a view to evaluate the efficacy of different treatments against *Alternaria porri and Stemphylium vesicarium* causing purple blotch complex of onion for seed production. The onion variety, 'Taherpuri' was used in the experiment. The foliar spray experiment was laid out in a RCBD with three replications. Eight treatments, Viz. T_0 = Control (No spraying); T_1 = Foliar spraying with Alamanda leaf extract 1:2 (W/V); T_2 = Foliar spraying with Neem leaf extract 1:2 (W/V); T_3 = Foliar spraying with Score 250 EC @ 0.2%; T_4 = Foliar spraying with Rovral 50 WP @ 0.2%; T_5 = Foliar spraying with Dithane M-45 @ 0.45%; T_6 = Foliar spraying with Tricost 1 % WP- @ 0.3% (*Trichoderma harzianum*); T_7 = Foliar spraying with Micronutrients ($ZnS0_4$ + Borax) were explored in the experiments. A significant variations were observed in respect of disease incidence and severity of purple blotch complex, seed yield, yield contributing characters, seed germination and prevalence of seed borne pathogens of harvested seeds as influenced by the treatment of different selected spray. The highest seed yield 707.45 kg/ha was obtained in case of treatment T_4 where foliar spraying were applied with Rovral 50 WP @ 0.2%. The lowest percent leaf infection, percent disease index (PDI-leaf), percent infected seed stalk, percent Stalk Area Disease (%SAD) were recorded in foliar spray with Rovral 50 WP followed by Score 250 EC. The highest seed germination by 93.5 % and the lowest prevalence of *Alternaria porri* by 0.25 % & *Stemphylium vesicarium* by 0.75 % and also total pathogenic infection by 8.5 % were recorded on harvested onion seed from Rovral 50 WP treated experimental plot followed by Score 250 EC.

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CHAPTER I

INTRODUCTION

The onion (*Allium cepa*), is the most widely cultivated species of the genus *Allium.* It belongs to the family Alliaceae. Onion (*Allium cepa* L.) rightly called as "queen of kitchen" is one of the oldest and an important spice crop grown in Bangladesh as well as in the world. Spices are important constituents of food items. A good number of spices crops are grown in Bangladesh. The major ones are Onion, Garlic, Zinger, Turmeric, Coriander, Chili, etc. Spices have a broad term used to describe herbal by-products that add flavor and aesthetic, aromatic and therapeutic treatments to food, drink and other items. (Kumar *et al.*, 2011).

Onion has manifold uses as spice, vegetable, salad, dressing etc. It is also used as condiments for flavoring a number of foods and medicines (Vohora *et al*., 1974; Hossain *et al*., 2006). The most important spicy character is its flavor, which increases the taste of food. It has common uses as an ingredient of curry, sauce, soup, pickles, seasoning food etc. To a lesser extent, it is used by processing industry for dehydration in the form of onion flakes and powder, which are in great demand in the world market. Besides, it has also preservative and medicinal values. It is used to relieve insect bites and sore throat. Onion bulb provides quite vitamin C 19.7%, fiber 10.8%, molybdenum 10.6%, manganese 10.5%, vitamin B 69.5%, potassium 6.6 %, and tryptophan 6.2%. Onions are very low in calories (just 40 cal per 100 g) and fats; but rich in soluble dietary fiber. Presently 109 kinds of spices are cultivated in the world but in Bangladesh we use only 27 and produce 17 types. On the basis of area, yield, demand and availability, spices are divided into three categories viz. major, minor and exotic. Major spices are regularly used in daily diet at large amount such as chili, onion, garlic, turmeric and ginger.

Onion used as important and popular vegetables in Australia, Belgium, India, Japan, United Kingdom, USA and many other countries. Out of 15 important vegetables and spice crops enlisted by FAO, onion stands second in terms of annual world production (Anon. 1997). Onion is an important spice crop in Bangladesh. It covers almost 36% of the total areas under spices. It grows extensively during winter season in Bangladesh but at present it also grows in the summer season. It ranks first in production 18.66 lakh metric tons and second in area 1.86 lakh hectares against its demand of 22 lakh tones among the spices crop in Bangladesh (BBS, 2018). It is reported that, onion productions during 2018-2019 were 23.3 lakh metric tons with a demand of 36 lakh metric tons. As such the annual production deficit in Bangladesh is 12.7 lakh metric tons (Sobhan, 2019). Bangladesh is 8th largest onion producer country in the world (FAO, 2018). This is particularly important for Bangladesh because existing mean yield of onion is very low estimated at 8.69 t/ha as compared to the world average of 18.33 t/h (FAOSTAT, 2015).

The major onion growing areas of the country are Faridpur, Cumilla, Manikganj, Dinajpur, Jessore, Pabna, Rajshahi, Mymensingh, Jamalpur, Patuakhali, Kishorganj, Tangail, Borishal, Bandarban, Khagrachari, Sylhet, Bogra, Rangamati, Kustia, Dhaka, Chittagong, and Rangpur. The highest yield (385669 metric ton) was in Faridpur in 81970 acre of land (BBS, 2018). The local varieties namely Faridpuri and Taherpuri are commonly grown in Bangladesh. The demand of bulb onion as well as the onion seeds is increasing every year in Bangladesh and the price of the true seed remains fairly high in each season. Several constraints affect the onion production in our country. The use of low quality seeds, imbalanced fertilizers, improper irrigation and attack of various insect-pests and diseases are the reason to lower the production.

In the world, onion is attacked by 66 diseases including 10 bacterial, 38 fungal, 6 nemic, 3 viral, 1 mycoplasmal, 1 parasitic plant and 7 miscellaneous diseases and disorders (Schwartz and Mohan, 2008; Schwartz, 2010). Onions are attacked by ten diseases in Bangladesh caused by various pathogens (Ahmed and Hossain,

1985; Bose and Som, 1986). Most of the disease caused by the fungi and among the fungal diseases, the most important and damaging one purple blotch that is causes seed rot, germination failure, black mould, and white rot.

Among the foliar diseases, purple blotch is one of the most destructive disease prevailing in almost all onion growing pockets of the world, which causes heavy loss under field conditions. The name "Purple blotch" for this disease was proposed by Nolla (1927). He named the causal organism as *Alternaria alli* which was later amended to *Alternaria porri.*

Purple blotch of onion is noted as a major disease throughout the world including Bangladesh (Ahmed and Hossain, 1985; Meah and Khan, 1987; Bose and Som, 1986; Castellanos-Linares *et al*., 1988 and Islam *et al.,* 2001). In India purple blotch of onion is considered as a major, devastating and widespread disease and causes serious yield reduction (Ahmed and Goyal, 1988). The disease is also a threat for seed production of onion (Gupta *et al*., 1986; Rahman *et al*. 1988 and Yazawa, 1993).

In Bangladesh the common diseases of onion are purple leaf blotch (*Alternaria porri*), stemphylium blight (*Stemphylium vesicarium)*, downey mildew (*Peronospora destructor*), basal/stem rot (*Fusarium* sp.; *Sclerotium* sp.; *Rhizoctonia* sp.), and damping off that may damage the crop and reduce the yield upto 100% (Brewster and James, 2008).

Nowadays *Stemphylium vesicarium*, the causal agent of white blotch of onion are being considered as an organism involved indirectly with the causation of purple blotch of onion. It is considered that *Stemphylium vesicarium* initiate the infection which facilitates subsequent infection of *Alternaria porri* causing purple blotch and hence the disease is designated as purple blotch complex (Nainwal and Vishunavat 2016; Akter *et al.*, 2015; Ali *et al.,* 2016).

Onion seed production is severely affected by purple blotch complex of onion because the disease causes breaking of floral stalks that results failure of seed production of onion (Munoz *et al*., 1984; Ashrafuzzaman and Ahmed, 1976). The infected seed stalks break down at the point where the blotch lesion is

developed (Singh, 1987). Bulb and seed yields of onions cv. “Nasik Red” were significantly reduced as a result of purple blotch caused by *Alternaria porri* (Gupta and Pathak, 1988). About 20 to 25% losses in seed yield have been recorded in India (Thind and Jhooty, 1982) and 41- 44% in Bangladesh (Hossain and Islam, 1993; Fakir, 2002).

Purple blotch and Stemphylium blight seriously affect the seed stalk that drastically reduce the seed production (Ara, 2013). In Bangladesh the cultivars Faridpuri and Taherpuri are susceptible to the disease (Rahman *et al*., 1988; Islam *et al*,. 2001). Temperature and humidity are the most predominant factors for the development of purple blotch disease. The disease is favored by moderate temperature (24-30°C) and high relative humidity (Gupta and Pathak, 1986; Evert and Locy, 1990 and Rodriguez *et al*., 1994).

Generally, onion seed is produced from planting mother bulbs or by sowing seed directly. The demand of quality true seeds are increasing day by day. The price of true seeds is also high. The seeds available in the market are poor in quality.

The total production of onion seed in Bangladesh is about 150 tons/year but the requirement is more than 900 tons (BBS, 2016). The unavailability of good quality onion seeds is partly responsible for low yield in Bangladesh (Bokshi *et al*., 1989). With the gradual increase of population, the demand of onion in Bangladesh is increasing day by day. So to meet the demand of Bangladesh the rest of the requirement is fulfilled by importing seeds from the neighboring countries such as India, China, Pakistan every year in exchange of huge foreign currency. (Hossain and Islam, 2006).

Moreover, the quality of the imported seeds did not find up to the mark in most cases. Increase of onion production depends on proper care and management of plant diseases.

Nowadays farmers are discouraged for onion cultivation that affects the national yield, so that which make the country has to importing enormous amount of onion bulb every year at the cost of huge foreign exchange. Unstable price of onion in the local market, especially in the month of Ramadan and in off season

are mainly due to the shortage of onion production, unavailability of quality seed, lack of information about the disease problems for seed production. The Government need to have the picture about disease problems of farmers for quality seed production and national yield status of onion prior to harvesting the crops to take necessary step to meet up the national demand. In Bangladesh, the productivity of onion varies from 370 to 500 kg of seeds/ha (Hossain *et al.*, 2017) leaving a wider scope to increase to make it comparable to those of the world onion seed production of 1,000-1,200 kg/ha.

Keeping all these facts in mind the present study was undertaken to achieve the following objectives:

- To isolate and identify the causal pathogens of purple blotch complex of onion.
- To find out the eco-friendly options for the management of purple blotch complex of onion for seed production.
- To determine the prevalence of pathogen in harvested onion seeds.

CHAPTER II

REVIEW OF LITERATURE

Onion is one of the most important spice crops, which received much attention of the researchers throughout the world. Onion (*Allium cepa*) is one of the most important and widely used vegetable and spices crops in Bangladesh as well as many countries all over the world. Researcher all over the world has been carrying out their research on management of purple blotch complex of onion for seed production. In Bangladesh very few works have been done in this respect. The available information in this connection over the world has been reviewed in this chapter.

2.1. Symptom of purple blotch of onion

Nuchnart Jonglaekha *et al.* (1982) observed that symptoms of purple blotch disease appearing on onion, shallat, multiphir onion, leek and garlic were similar except that the levels of susceptibility were different. They also observed that most of the conidia produce germ tubes and penetrate through wounds on leaves within 8 hrs. of inoculation. The conidia observed were club-shaped with transverse and longitudinal septa. This fungus produces spores when the temperature lies between 18-19^{0}c.

Munoz *et al*. (1984) reported that onion seed production is severely affected by purple blotch complex of onion because the disease causes breaking of floral stalks.

Gupta *et al.* (1991) reported that the purple blotch disease is characterized with small water-soaked lesions initially produce on leaves and seed stalk that quickly develop white centers. As lesions enlarge, they become zonate, brown to purple, surrounded by a yellow zone and extend upward and downward for some distance. Under humid condition, the surface of the lesion may be covered with brown to dark gray structure of fungus. A few large lesions have been formed in a leaf or seed stalk which may coalesce and girdle the leaf or seed stalks. Usually the affected leaves or seed stalks break down and die within 4 weeks if the environment favors the disease.

Sharma (1999) observed that leaf blight symptoms on onion first appeared on leaves or inflorescence as small (2-3 mm in diameter) water soaked lesions that quickly developed into white centers under favorable conditions. These lesions enlarged, coalesced, became zonate and turned brown to purple extending upwards and downwards. In moist weather, the surface of the lesions may be covered with black bodies of the fungus.

Basallotte Ureba *et al.* (1999) reported that early symptoms appear on the older leaves as white flecks. Under suitable environmental conditions the white flecks expand and produce sunken purple lesions that are often elliptical with a yellow to pale-brown border

2.2. Varietal resistance

Thirumalachar *et al.* (1953) reported about the existence of some varietal resistance and they stated that the fungus *Alternaria porri* (purple 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 *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

Alves *et al.* (1983) studied the incidence of purple spot (*Alternaria porri* EII. Cif.) on onion cultivars and hybrids in Manaus, Amazonia. Plants were divided into five classes on the basis of natural infection in the field. Incidence was 30-50% (class II) in most cases; only the hybrids Px76 having plants in class I (0-10%)

Ariosa-Terry and Herrera-Isla (1986) measured the damage of onion due to 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.

Gupta and Pathak (1988) studied 21 indigenous and exotic cultivars screened at 2 locations in India under artificial condition. All the exotic lines except 2 from the Sudan were highly resistant to *Alternaria porri* while all the indigenous lines were found susceptible. It is suggested that susceptible cultivars should be replaced by the resistant Pusa Red.

Bhonde *et al.* (1992) conducted a field trial during 1987-1988 on 8 onion cultivars (Agrifound Light red, Arka Niketan, L-102-1, Nasik Red and Pusa Red, Agrifound Dark Red, Arka Kalyan and Kharif Local). Agrifound Light Red had a good yield and had the highest DM content.

Sharma (1997) studied onion genotypes grown in Himachal Pradesh, India, for resistance to *Alternaria porri* during 1991-92. The lines IC48059, IC48179, IC39887, IC48025 and ALR found resistant.

Das (2010) reported that at seedling stage in net house no disease incidence of white blotch of onion (*Stemphylium vesicarium*) were recorded in case of BARI piaz-3, Indian big and Indian small. The lowest disease incidence and highest yield also recorded in BARI piaz-3, Indian big and Indian small among nine onion cultivars viz. BARI piaz-1, BARI piaz-2, BARI piaz-3, Thakurgong local, Foridpur local, Manikgong local, Indian big, Indian small and Taherpuri. BARI piaz-1 showed lower performance in respect of all parameters.

Kibria (2010) reported that BARI piaz-3 gave lowest disease incidence and highest yield (12.67 t/ha) against purple blotch of onion (*Alternaria porri*) among nine onion cultivars viz. BARI piaz-1, BARI piaz-2, BARI piaz-3, Thakurgong local, Foridpur local, Manikgong local, Indian big, Indian small and Taherpuri. In case of disease reaction 8.00% observed in BARI piaz-3 and was graded as resistant.

Chethana *et al*. (2011) conducted the Screening of onion genotypes for purple blotch under field condition of onion revealed that, the genotype Arka Kalyan was found moderately resistant while the genotypes viz., Rampur Rose, Agrifound Rose, Arka Pragati, Arka Niketan, Arka Pitamber and Arka Bindu were found moderately susceptible to purple blotch of onion.

Kumar *et al.* (2012) conducted an experiment in the Department of Plant Pathology, Bihar Agricultural College, Sabour to locate the sources of resistance of *Alternaria porri*. 45 days old seedlings were inoculated by spraying the spore suspension (1 × 106 spores/ml) of *Alternaria porri*. Seedlings in pots were subjected to humid chamber for about 24 hours before and after inoculation. The variety Arka Kalyan appeared most resistant recording the least disease intensity (5.53 percent only), although being statistically *at par* with Arka Niketan and Agri. Foundation Dark Red recording 6.36 percent and 6.33 percent disease intensity.

Abu bakar and Ado (2013) conducted an experiment on five onion cultivars Red Creole, Kaharda, Koumassa, Sokoto local and ori to find out the variability pattern for resistance to purple blotch disease of onion. Analysis of the variance component for the combined seasons and locations indicated that genotypic variance was greater than the environmental variance for all characters under consideration with exception of bulb weight. Disease incidence recorded 31.20%, 30.58% and 5.42% as phenotypic, genotypic and environmental coefficients of variability. Disease severity recorded 34.96%, 32.84% and 11.00% as phenotypic, genotypic and environmental coefficients of variability. With respect to fresh bulb yield 94.90%, 93.53% and 15.78% were observed as phenotypic, genotypic and environmental coefficients of variability for the genotypes. Cured bulb yield recorded 103.47%, 102.27% and 14.96% respectively as phenotypic, genotypic and environmental coefficients of variability. Similarly 29.43%, 24.79% and 17.91% were observed for days to maturity, as phenotypic, genotypic and environmental coefficients of variability.

Mansha *et al.* (2019) studied the potential of 25 onion genotypes were evaluated against purple blotch and their yield response during two years (2014–15) under field conditions. Five varieties (Phulkara, Sunset, Ceylon, TI-172, XP-Red) showed resistant response while Desi Red, Early Red, Robina, Dark Red and Mirpurkhas exhibited moderately resistant response. VRIO-6, VRIO-1, VRIO-4, Red Nasik and Desi Black were found moderately susceptible against the

disease. VRIO-9, Pak-10321, Fsd Red, Pusa Red and Red Imposta gave susceptible response, while VRIO-3, VRIO-5, VRIO-8, VRIO-7 and VRIO-2 exhibited highly susceptible response.

Bal *et al.* (2019) studied the 23 genotypes of onion were screened against purple blotch disease where none of the genotypes were found to be immune. Five genotypes viz., Akola Safed, Arka Niketan, Punjab Naroya, Arka Lalima, Arka Kirtiman exhibited resistance to this disease. Eight genotypes viz., Bhima Subhra, Arka Bheem, PRO-6, Bhima Raj, Kalyanpur Red Round, L-28, Bhima Dark Red, Bhima Shakti were found to be moderately resistant with 11-20% leaf area infected. A total of ten genotypes were grouped under moderate susceptible category (21-40% leaf area infected).

2.3. Epidemiology and its management

Khare and Nema (1981) studied on the sporulation of *Alternaria porri* . They observed maximum sporulation at 8-00 a.m. under field condition. A seasonal periodicity was also noted, indicating maximum sporulation immediately after rains. Under laboratory conditions maximum sporulation was at 22^0 c at 90% RH followed by 30^0c. They also reported that temperature, humidity and nutrients seemed to play an important role for ensuring infection of *A. porri* on onion. Cent percent spore germination occurred *in vitro* within 4 hours at 22^0c, while maximum germination was recorded within 6 hrs at 25^0c on the host 8 surface.

Khare and Nema (1982) also reported that the temperature ranged between 22^0cto 25^0c was not only suitable for growth and sporulation of *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 *Alternaria porri* in onion. Percent (100%) spore germination occurred *in vitro* within 4 hrs. at 22^0c, while maximum germination was recorded within 6 hrs. at 25^0c on the host surface.

Nuchart Joglaekha *et al*. (1982) observed that most of the conidia produced germ tubes and penetrated 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 18^0c- 26^0c.

Miller (1983) reported that measurements of infected leaves were taken weekly from bulb initiation to bulb maturity. They observed that the leaf damage levels were significantly lowered on younger than older leaves. Leaves emerging 9, 8, 7, 6 and 5 week before bulbing maturity required $5^1/_2$, 5, $4^1/_2$, $3^1/_2$ and $2^1/_2$weeks respectively to reach 50% damage.

Khare and Nema (1984) conducted an experiment to determine the effect of temperature and humidity of the development of symptoms of purple blotch of onion incited by *Alternaria porri* and noted that temperature between 22^0c to 25^0c and relative humidity 90% are the best for the development of leaf blotch symptom.

Gupta and Pathak (1988) reported that bulb and seed yields and 1000 seed weight of Nashik Red onion were significantly reduced by *Alternaria porri* infection. Disease severity was computed in terms of the co-efficient of disease index (Codex). A linear relationship was found between yield and Codex.

Everts and Lacy (1990) examined formation of conidia by *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 25^0cand conidia formed repeatedly (up to eight cycles) on lesions to alternating low RH (35-50%) and high (100%) RH.

Rodriguez *et al.* (1994) studied the intensity and dynamics of *Alternaria porri* conidial germination in different temperatures (5-40^0c) and RH (76-100%). Conidia developed at 5-37.5^0c, with an optimum temperature of 30^0c.

Germination started within 1 hr of incubation at 20-35^{0}c and 50% of the conidia had germinated at 4 hr. of incubation.

Srivastava *et al*. (1994) reported the high incidence (2.5 - 87.8%) of purple blotch (*Alternaria porri*) in both the kharif and rabi onions, when high humidity prevailed, during the 5 years of the survey (1988-93).

Srivastava *et al.* (1996) conducted 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 porri*) and *Stemphylium* blight (*S. vesicarium*) on onions in Haryana, 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 porri* and *Stemphylium vesicarium* during ploughing of infected soil was higher during the winter.

Everts and lacy (1996) studied the factors influencing infection of onion leaves by *Alternaria porri* and subsequent lesion expansion. Conidia deposited on onion leaves formed single to several germ tubes and appressoria and often penetrated at more than one locus under conditions favorable. After 3 hrs in the dew chamber at 24^{0}c following inoculation of onion leaves, 73% of conidia had germinated and 5% had formed appressoria. Infection hyphae were not observed until 6 h following inoculation, at which time 2% of conidia had formed infection hyphae and 0.5% of conidia had caused visible lesions. Length of dew period was 0 9. Significantly and positively correlated with lesion numbers but not with lesion size.

Gupta *et al*. (1996) stated Stemphylium blight (*Stemphylium vesicarium*) and purple blotch (*Alternaria porri*) are important diseases causing considerable damage to onion crops in India. Diseases are found severe during the rainy season especially when thrips are also associated with the crop.

Lakra (1999) found numerous purple spots / blotchs on older leaves and scapes when fortnightly dew fall was >1.0 mm, mean maximum relative humidity >

75% and mean maximum temperature 20-30^{0}c with > 18 hr favourable temperature (10-30)^{o}c duration. Exposure of leaf and/or scape to wetness for 8 hr. was a prerequisite for conidial germination with increasing disease intensity, every tield 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/umble, 1000-grain weight, number of seeds/plant and the seed yield/plant by 28.7, 74.5, 89.9, 41.7, 35.7, 95.7 and 97.3% respectively compared with healthy plants.

Suheri *et al.* (2000) studied the infection of onion by *Alternaria porri* and *Stemphylium vesicarium* under a range of controlled temperatures (4-25)^{0}c and leaf wetness periods (0-24h). Conidia of *Alternaria porri* and *Stemphylium vesicarium* germinated within 2 h when incubated at 4^{0}c. Terminal and intercalary appressoria were produced at similar frequencies at or above 1^{0}c. The maximum number of appressoria was produced after 24h at 25^{0}c. Penetration of leaves by both pathogens was via the epidermis and stomata, but the frequency of stomatal penetration exceeded that of epidermal penetration. There was a strong correlation (R2>90%) between appressorium formation and total penetration at all temperatures. Infection of onion leaves occurred after 16h of leaf wetness at 15^{0}c and 8h of leaf wetness at 10- 25^{0}c and infection increased with increasing leaf wetness duration to 24h at all temperature. Interruption of a single or double leaf wetness period by a dry period of 4-24h had little effect on lesion numbers. Conidia of *Alternaria porri* and *Stemphylium vesicarium* separately or in mixtures caused similar number of lesions. *Alternaria porri* and *Stemphylium vesicarium*, both potentially important pathogens in winter grown *Allium* crops. Purple leaf blotch symptoms were considered to be a complex caused by both pathogens.

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, Alternaria, Stemphylium* and *Aspergillus* spp.

Premchand *et al.* (2017) carried out a field experiment to understand the diseases development of purple leaf bloch of onion with respect to weather conditions during the year 2016 starting from 14th standard week to 22nd standard week at horticultural research station, haveli farm, Bagalkot dt., Karnataka, India. The results of this study revealed that the PDI value ranging from 0 to 73.33, Apparent infection rate at seven days intervals showed the range between -0.0000303 to 0 was observed and AUDPC value is recorded 2052.195 and it given the initiative that the delay in the sowing increases the severity of onion purple blotch, reduces seed test weight and yield of crop.

Ahmed and Hossain (1985) recorded purple blotch of onion from all onion growing regions of Bangladesh. Ashrafuzzaman and Ahmed (1976) also reported that the damage of foliage and breaking of floral stalks due to the disease resulting in failure of seed production are common.

The efficacy of six fungicides was evaluated by Rahman *et al*. (1988) for controlling leaf blotch of onion (*Alternaria porri*). Rovral and Dithane M-45 were found to be the best both in laboratory and field conditions. Under field conditions, all the test fungicides gave significant reduction of disease severity but significant increase of onion yield was achieved with Rovral, Dithane M-45 and Bordeaux mixture that gave 61, 35 and 29% yield increases, respectively.

Rahman *et al.* (1989) evaluated six fungicides viz. Antracol (Propineb) 65 WP, Bordeaux mixture (copper sulphate and lime), Cupravit (copper oxychloride), Dithane M-45 (Mancozeb), Rovral (Iprodione) and Trimiltox forte (Cu-salts and Mancozeb) for their efficacy against leaf blotch (*Alternaria porri*) of onion in laboratory and field condition. All the fungicides gave significant reduction of mycelial growth and disease severity. Increase of onion yield was achieved with Rovral, Dithane M-45 and Bordeaux mixture. Maximum yield increase was achieved with Rovral (61%) followed by Dithane M-45 (36%) and Bordeaux mixture (29%).

Prateung and Sangawonge (1991) conducted a field trial to determine the efficacy of nine (9) fungicides for controlling purple blotch of onion caused by

Alternaria porri during January-April 1989. The first spray was made 40 days after transplanting the onion seedlings, and the second and third sprays at weekly intervals. The fourth spray was made 12 days after the third. The results after 2nd applications of fungicides indicated that rnyclobutanil, iprodione, and imazalil gave the lowest percentage of disease infection. Triphenyl tin acetate and myclobutanil + mancozeb gave the second best result.

Perez- Moreno *et al*. (1992) observed that Iprodione gave the best control of purple spot and downy mildew followed by Fosetil. Fosetil gave the best control of the disease in the fresh market cultivars whereas; Iprodione gave the most effective disease control in the hybrids (USA origin). Iprodione gave the highest yield followed by Fosetil.

Hossain *et al.* (1993) reported that 41-44% loss of seed crop in Bangladesh due to purple blotch of onion. Under favorable environmental conditions of the disease, complete failure of onion seed crop was observed (Sharma, 1986). The disease causes 20-25% loss in seed yield in India. (Thind and Jhooty, 1982).

During 1992-93 and 1993-94 in Haryana, India, total failure of onion seed crop occurred due to *Stemphylium* blight (*Stemphylium vesicarium*) and purple blotch (*Alternaria porri*). To overcome this alarming situation Srivastava *et al.* (1995) conducted trials with Iprobenfos (Kitazin), Iprodione (Rovral), Fosetyl (Aliette), Kavatch, Thiophanate-methyl (Topsin M), Benomyl, Metalaxyl (Ridomil) and Mancozeb. Observation on disease intensity/PDI was recorded at fortnightly intervals, just before each spray, and a total of 5 sprays were applied. They recommended that seed growers in North India should apply fortnightly sprays of 0.25% Mancozeb or 0.25% Iprodione to control onion seed diseases caused by *Stemphylium vesicarium* and *Alternaria porri*.

Sugha (1995) conducted a field trial on the management of purple blotch of garlic caused by *Alternaria porri* during winter season of 1989-90, 1990-91 and 1991-92 and reported that three foliar sprays of Iprodione @ 0.1% alone or in combination with Copper oxychloride 0.1% and Mancozeb 0.1% at 15- days intervals resulted in 53.5 to 62% protection to the crop. Clove dip in Iprodione

0.25% for 1 hr before sowing followed by 2 sprays of Metalaxyl + Mancozeb (Ridomil MZ @ 0.25%) or Iprodione @ 0.2% proved highly effective, giving 79.6-84.9% control of the disease. Iprodione and Metalaxyl + Mancozeb were superior to Chlorothalonil, Copper oxychloride, Mancozeb and Zineb improving protection to garlic crop from purple blotch.

Islam (1995) evaluated seven fungicides against *Alternaria porri* causing purple blotch of onion. Score (Difenconazole) was found as the most effective fungicide followed by Rovral (Iprodione), Tilt 250 EC (Propiconazole) and Folicur (Tebuconazole). Percentage of reduction in disease index varied from 48.34 to 65.44 in score, 45.48 to 64.02 in Rovral, 34.90 to 47.24 in Tilt 250 EC and 32.93 to 46.34 in Folicur. Fungicidal treatments increased bulb yield by 10.53% to 95.53% over unsprayed control.

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Datar (1996) tested eight fungicides, viz. Carbendazim, Copper oxychloride, Zineb, Mancozeb, Iprodione, Thiophanate methyl, Dithianon and Ziram at 100, 250 and 500 ppm which significantly reduced the conidial germination of *Alternaria porri* on onion cv. N-53-1 over control.

Gupta *et al*. (1996) reported that Purple blotch (*Alternaria porri*) and Stemphylium blight (*Stemphylium vesicarium*) are 2 rnajor diseases causing serious losses of onion crops in india. To determine effective control measures of the diseases, studies were undertaken in Karnal, Haryana, India, during kharif, 1993, 1994 and 1995. Three sprays each of iprodione (as Rovral), fosetyl (as Aliette), chlorothalonil, metalaxyl (as Ridomil), iprobenfos (as Kitazin) and

benomyl and 4 sprays of mancozeb (as a control) were applied, after disease onset. Results from a 3-years study revealed that 3 sprays of chlorothalonil (0.2%) or iprodione (0.25%) were alternatives for controlling both *Alternaria porri* and *Stemphylium vesicarium*.

Ahmed *et al*. (1999) conducted an experiment to evaluate the efficacy of six fungicides against the purple blotch of onion caused *Alternaria porri* viz. Rovral 50WP (0.2%), Ridomil MZ -72 (0.2%), Folicur 250EC (0.1%), Dithane M-45 (0.2%) and Tilt 250EC (0.1%). Among the fungicides, Rovral 50WP and Ridomil MZ -72 found to be effective in controlling the disease incidence and disease severity with corresponding increase in seed yield by 100% when they were used alone or in combination of 1:1.

Islam *et al.* (1999) evaluated seven fungicides against *Alternaria porri* causing purple blotch of onion. Score (Difenconazole) was found as the most effective fungicide followed by Rovral (Iprodione). Tilt 250 EC (Propiconazole) and Folicur (Tebuconazole). Percentage of reduction in disease index varied from 48.34 to 65.44 in score. 45.48 to 64.02 in Rovral, 34.90 to 47.24 in Tilt 250 EC and 32.93 to 46.34 in Folicur. Fungicidal treatments increased bulb yield by 10.53% to 65.53% over unsprayed control.

Islam *et al.* (2001) conducted an experiment to evaluate the efficacy of eight fungicides viz. ‘Score (Difenconazole), Tilt 250 EC (Propiconazole),Folicur (Tebuconazole), Rovral 50wp (Iprodione), Knowin (carbendazim), Macuprax.(Borcleaux mixture + curfanex), Bavistin 50WP(carbendazim), Ridornil MZ-72 (Metalaxial + Mancozeb) against the purple blotch of onion caused by *Alternaria porri* Among the fungicides, Rovral 50WP was the most effective fungicide next to score in reducing radial mycelial growth of *Alternaria porri* in in-vitro and disease incidence and severity of purple blotch of onion in field.

Islam *et al*. (2003) evaluated the relative efficacy of ten fungicides against *Alternaria porri* causing purple blotch of onion. Rovral and Ridomil reduced disease incidence and severity and incurring higher seed yield.

Islam (2003) reported the relative efficiencies of seven plant extracts (dhatura, Garlic, Ginger, Marigold, Neem, Dholkalmi, , Nymbicidine) which was tested in the field condition. Nymbicidine showed significantly the best performance in reducing the disease incidence and giving higher yield.

Prodhan (2005) evaluated thirteen fungicides to control purple blotch of onion. All the tested fungicides reduced the severity of the disease. The performance of Rovral, Controll, Contaf and Pharzeb were the best in reducing mean severity of the disease and increased bulb yield compared to control.

Rahman (2004) observed the effect of three fungicides viz., Ridomil, Rovral and Tilt 250 EC (0.2%) comprising 13 treatments in field experiment. Eight sprays of Rovral or Ridomil at 7 days interval minimized disease incidence and increased yield. Rovral 0.2% sprayed at 7 days interval was the best, which gave the highest reduction in disease incidence and severity of leaf blotch and eventually increased the yield of onion.

Uddin (2005) reported bulb treatment followed by six foliar spraying at 10 days interval starting from 20 days after bulb sowing with Dithane M-45 (0.45%) or Rovral (0.2%) minimized disease incidence and severity and increased seed yield. The least seed infection by *Alternaria porri* and the highest seed germination was recorded in the seed sample picked up from Dithane M-45 and Rovral 50WP treated plot in a post-harvest seed health test.

Akter (2007) conducted a field experiment at the research farm of Sher-e-Bangla Agricultural University, Dhaka during the rabi season of 2006-2007 to study the management of purple blotch of onion through chemicals and plant extracts. Eleven treatments comprising Dithane M-45, Rovral 50WP, Bavistin 50WP, Cupravit 50WP, Proud 250EC, Champion, Tilt 250EC, Ridomill Gold, Neem leaf extract, Allamanda leaf extract and Control were explored in the experiment. The highest bulb yield (8.767 t/ha) was obtained with Rovral 50WP treated plot.

The percent plant infection, percent leaf infection, percent Leaf Area Diseased (% LAD) and Percent Disease Index (PDI) were the lowest in foliar spray with Rovral 50WP and the highest in control treatment. Neem extract performed better than Allamanda extract.

Ali (2008) reported that Rovral 50WP @ 0.2% reduced the highest mycelial growth of *Alternaria porri* and *Stemphylium vesicarium* followed by Ridomill Gold MZ-72 @ 0.2% and Dithane M-45 @ 0.45% compared to control. In the field experiment, the treatments showed significant effect in respect of disease incidence, disease severity, seed yield and yield contributing characters. The lowest disease incidence and disease severity were observed in Rovral 50WP @ 0.2% + micronutrients followed by Rovral 50WP @ 0.2% alone, Dithane M-45 @ 0.45% + micronutrients and Dithane M-45 @ 0.45% alone. The highest disease incidence and disease severity were recorded in control treatment.

Sultana *et al.* (2008) conducted an experiment in the field of Plant Pathology Division, BARI, Joydebpur to assess yield loss of onion bulb due to purple blotch disease. The design was paired plot technique having 5 replications using variety Taherpuri. Result indicate, 71.95% disease reduce in the fungicide spraying plot over control. Weight of l0 bulb (g) and yield/plot (kg) also increased 10.6% and 50.9% in fungicide sprayed plot over control.

Sobhy *et al.* (2013) evaluate the efficacy of certain plant extracts against the two identified pathogens of onion purple blotch and *Stemphylium* blight diseases, *in vitro* and under greenhouse condition. Antifungal activity of some aqueous plant extracts (*Azadirachta indica, Cydonia oblonga, Datura stramonium, Eucalyptus globulus, Foeniculum vulgare, Ocimum basilicum, Rosmarinus officinalis* and *Salix mucronata*) was assayed *in vitro* by dry weight technique. Under greenhouse conditions, application of the aqueous extract of *A. indica* either before or after 48 h *A. porri* inoculation produced the highest reduction in disease severity comprising 70 and 74.7%, respectively. On the other hand, the highest percentage of disease reduction before and after 48 h *S. vesicarium* inoculation was produced by Ridomil gold plus reached to 84.4 and

95.8% respectively, followed by the aqueous extract of *A. indica* (74.1 and 89.7, respectively).

Wanggikar *et al*. (2014) conducted a research in Department of Plant Pathology, College of Agriculture, Latur, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India, during 2011 to control Alternaria porri causing Alternaria blight of onion with fungicides, botanical and bio-agents. *in vitro* result revealed that in hexaconozole percent (100.00 %) inhibition was observed, followed by difenoconazole (83.91 %), mancozeb (63.58%), P. florescence (58.94 %) and T. viride (54.45%). The minimum percent inhibition was observed in chlorothalonil (31.40 %) followed by plant extract NSKE (43.92 %), copper oxychloride (46.87 %) and carbandazim (47.11 %). *In vivo* results revealed that hexaconozole (0.1%) was found most effective and recorded significantly least mean disease incidence (6.03 %) and intensity (13.33 %) with corresponding significantly increased bulb yield (438.00 q/ha) followed by mancozeb (@ 0.2%) and copper oxychloride (0.25%) which recorded significantly mean disease incidence of 6.83 and 8.53 per cent and intensity, 15.00 and 20.00 per cent, respectively and gave correspondingly bulb yield, respectively of 375.00 and 429.00 q/ha. The botanical tested, *A. indica* (@ 5%) was found antifungal against *A. porri* and recorded significantly disease incidence (7.96 %) and intensity (27.00 %), and gave the bulb yield (290.00 q/ha).

Harun *et al.* (2015) conducted a field experiment at spices Research Centre, BARI, Shibgonj, Bogra to determine the integrated approach for the management of purple blotch of onion for seed production. The treatment of the expeirment were Rovral wp @ 0.2% (T_1), Rovral wp @ 0.1% + Provax 0.25% (T_2); Trichoderma 5x106 spore/ml @ 100 ml/plant (T_3); Rovral wp @ 0.1% + Bavistin 0.5% (T_4); Neem leaf extract 1:6 (w/v) (T_5); Rovaral wp @ 0.1% +Evaral @ 0.1% (T_6); Rovaral wp @ 0.1% +Ridomil gold MZ-72 @ 0.1% (T_7); Rovaral wp @ 0.1% +Secure @ 0.05% (T_8) and control (T_9). Different treatment has effect on inhibition of mycelial growth of the fungus. The lowest mycelial growth was recorded in case of T_7 followed by T_2 where the highest mycelial

growth was recorded in case of control (T9). The highest plant height (81.40cm) and the yield (1.24 ton/ha) were recorded in treatment T_7 whereas the lowest plant height (61.20cm) and seed yield (0.82. ton/ha) were recorded in control (T9).

Mansha *et al.* (2019) investigated the induced defense response and protective effects of onion against *Alternaria porri* by application of salicylic acid (SA) and benzothiadiazole (Bion®) through foliar application and seedling root dipping method in onion for two years under greenhouse conditions. The results of this study provide evidence that application of simple non-toxic chemical solutions like SA and Bion® can control purple blotch of onion by the modification of biochemical attributes.

Paneru *et al.* (2020) conducted a field experiment in Chaurjahari Municipality, Rukum-west, Nepal during the rabi season of 2019 to study the management of purple blotch complex of onion through chemicals and bio fungicides. Six different chemical fungicides; Hexaconazole, Tebuconazole, Mancozeb+Cymoxanil, Dimethomorph, Chlorothalonil, Carbendazim and one biological fungicides *Trichoderma* were evaluated in field condition against *Alternaria porri* and *Stemphylium vesicarium* for effective control of purple blotch complex. The highest yield 878.7 kg/ha and thousand seed weight 3.72gm were recorded from Hexaconazole treated plot followed by Mancozeb + Cymoxanil with yield and thousand seed weight of 878.3kg/ha and 3.64gm respectively.

2.4 Prevalence of pathogen on onion seed

Wu (1979) surveyed on the seed-borne diseases of vegetables. Results of the survey on onion showed that *Alternaria porri* and *Stemphylium botryosum (Pleospora herbarum)* reduced germination of onion seeds.

Miura (1985) found that *Alternaria porri, A. alternata* and *Fusarium spp*. are predominated among the fungi isolated from onion seeds. *In vitro* products based on iprodione gave the best results resulting 97.4% control of the fungi with 81.4% germination against 54.8% germination of untreated seeds.

Khanam (1992) studied on seed borne fungi of onion and their effect on germination in sind, Palistan. Shes observed that 1.9% infection in onion seeds and a high percentage of fungal infestation reduced seed germination.

Koycu *et al.* (1997) were obtained Samples of onion (*Allium cepa*) seeds from seven regions in Turkey. Among the fungi determined, *Aspergillus alutaceus Berk. and Curt., Beauveria bassiana (Bals.) Vuill., Cladosporium cladosporioides (Fres.) de Vries, Geotrichum sp., Humicolafuscoatra Traaen, Trichoderma harzianum Rifai and T. pseudokoningii Rifai* in onion seeds, and *Fusarium culmorum (W.G.Sm.) Sacc., E graminearum Schwabe and E sambucinum Fuckel* in onion sets, were recorded for the first time. *Aspergillus niger v. Tieghem* was found at the highest rate in seed samples (especially in the seed coat), and in bulbs and roots of onion sets that developed from these seeds.

Hossain (1999) observed a total of 10 fungi representing five genera viz, *Aspergillus sp., Penicllium sp., Fusarium sp., Rhizopus sp., Curvularia sp.* in onion seeds. The detected fungi was *Alternaria porri, Alternaria tennuis, A. niger, A. flavus, F. oxysporum, F. Culmorum, F. Poae, Curvularia lunata, Penicllium atramentosum and Penicillium pinophilum.*

Alam (2001) detected for species *Aspergillus*, *Penicillium* and *Rhizopus* in onion seeds by blotter method.

Fakir (2001) listed ten seed-borne pathogens causing six diseases on onion in Bangladesh. The diseases along with their pathogens were- purple blotch (*Alternaria porri*), seed rot (*Alternaria* sp. and *Fusarium* sp.). Germination reduction (*Aspergillus flavus*, *A. glaucus* and *Rhizopus* sp.), Black mold (*Aspergillus niger*), germination failure (*Fusarium* sp.) and white rot (*Sclerotium cepivorum).*

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 out breaks on seed stalks resulting in low seed recovery and poor seed health and vigour. They detected *Fusarium, Alternaria*, *Stemphylium* and *Aspergillus sp.* in

the onion seeds of N-53, ADR and PRR, Punjab selection, Punjab white, Punjab naroya and Punjab 48 cultivars.

Some (2002) studied seed health of onion seeds collected from different sources like BADC, Private Seed Company, Local seed trader and farmers. Four fungi such as *Aspergillus sp., Penicllium sp., Fusarium sp., Rhizopus sp.,* were detected in onion seeds. Among the fungi, *Aspergillus sp.* was found in all samples. He also stated that prevalence of *Aspergillus sp.* was the highest and Fusarium sp. had the lowest incidence.

In Bangladesh, Karim (2004) conducted experiments on the effect of harvesting, processing and storage on quality of onion seeds and identified the fungi associated with seeds. He found the same 10 fungi in onion seeds as observed by Hossain (1999)

du Toit *et al.* (2004) studied of 12 onion seed lots harvested in the semi-arid Columbia Basin of Washington in 1999 or 2000, 8 were infected and 10 infested with *Botrytis aclada* at incidences of 1 to 10% and 2 to 26%, respectively. Twenty to forty plants were sampled from each of nine direct-seeded, biennial seed crops in April, June, and July 2001 and assayed for *Botrytis sp.* Six direct-seeded crops were sampled in October and November 2001 and April, June, and July 2002. One bulb-to-seed crop was sampled in April, June, and July 2002. The incidence of *B. aclada* increased through each season, reaching 100% in most fields by July. Infections were primarily asymptomatic, with no apparent relationship between plant infection and infection of harvested seed. *B. cinerea, B. squamosa, and B. porri* were detected in 16, 4, and 4% of the fields, respectively, at lower incidences than *B. aclada*. Harvested seed from 15 of the fields were infected with *B. aclada* at 1 to 28%. *B. cinerea*, *B. porri*, and *B. squamosa* were detected in three, three, and none of the harvested lots, respectively.

Jidda *et al.* (2016) the Blotter method was used to assess the presence of fungi on seed samples of Monguno Red variety. More than 60% of the seed samples were associated with various species of fungi. The most frequently isolated

species were *A. flavus* and *A. niger* which together contaminated more than 43% of the seed samples. *A. alternata* was associated with about 30% of the seed samples. The least percentage (2.7) contamination of the seed samples was by *Botrytis allii.*

Dabire *et al*, (2016) studied to assess the seed-borne fungi of onion in Burkina Faso. Eighteen onion seed samples were collected from local farmers and wholesalers of vegetable seeds in the country and were investigated for fungi. The investigation was done using the "blotter method" on dry seeds and on seedlings. Fungal contamination was detected in all 18 tested samples. Seventeen fungal species belonging to 11 fungal genera were identified in the seed samples: Aspergillus was detected in 17 samples, *Fusarium sp.* and *Rhizopus sp.* in 15 samples, *Cladosporium sp.* in 14 samples and *Penicillium sp.* in 13 samples. The infection rates by the fungal species varied from 0 to 90.3% for *A. niger* and from 0 to 13.5% for *F. oxysporum*. *Alternaria porri*, the causal agent of purple blotch disease was recorded lowly on two seed samples at infection rates of 0.5 and 1%.

CHAPTER III

MATERIALS AND METHODS

The details of the materials and methods of this research work were described in this chapter under the following headings and sub-headings:

3.1 Experimental sites

The experiment was conducted in the central farm of Sher-e-Bangla Agricultural University, Dhaka. The experimental field is located at the $23^0$74 N latitude and 90^0 35 E longitude with an elevation of 8.2 meter from sea level.

3.2 Experimental period

The experiments were carried out during the Rabi season from November 2019 and to March 2020. Seeds were sown on 15th November, 2019 and were harvested on 21-25th March 2020.

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 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 (Appendix-II).

3.4 Weather

The monthly mean of daily maximum, minimum and average temperature, relative humidity, monthly total rainfall and sunshine hours received at the experimental site during the period of the study have been collected from Bangladesh Meteorological Department, Agargaon, Dhaka and presented in (Appendix-III).

3.5 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. Finally the land was properly leveled before seed sowing. and plots were prepared as per the Experimental design.

3. 6 Application of Fertilizers

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. Fertilizers were applied as per recommended doses (BARC, 1997).

Doses of chemical fertilizers

Name of the nutrient element	Name of the Fertilizer	Fertilizer dose (kg/ha)	Fertilizer applied during final land preparation (kg/174 m^2 land)	Rest installments (Urea) (kg/174 m^2 land)		
				1st	2nd	3rd
N	Urea	320	1.39	1.39	1.39	1.39
P	TSP	415	7.22	-	-	-
K	MP	168	2.92	-	-	-
S	Gypsum	100	1.74	-	-	-
Zn	ZnO	5	0.09	-	-	-
B	Boric Powder	5	0.09	-	-	-
Manure		10000	174	-	-	-

3.7 Experimental design and layout

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

- Total plot area : 174 m2
- Number of plot : 24
- Plot size : 4 m2
- Block to block distance : 1.0 m
- Plot to boundary distance : 0.5 m
- Plot to plot distance (Lengthwise) : 0.5 m
- Plot to plot distance (breath wise) : 0.5 m
- Plant to plant spacing : 15 cm
- Row to row spacing : 25 cm

3.8 Multiple treatment experiments

Multiple treatments were applied in the experiments. Altogether treatments were applied comprising different number of sprays as follows.

T_0 = Control (No spraying)

T_1 = Field spraying with Allamanda leaf extract 1:2 (W/V)

T_2 = Field spraying with Neem leaf extract 1:2 (W/V)

T_3 = Field spraying with Score 250 EC @ 0.1%

T_4 = Field spraying with Rovral 50 WP @ 0.2%

T_5 = Field spraying with Dithane M-45 @ 0.45%

T_6 = Field spraying with Tricost 1 % WP- @ 0.3% (*Trichoderma harzianum*)

T_7 = Field spraying with of Micronutrients ($ZnSO_4$ + Borax)

3.9 Variety Selection:

The experiment was conducted with a local onion variety “Taherpuri”. This onion variety is most popular in Bangladesh and its quality is more standard than other local or high yielding variety.

A

B

Plate 1: A. Onion bulb, Taherpuri for planting purpose
B. Field ready for planting

3.10 Collection of onion bulb

Bulb of onion were collected from Gourango bazar, Manikgonj.

3.11 Planting date of onion bulb

Uniform seed (mother) bulbs were planted in the experimental plot in 15th November 2019.

3.12 Planting procedure:

The healthy bulbs were selected for planting in experimental plots. The bulbs were planted maintaining row to row distance 25 cm and plant to plant distance 15 cm. The bulbs were planted, as per design and spacing.

3.13 Intercultural operations

3.13.1 Irrigation

Irrigation was given as per requirement of the land with regular intervals. First irrigation was given after 7 days of sowing of bulbs and continued up to harvesting of crop. Water cane with perforated mouth piece was used for soft discharged of water. Irrigation was generally followed the each weeding of the crops.

3.13.2 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 plants undisturbed.

3.14 Preparation of spray solutions

3.14.1 Preparation of plant extracts

Table 1. Plant extract used in experiment

Local name	Scientific name	Plant parts used	Concentrations
Allamanda	*Allamanda catherica*	Leaf	1:2 (w:v)
Neem	*Azadirachta indica*	Leaf	1:2 (w:v)

For the preparation of Allamanda leaf extract and Neem leaf extract, required amount of respective plant parts of each plant was collected, weighted in an electric balance and washed in the tap water. Then the plant parts were chopped into small pieces. For getting the extract, the chopped materials were blended in an electric blender and equal amount of sterile water was added. The blend was filtered through sterile cotton cloth. The supernatant was diluted in equal amount of sterile water for getting 1:2 ratio in the solution for Allamanda leaf extract and Neem leaf extract.

3.14.2. Preparation of Fungicide solutions

Table 2. Fungicides used in the experiment

Trade name	Active ingredients	Doses used
Score 250 EC	Difenoconazole(25%)	0.1 %
Rovral 50 WP	Iprodione (50%)	0.2 %
Dithane M-45	Mancozeb (80%)	0.45 %

Fungicide solutions were prepared by taking requisite amount of fungicide, then mixed with one litre sterile water for optimum concentration.

3.14.3. Preparation of *Trichoderma harzianum* spore suspension

3 gm formulated Tricost 1 % WP (2×10^6 CFU of *Trichoderma harzianum* per gm) was mixed with one litre sterile water to prepare the optimum spore suspension.

3.14.4. Preparation of Micro nutrient spray solutions

Table 3. Micronutrients used in the experiment

Name	Chemical name	Doses used
Borax	Boric acid (H_3BO_3)	0.3 %
$ZnSO_4$	Zinc Sulphate Monohydrate ($ZnSO_4,H_2O$)	0.5 %

3 gm Boric acid powder and 5 gm zinc sulphate monohydrate were mixed with one litre sterile water separately to prepare the micro nutrient spray solution.

3.15. Application of sprays:

The experiment was monitored regularly to observe the on-set of purple blotch disease. Spraying was started from 20 days after planting. Totally 8 sprayings were done with a hand sprayer. Two liter of suspension of each plant extract, fungicide, *Trichoderma harzianum* was used to spray the plants under each treatment. To avoid the drifting of the fungicides during application, temporary fencing was made with polythene sheet surrounding the unit plot at the time of spraying. A control treatment was maintained in each block where spraying was done with plain water.

3.16 Tagging and data collection:

Randomly ten (10) plants were selected from each plot and tagged for data collection and mean values were determined to get rating score of each treatment.

3.17 Collection of leaf diseased samples

Onion leaves having typical leaf spot symptoms (plate-2) were collected from central farm of Sher-e-Bangla Agricultural University, Dhaka. The diseased leaves were cut from the plants and put into a brown paper envelope. Then the brown paper envelopes of each collection sample were taken to the laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for isolation.

3.18 Preparation of culture medium and culture plates

The standard Potato Dextrose Agar (PDA) media was used in this experiment for culturing isolated organism (Table 4).

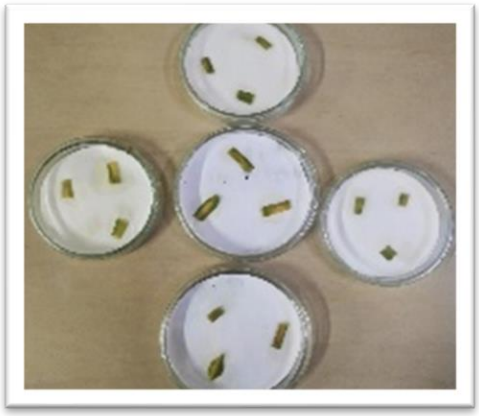
Table 4. Composition of Potato Dextrose Agar (PDA) media.

Ingredients	Amount
Potato (Peeled and Sliced)	200g
Dextrose	20g
Agar	20g
Water	1000 ml

At first cleaned and peeled potato tubers were sliced into pieces. Then the pieces were boiled in distilled water to collect the extract by sieving with a fine piece of cloth. Dextrose and Agar were dissolved in the potato extract and the volume was made up to 1000 ml by adding distilled water. After preparation, the media was poured into 500 ml Erlenmeyer flasks, plugged with cotton and wrapped with aluminum foil. The flasks containing media were sterilized in the autoclave at 121^0C under 15 (PSI) pound per square inch for 20 minutes. The media were acidified with 30 drops of lactic acid per 250 ml medium to inhibit the growth of bacteria. 20 ml of PDA medium was poured into each petri dishes (9 cm

diameter) inside Laminar air flow (LAF) cabinet with proper cautions and then allowed to solidify.

A



B

C

Plate: 2. A = Leaf samples showing typical symptom of purple blotch complex of onion
B = Tissue planting in Blotter paper
C = Tissue planting in PDA plate

3.19 Isolation and identification of pathogens

Isolation and identification pathogen were made in two ways-

a) By direct inspection of diseased sample

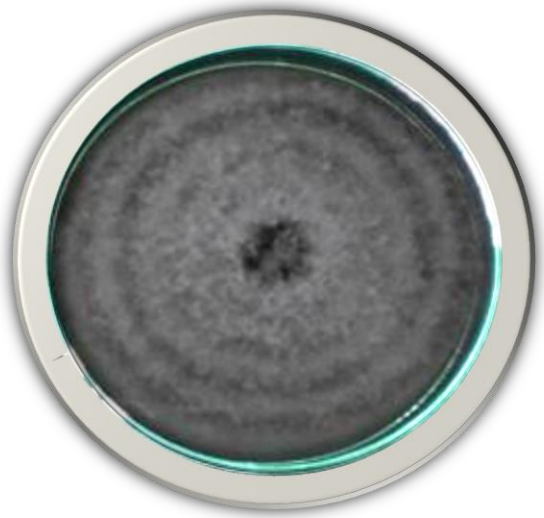
b) By growing on PDA following microscopic observation

(a) By direct observation

The diseased leaves of onion plants were collected and kept in ploythene bags and tagged. The samples were then taken to the laboratory. Then slides were prepared from the diseased samples, observed under microscope and identified the pathogen according to CMI Description.

(b) By growing on Potato Dextrose Agar (PDA) medium

Pathogen was isolated by tissue planting method (Hasan, 2008). The surface of the working clean bench was sterilized with ethanol (70%). Then the infected onion samples were taken into the clean bench and cut into small pieces (0.5-1.0cm). The cut pieces were sterilized in $HgCl_2$ solution (1:1000) for 1 and half minutes and then taken out with the help of sterile forceps and put on sterile distilled water to wash the samples and washing was repeated 3 times. After washing, the cut pieces were placed on sterilized blotter paper (Whatman No. 1) in petriplates and also placed onto the PDA plates, incubated at 25^0C under near ultraviolet light following ISTA rules (ISTA, 1996). Five to ten days after incubation the fungal culture were studied under stereoscope (Model: Motic, SMZ-168) and compound microscope (Model: Omano, OMTM-85) for identification of the desired pathogens.



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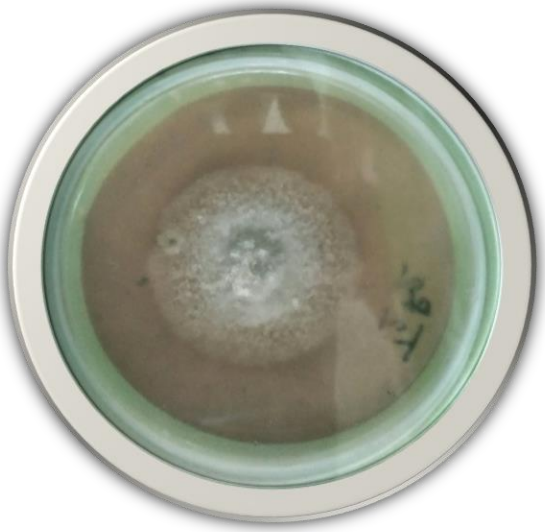


B

Plate 3. Pure culture of *Alternaria porri* (A); *Stemphylium vesicarium* (B)

3.20 Collection of data

The following parameters were considered for data collection.

Disease incidence and severity

a. Percent leaf infection

b. Percent leaf area diseased (% LAD)

c. Percent stalk infection

d. Percent stalk area diseased (% SAD)

Yield and yield contributing characters

a. Seed stalk height

b. Number of stalk/hill

c. Number of umbel/plot

d. 1000-seed weight (g)

e. Yield (kg/ha)

Harvested seed

a. Percent seed germination

b. Percent seed infection

3.21 Procedure of data collection

3.21.1 Number of leaf / plant

Number of leaves per plant was counted from randomly selected 10 plants from each plot at different dates as scheduled

3.21.2 Number of infected leaf / plant of different treatment

Number of leaves infected per plant were recorded and used for calculation of diseased incidence.

3.21.3 Percent leaf infection

Ten plants per plot were selected and tagged for collection of data. Data on percent leaf infection were recorded at 35, 50, 65 and 80 days after planting by visual observation of symptoms. Percent leaf infection was calculated by the following formula.

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

3.21.4 Percent disease index (PDI) of leaf

Percent disease index (PDI) was measured by the following formula-

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of total disease ratting}}{\text{Total no. of observation X Maximum grade in the scale}} \times 100$$

Disease severity scale:

Using "0-5" scale (Horsfall and Barratt, 1945) we calculated the disease severity. "0-5" scale is given bellow-

% Leaf Area Diseased (LAD)	Grade / rating
0	0
0.1 - 5.0	1
5.1 - 12.0	2
12.1 - 25.0	3
25.1 - 50.0	4
>50.0	5
Total	

3.21.5 Percent stalk infection

Data on percent stalk infection were recorded at 80, 95 and 110 days after planting by visual observation of symptoms. Percent stalk infection was calculated by the following formula.

$$\% \text{ Stalk infection} = \frac{\text{Number of infected stalk}}{\text{Number of total inspected stalk}} \times 100$$

3.21.6 Percent stalk area diseased

Data on percent stalk area diseased were recorded at 80, 95 and 110 days after planting by visual observation of symptoms. Percent stalk area diseased was calculated by the following formula.

$$\% \text{ Stalk infection diseased} = \frac{\text{Stalk area infected}}{\text{Total stalk area inspected}} \times 100$$

3.21.7 Stalk height

A Stalk height was measured by a meter scale. Data were also recorded as the average of randomly selected ten (10) stalk from each plot. The mean height was expressed in cm.

3.21.8 Number of stalk per hill

Number of stalk per hill was recorded as the average of randomly selected ten (10) hill from each plot.

3.21.9 Number of umbel per plot

Total number of umbel per plot was recorded.

3.21.10 1000-seed weight (g)

One thousand grains were randomly counted and selected from the stock seed and weighed by digital electric balance. It was expressed as 1000-seed weight in gram (gm).

3.21.11 Seed Yield (kg/ha)

Seed yield was recorded from each plot. After harvesting, the umbels were sun-dried and threshed. Seeds were properly sun-dried and weighed. Seed yield was then converted to kg/ha.

3.22 Testing of Seed health status, prevalance of purple blotch complex pathogens and others seed borne pathogen of onion by blotter method

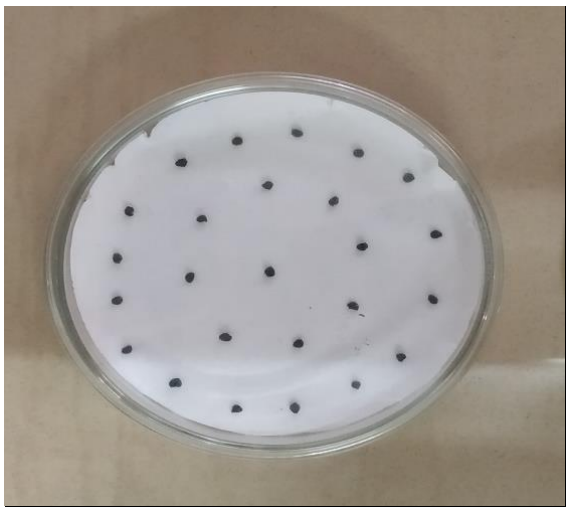
For germination and seed health test the presence of *Alternaria porri*; *Stemphylum vesicarium* and others seed borne pathogen in the harvested seed, 400 seeds were randomly drawn from each sample and were tested by the standard technique (ISTA, 2001).

Seeds were placed on three layers of moist blotting paper (Whatman no. 1) contained in petridishes. In each petridish, 25 seeds were placed in equidistance. All the plates with seeds were incubated at room temperature (25+/-2^{0}C) under 12 hours cycle of alternate Near Ultra Violet (NUV) light and darkness for 7 days. Watering was done as and when required. After 7-10 days of incubation the plates were examined under sterio-microscope for detection of fungi. Each individual incubated seed was observed under sterio-microscope at 10X and 25X magnifications in order to record the incidence of *Alternaria porri*; *Stemphylum vesicarium* and other pathogens. Most of the associated fungi were detected by observing their growth characters on the incubated seeds on filter paper following the keys obtained by Ram Nath *et al.* (1970), Booth (1971), Barnette and Barry (1972) and Mathur and Kongsdal (2003).

For proper identification of fungi temporary slides were prepared from fungal colony and observed under compound microscope, and identified with the help of keys suggested by Ram Nath *et al.* (1970), Ellis (1971), Barnette and Barry (1972) and Mathur and Kongsdal (2003). The fungi were identified to species level wherever possible. The germination and seed infection occurred by incidence of *Alternaria porri*; *Stemphylum vesicarium* and others seed borne pathogen were recorded and expressed in percentage.

$$\text{Germination (\%)} = \frac{\text{No. of germinated seeds}}{\text{Total No. of seeds observed}} \times 100$$

$$\text{Incidence of pathogen (\%)} = \frac{\text{No. of infected seeds associated with the seeds}}{\text{Total No. of seeds observed}} \times 100$$



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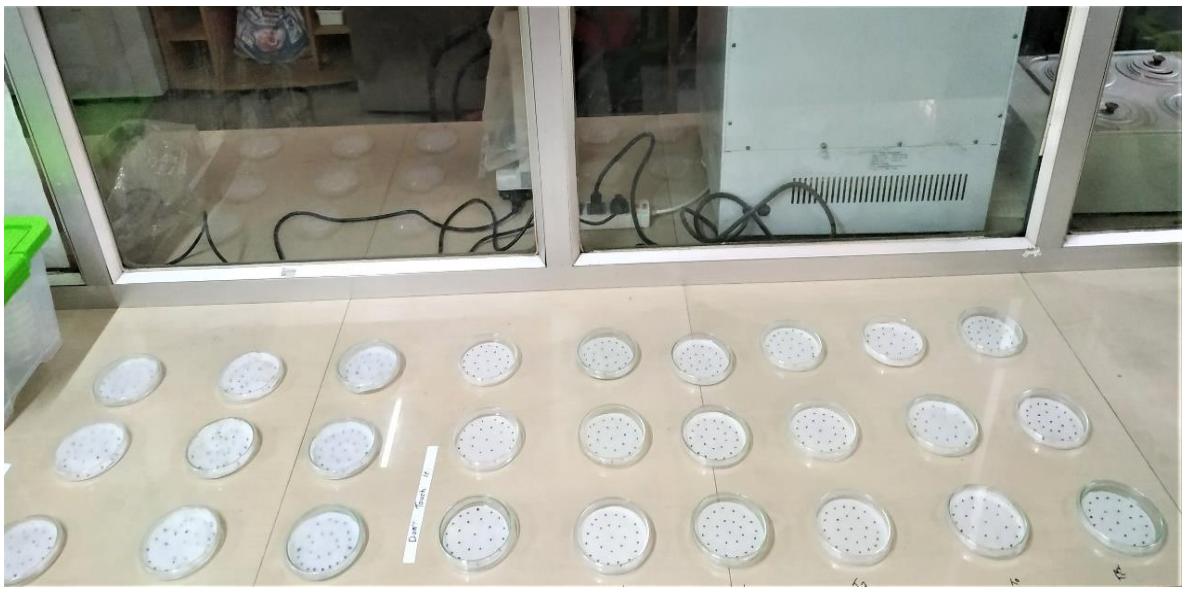


B

Plate 4. A. Seed health testing: Plating of 25 onion seeds on blotter paper
B. Incubation of onion seeds placed on blotter paper

3.23 Experimental Design and Statistical analysis

All collected data for different parameters were tabulated and analyzed following standard procedure (Gomez 1984). The treatment means were compared by LSD (Least Significant Difference). Statistix 10 computer package was used for performing the statistical analysis. ANOVA table was presented in appendix- VI.

CHAPTER IV

RESULTS

The present experiment was conducted for the management of the Purple blotch complex of onion" through different treatment experiment. Data were recorded on % infected leaf, disease severity (leaf), % infected stalk, disease severity (stalk), yield contributing characters and yield of onion seed in field condition, After harvesting % seed germination, prevalence of purple blotch complex pathogen and others seed borne pathogens were recorded. The analyses of variance (ANOVA) of the data on different parameters were done (Appendix VI). The results have been presented, discussed and possible interpretations have been drawn in this chapter under the following headings:

4.1 Percent leaf infection

Data were recorded on the effect of different treatments on percent leaf infection of onion at 35, 50, 65 and 80 days after planting (DAP), summarized and presented in (Table 5). Different treatments had significant influence on percent leaf infection of onion (Taherpuri) at different days after planting (DAP) over control. The result showed that the spraying of Rovral 50 WP gave the lowest leaf infection irrespective of different days after planting that were 3.66 %. 5.5 %, 9.8 % and 17.59 %, respectively at 35 DAP, 50 DAP, 65 DAP, 80 DAP followed by Score 25 EC, Dithane M-45, Neem leaf extract and Tricost (*Trichoderma harzianum*). The moderate leaf infection was observed in the treatment Allmanda leaf extract followed by Micronutrients ($ZnSO_4$ + Borax). The highest leaf infection was observed in control irrespective of days after planting. It was noted that the percent leaf infection was gradually increased with the age of the crop and increasing rate was much slower in Rovral 50 WP, Score 25 EC, Dithane M-45, Neem leaf extract, Tricost (*Trichoderma harzianum*) treated plot compared to control.

Table 5. Effect of different treatments on percent leaf infection of onion at different days after planting (DAP)

Treatments	% Leaf Infection				% inhibition of leaf infection over control at 80 DAP
	35 DAP	50 DAP	65 DAP	80 DAP	
T_0 = Control	30.5 a	37.5 a	39.967 a	49.5 a	0.00
T_1 = Allamanda leaf extract	23.23 bc	27.1 bc	28.5 c	38.5 b	22.22
T_2 = Neem leaf extract	17.5 cd	22.4 c	23.5 d	30.5 cd	38.38
T_3 = Score 250 EC	8.0 ef	14.5 d	15.5 e	25.18 d	49.13
T_4 = Rovral 50 WP	3.667 f	5.5 e	9.8 f	17.593 e	64.45
T_5 = Dithane M-45	12.5 de	16.1 d	17.5 e	27.5 d	44.45
T_6 = Tricost 1 % WP- (*Trichoderma harzianum*)	16.5 d	23.5 c	25.5 cd	36.5 bc	26.25
T_7 = Micronutrients ($ZnS0_4$ + Borax)	23.633 b	31.0 b	34.5 b	41.3 b	16.56
LSD	5.8696	5.6667	4.5215	7.1487	
% CV	19.78	14.58	10.61	12.25	

In a column means having same letter(s) do not differed significantly at 5 % level.

4.2 Percent Disease Index (PDI)

Significant differences were recorded among the effect of different treatments on Percent Disease Index (PDI -leaf) of purple blotch complex disease of onion compared to control (Table 6). On the basis of performances of the treatments in reducing PDI of Rovral 50 WP showed the best performance followed by Score 25 EC, Dithane M-45, Neem leaf extract, Tricost (*Trichoderma harzianum*), Allmanda leaf extract and Micronutrients ($ZnS0_4$ + Borax). The results showed that the lowest PDI (0.41 %) was noted in treatment T_4 at 35 DAP, where Rovral 50 WP was sprayed, which was statistically similar to Score 250 EC (0.95 %). Furthermore, the effect of Dithane M-45 (1.24 %) were statistically similar to

Neem leaf extract (1.45 %). The treatment Tricost (*Trichoderma harzianum*) resulted moderate performance (2.13 %) in controlling Percent Disease Index followed by Allamanda leaf extract (2.32 %). The highest Percent Disease Index was observed in control treatment (3.95 %), preceded by Micronutrients (3.14 %). Similar trend of result were found at 50 DAP, 65 DAP and 80 DAP. At 80 DAP, the lowest PDI (3.64 %) was counted in case of Rovral 50 WP followed by Score 250 EC (7.86 %), Dithane M-45 (11.50 %), Neem leaf Extract (19.50 %). The highest disease severity (PDI-leaf) was conted in control (50.80%) preceded by Micronutrients (33.50 %), Allamanda leaf extract (27.50 %), Tricost (*Trichoderma harzianum*) (21.50 %). The result showed that with increasing the age of onion the percent disease index was gradually increased. But in every case, control treatment showed highest disease severity.

Table 6. Effect of different treatments on the severity of purple blotch complex disease of onion at different days after planting (DAP)

Treatments	Percent Disease Index (PDI)				%Reduction of disease index over control at 80 DAP
	35 DAP	50 DAP	65 DAP	80 DAP	
T_0= Control	3.95 a	9.32 a	37.50 a	50.80 a	0.00
T_1= Allamanda leaf Extract	2.32 c	4.53 c	16.59 c	27.50 c	45.86
T_2= Neem leaf extract	1.45 de	2.40 de	8.85 de	19.50 d	61.61
T_3= Score 250 EC	0.95 ef	1.35 ef	4.55 ef	7.86 ef	84.52
T_4= Rovral 50 WP	0.41 f	0.58 f	2.50 f	3.64 f	92.83
T_5= Dithane M-45	1.24 e	1.12 ef	5.58 ef	11.50 e	77.36
T_6= Tricost 1 % WP- (*Trichoderma harzianum*)	2.13 cd	3.25 cd	14.38 cd	21.50 d	57.67
T_7= Micronutrients ($ZnS0_4$ + Borax)	3.24 b	6.20 b	23.49 b	33.50 b	34.05
LSD	0.7026	1.3645	5.5632	5.4427	
% CV	20.46	21.68	22.40	14.14	

In a column means having same letter(s) do not differed significantly at 5% level

4.3 Percent stalk infection

Data recorded on the effect of different treatments on percent stalk infection of onion at 80, 95 and 110 days after planting (DAP) summarized and presented in (Table 7). Different treatments had significant effects on percent stalk infection of onion at different days after planting (DAP) over to control. The result showed that the spraying of Rovral 50 WP gave the lowest stalk infection irrespective of different days after planting that were 5.33 %. 12.88 %, and 16.21 %, respectively at 80 DAP, 95 DAP, 110 DAP followed by Score 25 EC, Dithane M-45, Neem leaf extract, Tricost (*Trichoderma harzianum*). The moderate stalk infection was observed in the treatment Allmanda leaf extract followed by Micronutrients ($ZnS0_4$ + Borax). The highest stalk infection was observed in control irrespective of days after planting. It was noted that the percent stalk infection was gradually increased with the age of the crop and increasing rate was much slower in Rovral 50, Score 25 EC, Dithane M-45, Neem leaf extract, Tricost (*Trichoderma harzianum*) treated plot compared to control.

4.4 Percent stalk area diseased (SAD)

There were significant differences among the effect of different treatments on Percent Stalk Area Diseased (SAD) of purple blotch complex of onion compared to control (Table 8). On the basis of effectiveness of the treatments in reducing SAD, Rovral 50 WP showed the best performance followed by Score 25 EC, Dithane M-45, Neem leaf extract, Tricost (*Trichoderma harzianum*), Allmanda leaf extract and Micronutrients (ZnSo4 + Borax) irrespective of different days after planting. The results showed that at 110 DAP, the lowest SAD (2.05 %) was found in treatment T_4, where Rovral 50 WP was sprayed, followed by Score 250 EC (3.58 %) and Dithane M-45 (5.75 %). The effect of Score 250 EC (1.35%) were similar with Dithane M-45 (5.75 %). Furthermore, the effect of Neem leaf extract (7.13 %) were statistically similar to Tricost (*Trichoderma harzianum*) (9.35 %). The Allamanda leaf extract (12.85 %) resulted moderate performance in controlling Percent Stalk Area Diseased (SAD) followed by Micronutrients (18.82 %). The highest Percent Stalk Area Diseased was

observed in control treatment (22.05 %), where only water was sprayed. The result showed that with increasing the age of onion the stalk disease area was increased. But in every case, control treatment showed highest disease severity. On the basis of performance of the treatment it was counted that Rovral 50 WP reduced (90.70 %) SAD over control followed by Score 250 EC (83.76 %), Dithane M-45 (73.92 %), Neem leaf extract (67.66 %) and Tricost (*Trichoderma harzianum*) (57.59 %).

Table. 7. Effect of different treatments on percent stalk infection of onion at different days after planting (DAP)

Treatments	% Stalk Infection			% inhibition of stalk infection over control at 110 DAP
	80 DAP	95DAP	110 DAP	
T_0= Control	32.80 a	45.89 a	75.80 a	0.00
T_1= Allamanda leaf extract	21.35 c	30.50 c	37.97 b	49.90
T_2= Neem leaf extract	15.80 d	24.23 cde	28.42 cd	62.50
T_3= Score 250 EC	8.91 f	17.56 ef	19.35 ef	74.47
T_4= Rovral 50 WP	5.53 g	12.88 f	16.21 f	78.61
T_5= Dithane M-45	11.50 e	20.51 de	23.98 de	68.36
T_6= Tricost 1 % WP- (*Trichoderma harzianum*)	16.60 d	26.80 cd	32.15 c	57.58
T_7= Micronutrients ($ZnS0_4$ + Borax)	25.60 b	37.61 b	42.57 b	43.43
LSD	2.3164	6.8374	5.0358	
% CV	7.66	14.46	8.32	

In a column means having same letter(s) do not differed significantly at 5% level.

Table. 8. Effect of different treatments on percent Stalk area diseased (%SAD) of onion at different days after Planting (DAP)

Treatments	% Stalk Area Diseased			%Reduction of stalk area diseased over control at 110 DAP
	80 DAP	95 DAP	110 DAP	
T_0= Control	3.11 a	12.05 a	22.05 a	0.00
T_1= Allamanda leaf extract	2.05 bc	8.85 b	12.85 c	41.72
T_2= Neem leaf extract	1.61 cd	4.13 cd	7.13 de	67.66
T_3= Score 250 EC	1.35 d	1.58 ef	3.58 fg	83.76
T_4= Rovral 50 WP	0.61 e	1.05 f	2.05 g	90.70
T_5= Dithane M-45	1.37 d	2.75 de	5.75 ef	73.92
T_6= Tricost 1 % WP- (*Trichoderma harzianum*)	1.79 cd	5.35 c	9.35 d	57.59
T_7= Micronutrients (ZnSo4 + Borax)	2.45 b	9.82 b	18.82 b	14.64
LSD	0.5689	1.6716	2.5410	
% CV	18.13	16.75	14.23	

In a column means having same letter(s) do not differed significantly at 5% level.

4.5.1 Height of onion seed stalk (cm)

The effect of different treatments on onion stalk height differed significantly that ranged from 63.19 cm to 76.03 cm (Table 9). The highest stalk height (76.03 cm) was recorded in the treatment of Rovral 50 WP, followed by Score 25 EC (74.85 cm), Dithane M-45 (74.75 cm), Neem leaf extract (73.11 cm), Tricost (*Trichoderma harzianum*) (72.28 cm). The moderate seed stalk height was recorded in the treatment Allamanda leaf extract (70.53 cm) followed by Micronutrient (68.12 cm). The lowest seed stalk height (63.19 cm) was recorded in the control treatment.

4.5.2 Number of onion seed stalk / hill

Number of onion seed stalk / hill differed significantly due to the application of different treatments that ranged from 1.63 to 2.33 (Table 9). The highest number

of onion stalk per hill (2.33) was noted in case of Rovral 50 WP followed by Score 25 EC (2.10), Dithane M-45 (2.05), Neem leaf extract (1.91). The effect of Tricost (*Trichoderma harzianum*) (1.85) was statistically similar with the treatment Allamanda leaf extract (1.78). The lowest number of onion seed stalk per hill (1.63) was recorded in the control treatment preceded by Micronutrients (1.73).

4.5.3 Number of umbel / plot

Number of onion umbel per plot differed significantly due to the application of different treatments that ranged from 106.75 to 135.25 (Table 9). The highest number of umbel per plot (135.25) was recorded in the treatment of Rovral 50 WP, followed by Score 25 EC (131.13), Dithane M-45 (128.51), Neem leaf extract (125.32) and Tricost (*Trichoderma harzianum*) (123.32). The moderate number of umbel was recorded in the treatment Allamanda leaf extract (116.18) followed by Micronutrients (114.25). The lowest number of umbel (106.75) was recorded in the control treatment.

4.5.4 1000-Seed weight (g)

Thousand seed weight differed significantly due to the application of different treatments (Table 5). The treatment Rovral 50 WP gave the maximum 1000-seed weight (3.472 g) which was statistically similar with the treatment Score 250 EC (3.148 g). The treatment Dithane M-45 (3.391 g)) was statistically similar with the treatment Neem leaf extract (3.328 g) followed by Tricost (*Trichoderma harzianum*) (3.261 g). The effect of Allamanda leaf extract (3.141 g) was statistically identical with Micronutrients (3.119 g). The minimum 1000-seed weight (2.931 g) was recorded in case of control treatment.

4.5.5 Seed Yield (kg/ha)

The onion seed yield was found to increase significantly from 320.75 kg/ha (Control Plot) to 707.45 kg/ha (Rovral 50 WP) due to application of treatment (Table 9). The onion seed yield in case of Score 250 EC (675.50 kg/ha) treated plot was statistically similar with the Dithane M-45 (656.75 kg/ha). Neem leaf

extract (584.54 kg/ha) also gave promising seed yield followed by Tricost (*Trichoderma harzianum*) (555.17 kg/ha) and Allamanda leaf extract (505.07 kg/ha). The lowest seed yield (320.75 kg/ha) was recorded in the control treatment preceded by Micronutrients (414.75 kg/ha). The onion seed yield was increased by 120.56 % in case of Rovral 50 WP over control followed by Score 250 EC (110.60 %), Dithane M-45 (104.75 %) and so on. The lowest increase of onion seed yield was counted in Micronutreient (29.30 %) preceded by Allamanda leaf extract (57.47 %) and Tricost (*Trichoderma harzianum*) (73.08 %).

Table 9. Effect of different treatments on yield and yield contributing characters of onion

Treatments	Height of Onion seed stalk (cm)	No. of onion seed stalk/ hill	No. of umbel /plot	Thousand (1000) seed weight (g)	Seed yield (kg/ha)	% Yield increased over control
T_0 = Control	63.19 f	1.63 e	106.75 f	2.931 e	320.75 g	-
T_1= Allamanda leaf Extract	70.53 d	1.78 cd	116.18 e	3.141 d	505.07 e	57.47
T_2= Neem leaf extract	73.11 bc	1.91 c	125.32 cd	3.328 bc	584.54 c	82.24
T_3= Score 250 EC	74.85 a	2.10 b	131.13 ab	3.418 a	675.50 b	110.60
T_4= Rovral 50 WP	76.03 a	2.33 a	135.25 a	3.472 a	707.45 a	120.56
T_5= Dithane M-45	74.75 ab	2.05 b	128.51 bc	3.391 ab	656.75 b	104.75
T_6= Tricost 1 % WP- (*Trichoderma hargianum*)	72.28 c	1.85 cd	123.32 d	3.261 c	555.17 d	73.08
T_7= Micronutrients ($ZnS0_4$ + Borax)	68.12 e	1.73 de	114.25 e	3.119 d	414.75 f	29.30
LSD	1.6843	0.1359	4.6225	0.0866	22.910	
% CV	1.34	4.04	2.15	1.52	2.37	

In a column means having same letter(s) do not differed significantly at 5% level.

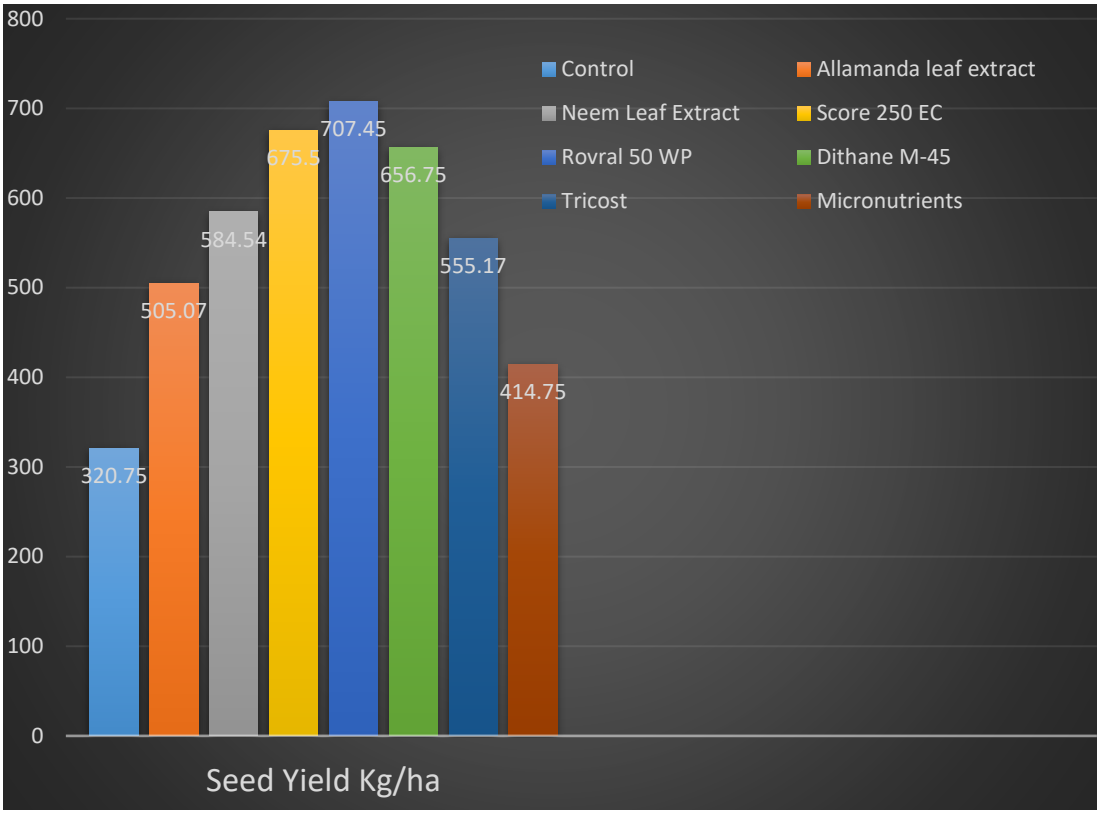


Plate 5. Effect of different treatments on onion seed yield

4.6. Seed health status and prevalence of seed borne pathogens on harvested onion seeds

4.6.1 Percent seed germination

Percent seed germination of harvested onion seeds from different treated plots were found to be differed that presented in (Table 10). Seed obtained from T_4 (Rovral 50 WP) treated plot showed the highest seed germination (93.5 %) followed by Score 250 EC (90.8 %), Dithane M-45 (88.5 %), Neem leaf extract (85.5 %), Tricost (*Trichoderma harzianum*) (81.5 %), Allmanda leaf extract (77.8 %) and Micronutrients (71.3 %). Seed germination obtained from control plot showed the lowest germination percentage (65.5 %).

Plate 6. Germinating onion seedlings on blotter paper

4.6.2. Total seed borne pathogenic infection

A total of 650 pathogenic infections were recorded from 3200 harvested onion seeds from experimental plots treated differently. The prevalence of total seed borne pathogenic infections varied in respect to harvested onion seeds treated with different treatments. The lowest number of seed borne pathogenic infections (34) were recorded on T4 (Rovral 50 WP) over control (160) preceded by Score 250 EC (48), Dithane M-45 (54), Neem leaf extract (74), Tricost (*Trichoderma harzianum*) (86), Allmanda leaf extract (88) and Micronutrients (106). Based on the total infection, the % pathogenic infection was calculated and it was varied from 8.5 % to 40.0 %. The lowest pathogenic infection was recorded in Rovral 50 WP (8.5 %) and the highest in control (40.0 %) (Table 10).

Table 10. Effect of different treatments on percent seed germination and incidence of seed borne pathogenic infection on harvested onion seed.

Treatment	% Seed germination	No. of pathogenic infection	% of total infection *
T0= Control	65.5	160	40.0
T1= Allamanda leaf extract	77.8	86	21.5
T2= Neem leaf extract	85.5	74	18.5
T3= Score 250 EC	90.8	48	12.0
T4= Rovral 50 WP	93.5	34	8.5
T5= Dithane M-45	88.5	54	13.5
T6= *Trichoderma harzianum*	81.5	88	22.5
T7= Micronutrients ($ZnS0_4$+ Borax)	71.3	106	26.5
Total		650	100

* No. of fungal infections was calculated on the basis of 400 seeds per treatment.

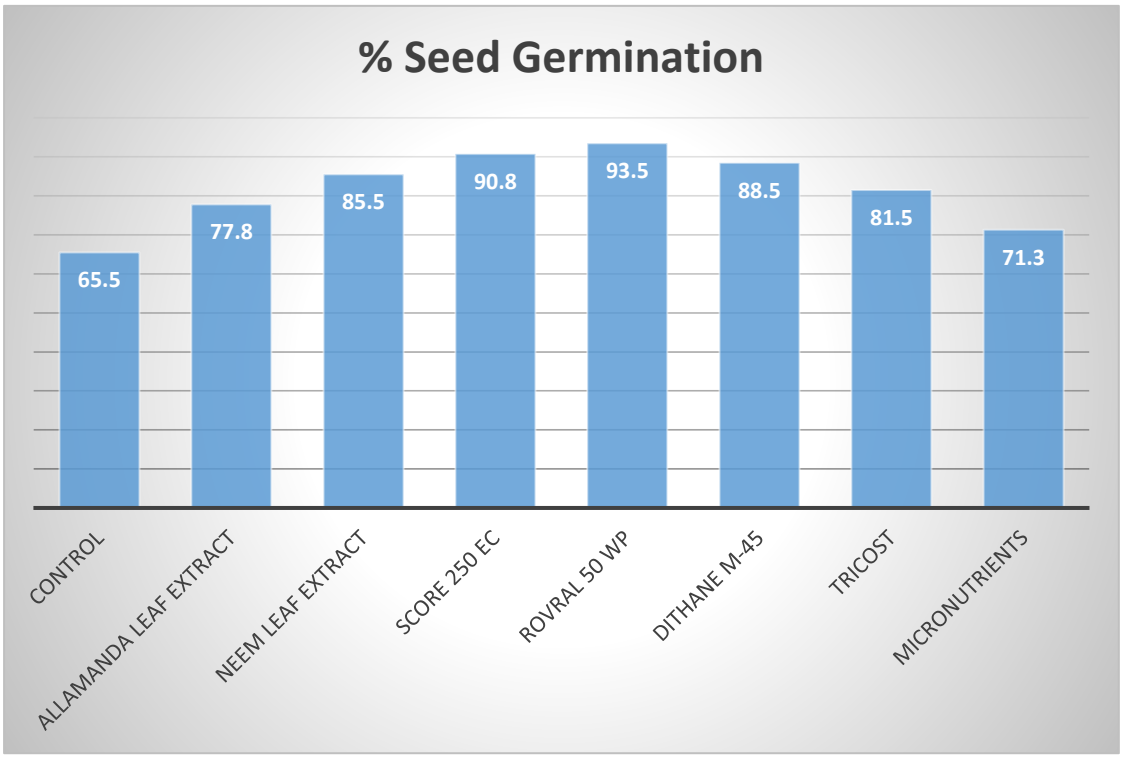


Plate 7. Effect of different treatments on percent onion seed germination

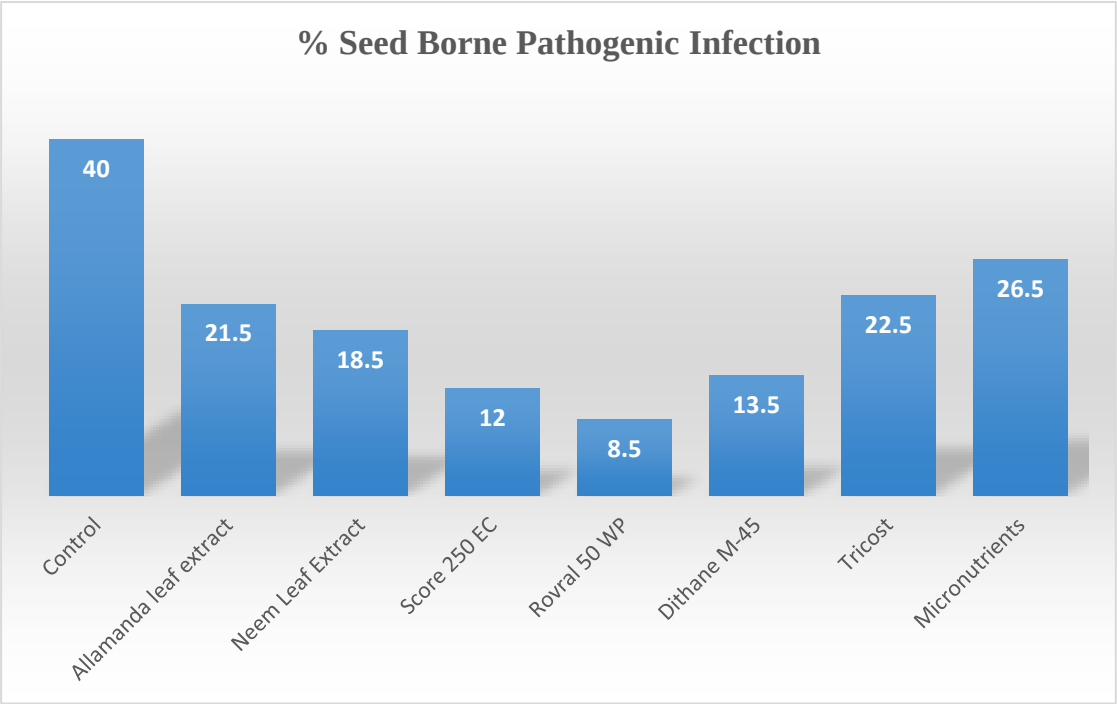


Plate 8. Effect of different treatments on Incidence of seed borne pathogenic infection on harvested onion seed.

4.6.3. Seed borne pathogens and their frequency on harvested seeds.

Ten species of seed borne microflora were identified out of 650 seed borne pathogenic infections recorded from harvested onion seeds of different experimental plots. The identified seed borne microflora were *Alternaria porri, Stemphylium vesicarium, Aspergillus flavus, Aspergillus niger, Botrytis cinerea, Fusarium* sp., *Penicillium* sp., *Rhizopus* sp., *Bacterial Ooze* and unknown (Plate 9-17).

Aspergillus flavus, Aspergillus niger, Botrytis cinerea, Penicillium sp., *Stemphylium vesicarium, Rhizopus* sp. and *Fusarium* sp. respectively, were constituted 23.38%, 21.53%, 13.84%, 10.76%, 8.30%, 6.15% and 5.38% of the total seed borne pathogenic infections. *Aspergillus spp.* occupied the highest frequency followed by other seed borne microflora. *Alternaria porri* possess 3.38 % total infection while *Stemphylium vesicarium* possess 8.30 % (Table 11).

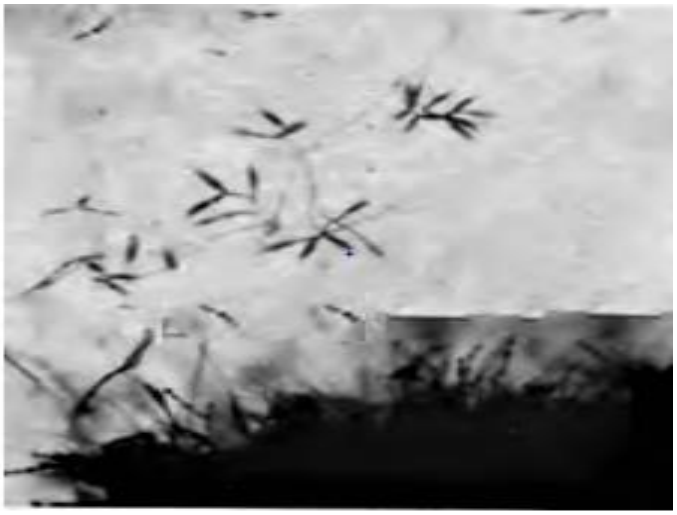
Again, regarded to percentage of seed yielding pathogen, *Aspergillus flavus* yielded 4.75 % infection followed by *Aspergillus niger* (4.37%), *Botrytis cinerea*

(2.81%), *Penicillium* sp. (2.18%), *Stemphylium vesicarium (*1.68%), *Rhizopus* sp. (1.25%), *Fusarium* sp. (1.09%), unknown (0.78%), *Alternaria porri* (0.69%) and Bacterial ooze (0.69%), respectively (Table 11).

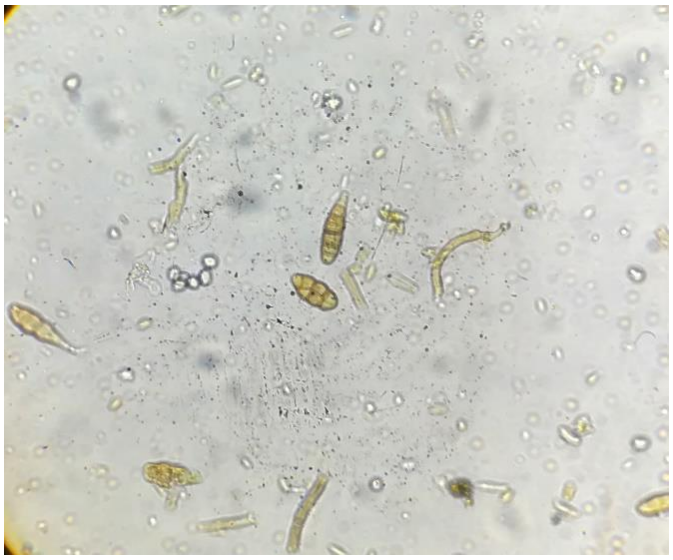
Table 11. Frequency of various pathogenic infection recorded on onion seeds harvested from different treatment experimental plots

Fungi	No. of pathogenic infection out of 3200 seed*	% of total infection	% of the seed yielding (calculated on the basis of 3200 seed)
Alternaria porri	22	3.38	0.69
Stemphylium vesicarium	54	8.30	1.68
Aspergillus flavus	152	23.38	4.75
Aspergillus niger	140	21.53	4.37
Botrytis cinerea	90	13.84	2.81
Fusarium sp.	35	5.38	1.09
Penicillium sp.	70	10.76	2.18
Rhizopus sp.	40	6.15	1.25
Unknown 1	25	3.84	0.78
Bacterial Ooze	22	3.38	0.69
Total	650	100	

* Out of 3200 seed (Treatments 8 × 400 seeds)

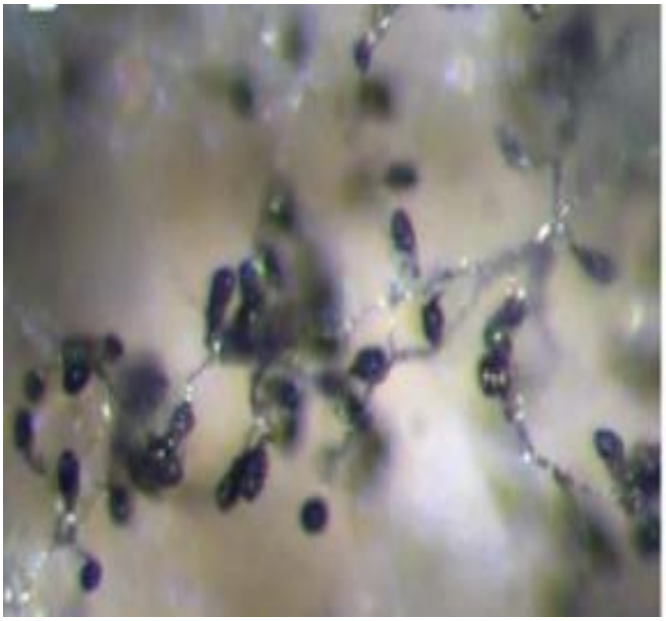


A

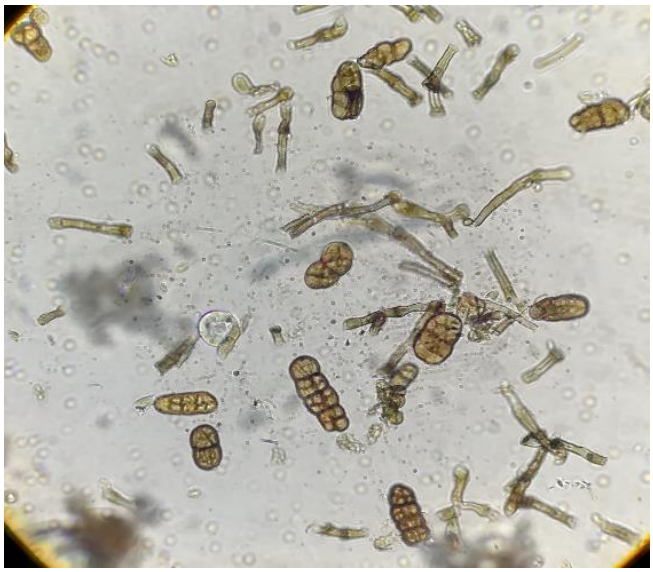


B

Plate 9. A. *Alternaria porri* growing on onion seed incubated on blotter paper
B. Conidia of *Alternaria porri* observed under compound microscope (40X)

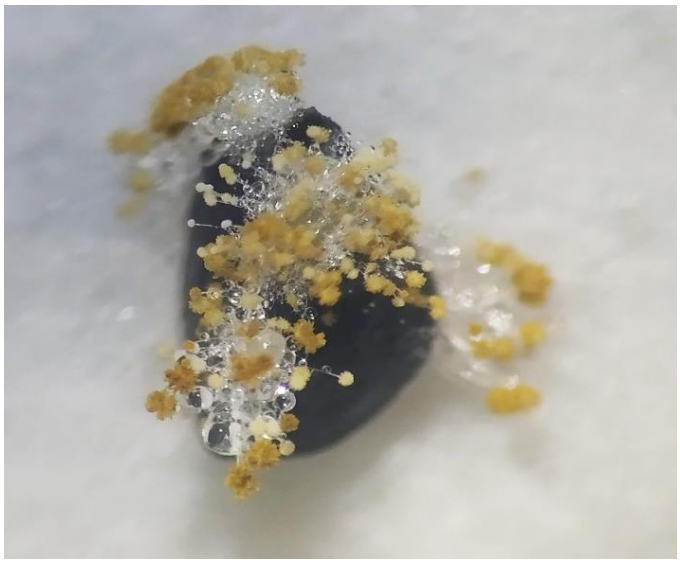


A



B

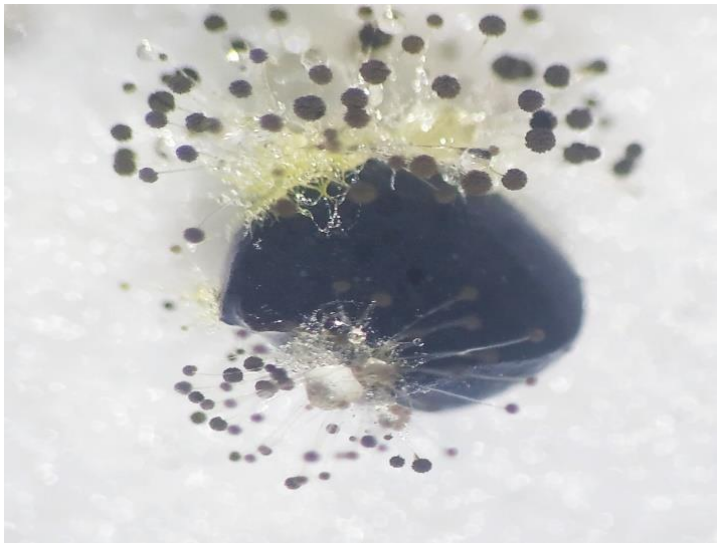
Plate 10. A. *Stemphylium vesicarium* growing on onion seed incubated on blotter paper
B. Conidia of *Stemphylium vesicarium* seen under compound microscope (40X)



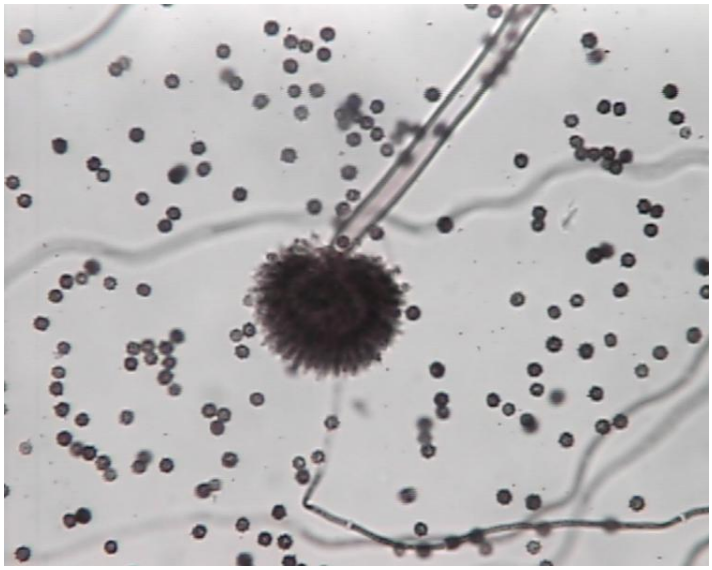
A

B

Plate 11. A. *Aspergillus flavus* growing on onion seed incubated on blotter
B. Conidia and Conidiophore of *Aspergillus flavus* seen under compound microscope (400X)



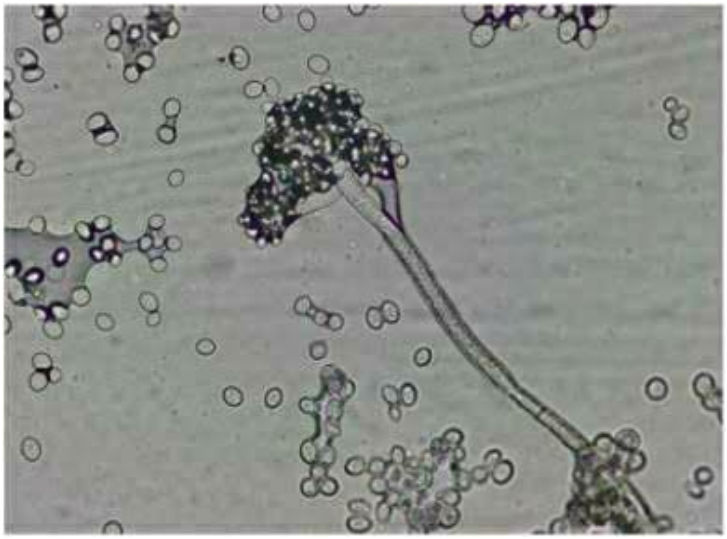
A



B

Plate 12. A. *Aspergillus niger* growing on onion seeds incubated on blotter paper
B. Conidia, conidiophore and conidial heads of *Aspergillus niger* seen under compound microscope (400X)

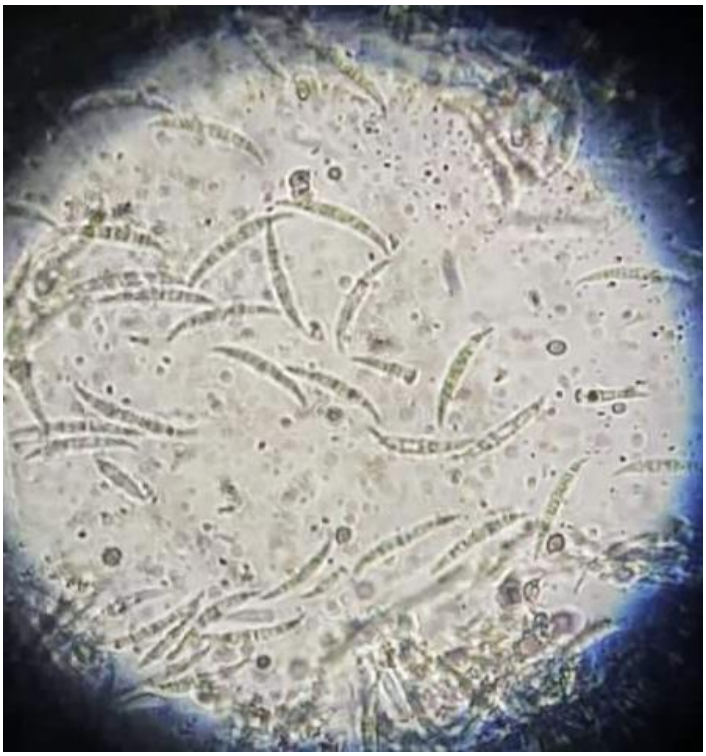
A



B

Plate 13. A. *Botrytis cinerea* growing on onion seed incubated on blotter paper
B. Conidia of *Botrytis cinerea* seen under compound microscope (400X)

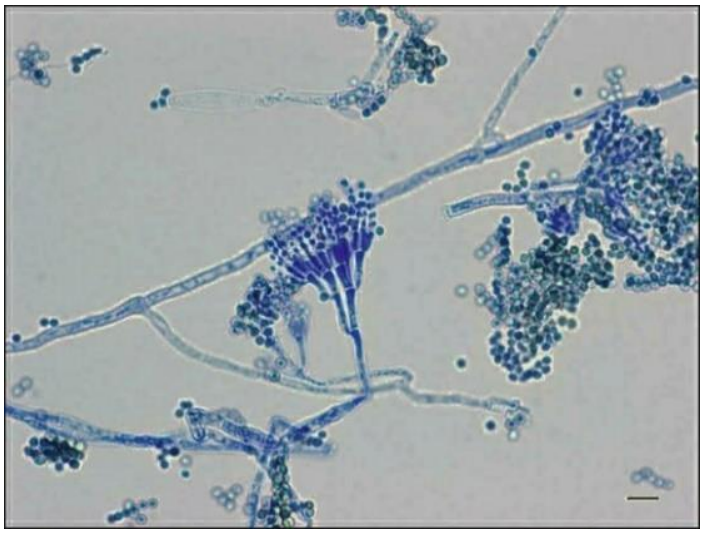
A



B

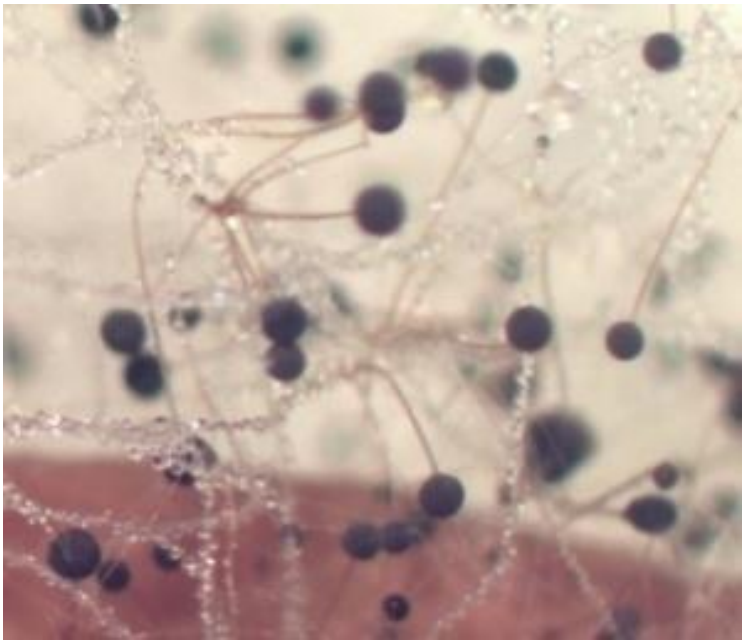
Plate 14. A. *Fusarium* sp. growing on onion seeds incubated on blotter paper
B. Conidia of *Fusarium* sp. seen under compound microscope (40X)

A



B

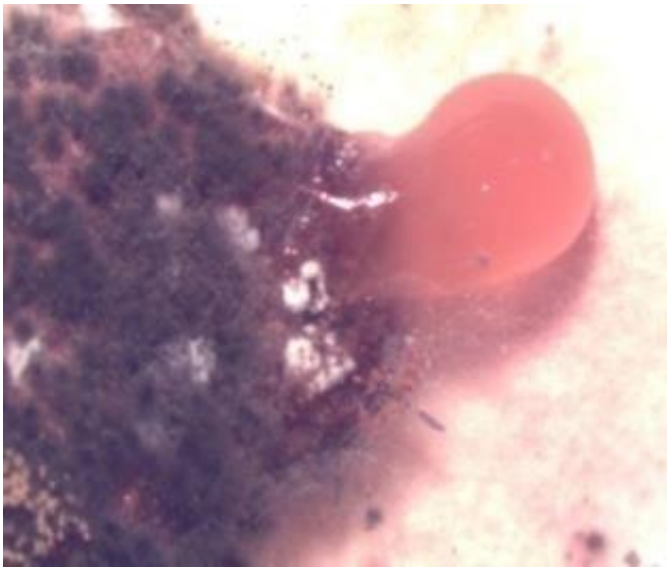
Plate 15. A. *Penicillium* sp. growing on onion seeds incubated on blotter
B. Conidia and brush like conidiophore of *Penicillium* sp. seen under compound microscope (40X)



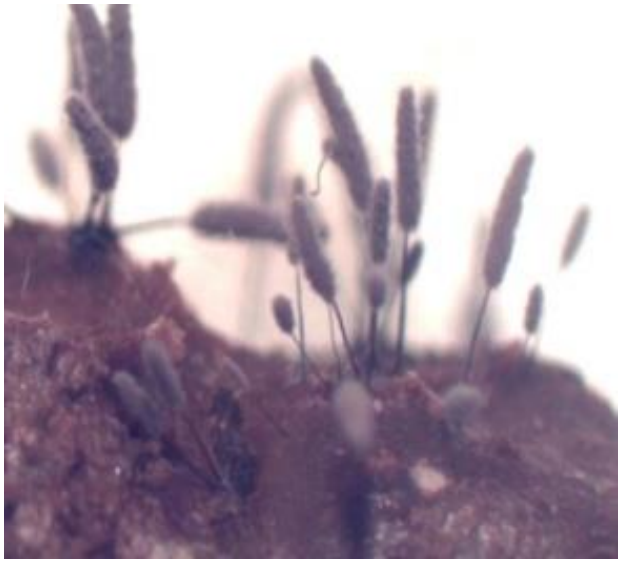
A

B

Plate 16. A. *Rhizopus* sp. growing on onion seeds incubated on blotter paper
B. Sporangia and sporangiophores of *Rhizopus* sp. seen under compound microscope (40X)



A



B

Plate 17. A. Bacterial Ooze formation on onion seed incubated on blotter paper
B. Unknown pathogen growing on onion seeds incubated on blotter paper

4.6.4. Prevalance of seed borne infections of individual pathogen on harvested onion seeds collected from different treatment experimental plots.

Prevalance of different seed borne microflora on harvested onion seeds were counted in respect of different treatments applied in the experiment and presented in (Table 12).

The results showed that the incidence of *Alternaria porri,* the causal organism of purple blotch of onion was only 0.25 %, where Rovral 50 WP was applied. The second lowest incidence (0.25 %) of *Alternaria porri* was recorded in case of Score 250 EC that was similar to *Trichoderma harzianum.* The third lowest incidence of *Alternaria porri* (0.75 %) was recorded conjointly in Dithane M-45 and Neem leaf extract. The highest (1.25 %) incidence of *Alternaria porri* was noted in control.

In case of the prevalence of *Stemphylium vesicarium*, the lowest incidence (0.75 %) was also counted in T_4 treatment, where Rovral 50 WP was applied. The second lowest incidence (1.0 %) of *Stemphylium vesicarium* was counted in T_3 treatment, where Score 250 EC was applied as foliar spray and this incidence was identical with T_5, where Dithane M-45 was applied. The highest (3.0 %) incidence of *Stemphylium vesicarium* was recorded in control preceded by Neem leaf extract (2.25 %), *Trichoderma harzianum* (2.25 %), Micronutrients (1.75 %).

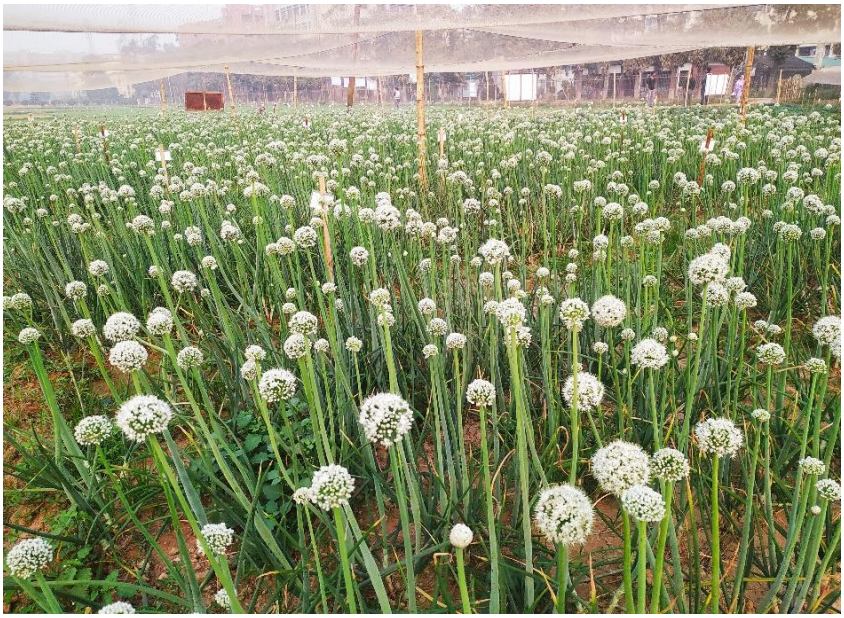
The incidence of different seed borne microflora other than *Alternaria porri* and *Stemphylium vesicarium* had no trend of occurrence in the tested samples. However, the prevalence of *Aspergillus flavous*, *Aspergillus niger* and *Penicillium* sp. were higher irrespective of treatments applied.

Table: 12. Prevalence of seed borne individual fungi detected on onion seeds from collected from different treatment experimental plot

% SEED YIELDING INFECTION										
Treatments	***Alternaria porri*** *	***Stemphylium vesicarium*** *	***Aspergillus flavus*** *	***Aspergillus niger*** *	***Botrytis cinerea*** *	***Fusarium* sp.** *	***Penicillium* sp.** *	***Rhizopus* Sp.** *	**Bacterial Ooze***	**Unkwon 1***
T_0 = Control	1.25	3.0	8.75	8.0	6.25	2.00	4.0	0.5	1.25	3
T_1 = Allamanda leaf extract	0.5	2.25	4.75	5.75	2.25	1.5	3.0	1.0	0.5	-
T_2 = Neem leaf extract	0.75	1.5	4.25	4.5	2.75	0.5	2.0	1.25	0.5	-
T_3 = Score 250 EC	0.5	1.0	3.25	2.90	1.5	0.5	1.0	0.5	0.5	0.25
T_4 = Rovral 50 WP	0.25	0.75	2.0	1.75	1.25	0.25	0.5	1.25	0.25	0.5
T_5 = Dithane M-45	0.75	1.0	3.2	3..75	2.0	0.5	1.0	0.5	0.75	0.25
T_6 = *Tricost 1 % WP-(Trichoderma harzianum)*	0.5	2.25	5.25	5.0	3.0	1.25	2.5	1.25	0.5	0.5
T_7 =Micronutrients ($ZnS0_4$+ Borax)	1.0	1.75	5.0	3.75	5.5	1.75	3.77	3.5	1.5	1.25

*Percentage of the seed yielding different pathogens was calculated on the basis of 400 seeds

A



B

Plate 18. A. Field view of experimental plot at 35 DAP
B. Field view of experimental plot of flowering stage of onion

A

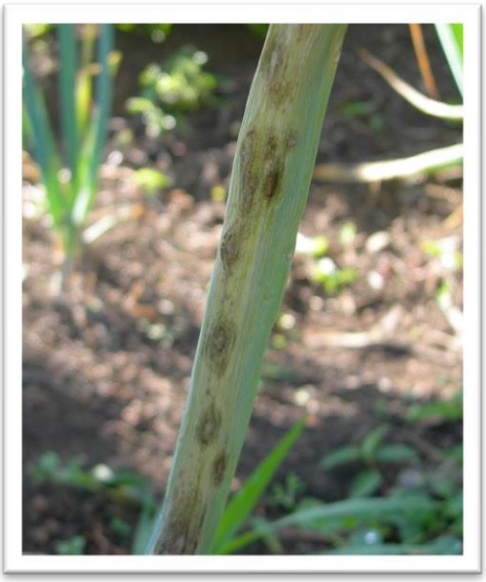
B

Plate 19. A. Field view of experimental plot at 100 DAP of full blooming of onion umbel
B. A mature umbel of onion ready for harvest

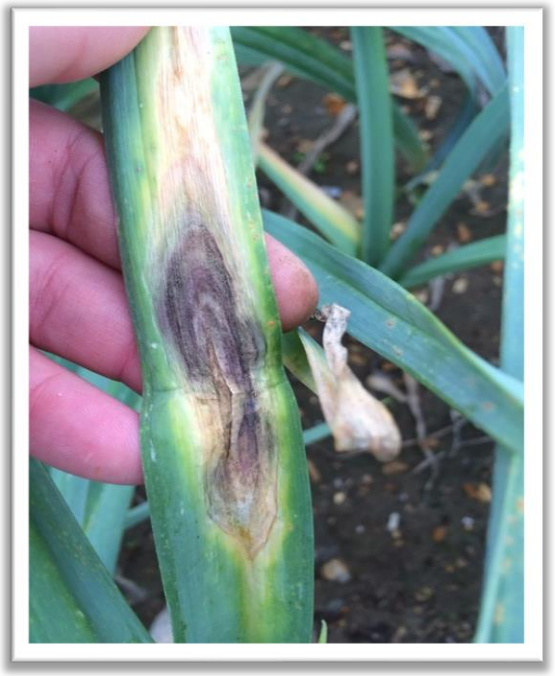
A

B

Plate 20. A. Drying of the harvested onion seeds
B. Sun drying and Packaging of the harvested onion seeds

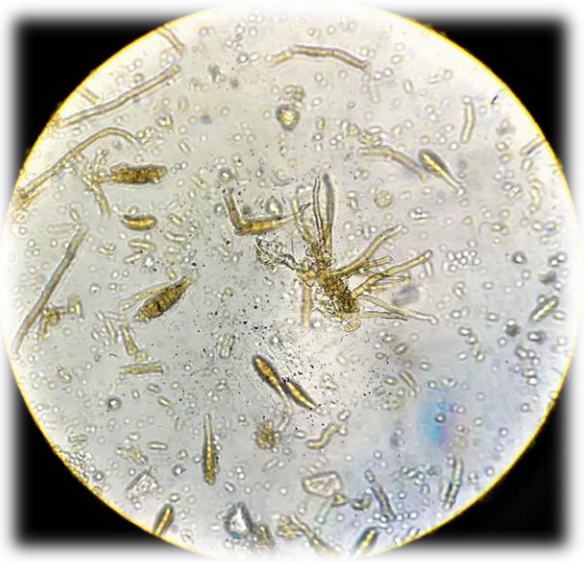


A **B**

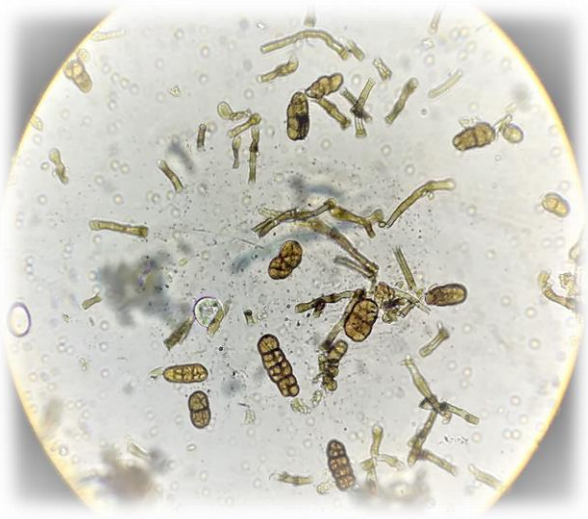


C

Plate 21: Typical symptoms of purple blotch complex of onion
A = White blotch (initial stage)
B = Purple blotch
C = Purple blotch complex



A



B

Plate 22. Conidia of *Alternaria porri* (A) and *Stemphylium vesicarium* (B) seen under compound microscope (40X)

CHAPTER V

DISCUSSION

The effect of treatments in controlling purple blotch complex of onion caused by *Alternaria porri* and *Stemphylium vesicarium* was assessed in respect of percent leaf infection, percent disease index (PDI), percent infected seed stalk, percent Stalk Area Disease (%SAD), height of seed stalk (cm), number of seed stalk per hill, number of umbel per plot, thousand (1000) seed weight (g), seed yield, percent seed germination and prevalence of seed borne pathogens on harvested onion seeds.

In the field experiments the application of different treatments had significantly variable effect in reducing the disease incidence, severity and increasing the seed yield. Among the treatments, the effect of Rovral 50 WP (0.2%) against purple blotch complex of onion in terms of percent leaf infection, percent disease index (PDI), percent infected seed stalk, percent Stalk Area Disease (%SAD), growth parameters, seed yield, seed germination and prevalence of pathogenic infection of onion was found promising.

In case of disease incidence, the minimum leaf infection and stalk infection was observed in the Rovral 50 WP treated plot irrespective at all days after planting (DAP) followed by Score 25 EC, Dithane M-45, Neem leaf extract, Tricost (*Trichoderma harzianum*). At 80 DAP, Rovral 50 WP reduced the highest leaf infection (64.45 %) over control while Score 25 EC (49.13 %), Dithane M-45 (44.45 %), Neem leaf extract (38.38 %), Tricost (*Trichoderma harzianum*) (26.25 %) over the control. At 110 DAP, Rovral 50 WP reduced the highest Stalk infection (78.61 %) over control while Score 25 EC, Dithane M-45, Neem leaf extract and Tricost (*Trichoderma harzianum*) reduced the stalk infection respectively by 74.47 %, 68.36 %, 62.50 % and 57.58 % over the control.

In terms of disease severity (PDI and % SAD), the result showed that Rovral 50 WP proved to be the best potential fungicide followed by Score 25 EC, Dithane M-45, Neem leaf extract and Tricost (*Trichoderma harzianum*). At 80 DAP,

Rovral 50 WP reduced the highest percent disease index (PDI) of leaf (92.83 %) over control while Score 25 EC (84.52 %), Dithane M-45 (77.36 %), Neem leaf extract (61.61 %) and Tricost (*Trichoderma harzianum*) (57.67 %) over the control. At 110 DAP, Rovral 50 WP reduced the highest stalk area diseased (SAD) (90.70 %) over control while Score 25 EC, Dithane M-45, Neem leaf extract and Tricost (*Trichoderma harzianum*) reduced the highest stalk area diseased respectively by 83.76 %, 73.92 %, 67.77 %, 57.59 % over control.

From the results of leaf infection and stalk infection it is revealed that the performance of Allamanda leaf extract and micronutrients used in the experiment was not up to the mark.

The data recorded on disease incidence and severity at different days after planting indicated that the preliminary disease development was more or less identical in all cases of treatments but the disease incidence and severity appeared to be distinct among the treatments gradually with the increase of time in compare to control due to consecutive spraying with the fungicides, plant extracts, *Trichoderma* solution and micronutrients. It indicates the demonstration of inhibitory effect of the treatments applied in controlling the disease.

The present findings was supported by the reports of the previous researches (Ahmed *et al.*, 1999; Sugha, 1995; Miura, 1985; Rahman, 2004; Srivastava *et al.*, 1994; Islam *et al.*, 2001 & 2003; Khatun, 2007 and Hoque, 2008). Ahmed *et al.* (1999) reported that the fungicides Rovral 50 WP (0.2%) and Ridomil MZ-72 (0.2%) were effective in reducing incidence and severity of purple blotch of onion. Sugha (1995) reported that Iprodione (0.2%) proved to be highly effective against purple blotch of onion resulting 79.6 - 84.9% control of the disease. Rahman (2004) reported that among 6 fungicides, Rovral 50 WP significantly reduced the disease severity of purple blotch of onion.

Miura, (1985) reported that 97.4 % control of *Alternaria porri* causes purple blotch of onion was achieved by apply Rovral 50 WP. Srivastava *et al.* (1996) observed that seedling dipped in Carbendazim and thiophanate methyl followed

by 4 sprays of Rovral 50 WP was effective against purple blotch of onion. Islam, (1995 & 2003) reported that fungicidal spray reduced disease severity (PDI) of purple blotch of onion up to 64.02%. Islam, *et al.* (2001) also reported that Rovral 50 WP gave promising effect in reducing the disease severity of purple blotch of onion. Khatun (2007) reported that 6 foliar spraying at 10 days interval starting from 20 DAP with Rovral 50 WP (0.2%) or Dithane M-45 (0.45%) successfully minimized disease incidence and severity of *stemphylium* blight of onion caused by *Stemphylium vesicarium.* Hoque (2008) also reported that the bulb treatment with Rovral 50 WP (0.2%) followed by foliar spraying with Rovral 50 WP at 7 days interval starting from onset of the disease minimized disease incidence and severity.

Ali (2008) reported that spraying of Rovral 50WP (0.2%) along with application of micronutrients remarkably reduced the incidence and severity of purple blotch of onion.

The treatments had remarkable effect on yield contributing characters like Seed stalk height, number of umbel per plot and 1000 seed weight that influenced the seed yield.

The highest seed yield (707.45 kg/ha) was obtained from the plot where Rovral 50 WP @ 0.2% was applied against the disease that increased seed yield by 120.56 % compared to control followed by Score 25 EC (675.50 kg/ha), Dithane M-45 (656.75 kg/ha), Neem leaf extract (584.54 kg/ha), *Trichoderma harzianum* (555.17 kg/ha) over control, where seed yield increased by 110.60 %, 104.75 %, 82.24 %, 73.08 % compare to control.

These findings were well supported by Barnoczki-stoilova *et al.* (1989) and Georgy *et al*. (1983). Hoque (2008) also reported that micronutrients along without spraying of fungicides had significant effect compared to control. Barnoczki-stoilova *et al.* (1989) conducted a field experiment spraying with fungicides at different blooming stages of flowers and reported that Rovral 50 WP (Iprodione) and Ridomil plus 50 WP (Methyl + Copper oxychloride) showed less harmful and effective in controlling disease in onion seed production.

Georgy *et al*. (1983) also reported that the Iprodione group and Ridomil MZ (Metalaxyl + Mancozeb) proved most effective in reducing the disease severity and increasing bulb and seed yield. Hoque (2008) reported that Rovral 50 WP effectively controlled Stemphylium blight of onion. Harun *et al*. (2015) observed that the highest plant height and the yield were recorded in treatment (Rovaral wp @ 0.1% +Ridomil gold MZ-72). Paneru *et al.* (2020) also reported that the highest seed yield and thousand seed weight were recorded from Hexaconazole treated plot followed by Mancozeb + Cymoxanil.

In the present investigation, seed health status and prevalence of seed borne pathogenic infection on collected harvested seeds of onion from multiple treatment experimental plots was studied. Under the present study, germination of harvested onion seeds varied significantly from 65.5 % to 93.5 %. The result showed that the highest seed germination (93.5 %) of harvested seed was recorded in Rovral 50 WP treated plot.

A total of 650 seed borne pathogens were recorded from 3200 seeds. Thus, the present study shows that around 21 % of the seeds were infected by pathogens. This indicates that the seeds of onion under study can quite often be infected by fungi.

The total as well as the individual pathogen encountered in harvested onion seeds varied in prevalence depending on the multiple treatment experimental plots. 10 species of seed borne pathogens were identified in the present study. The identified seed borne pathogens were *Alternaria porri, Stemphylium vesicarium, Aspergillus flavus, Aspergillus niger, Botrytis cinerea, Fusarium* sp., *Penicillium* sp., *Rhizopus* sp., *Bacterial Ooze* and Unknown 1. Where *Aspergillus flavus, Aspergillus niger, Botrytis cinerea* were predominant. *Alternaria porri, Stemphylium vesicarium* also found on collected harvested seed of onion from multiple treatment experimental plots.

The highest number of seed borne pathogenic infections (160) were recorded on the harvested seed of T_0 (control) plot which was 40.0 % of total seed borne pathogenic infection. The lowest number (34) of seed borne pathogenic infection

of harvested seed from Rovral 50 WP treated plot was recorded over control, which was 8.5 % of total pathogenic infection.

The lowest seed infection by *Alternaria porri* and *Stemphylium vesicarium* on harvested onion seeds from the experimental plot, respectively were 0.25 % and 0.75 % in case of Rovral 50 WP followed by Score 250 EC treated plot. It indicates that the purple blotch complex pathogens were potentially controled by foliar spraying of Rovral 50 WP and Score 250 EC.

The present findings corroborate with the findings of previous research report (Wu., 1979; Vannacci, 1981; Mannerucci *et al*, 1982; Gupta *et al*, 1984; Cristani, 1992; Javed *et al*, 1994; Boff *et al*, 1995; Beliard *et al*, 1998). Anonymous (1997) reported that foliar spray of Rovral significantly reduced the seed borne infection of *Alternaria porri* and increased germination percentage of onion seed. It was reported that, seed born infection of *Alternaria porri* was reduced above 90% and seed germination was increased above 9% over control while seed infection was reduced up to 18.8% with 3 times foliar spray of Rovral.

Wu (1979) surveyed on the seed-borne diseases of vegetables. Results of the survey on onion showed that *Alternaria porri* and *Stemphylium vesicarium (Pleospora herbarum)* reduced germination of onion seeds. Miura (1985) found that *Alternaria porri*, *A. alternata* and *Fusarium* spp. are predominated among the fungi isolated from onion seeds. *In vitro* products based on iprodione gave the best results resulting 97.4% control of the fungi with 81.4% germination against 54.8% germination of untreated seeds. Hossain (1999) observed a total of 10 fungi representing five genera viz, *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Rhizopus* sp., *Curvularia* sp. in onion seeds. Alam (2001) detected for species *Aspergillus, Penicillium* and *Rhizopus* in onion seeds by blotter method. In Bangladesh, Karim (2004) conducted experiments on the effect of harvesting, processing and storage on quality of onion seeds and identified the fungi associated with seeds. He found the 10 fungi in onion seeds as observed by Hossain (1999).

Dabire *et al*, (2016) reported that seventeen fungal species belonging to 11 fungal genera were identified in the onion seed samples: Aspergillus was detected in 17 samples, *Fusarium* sp. and *Rhizopus* sp. in 15 samples, *Cladosporium* sp. in 14 samples and *Penicillium sp.* in 13 samples. The infection rates by the fungal species varied from 0 to 90.3% for *A. niger* and from 0 to 13.5% for *F. oxysporum*. *Alternaria porri*, the causal agent of purple blotch disease was recorded on two seed samples at infection rates of 0.5 and 1%.

CHAPTER VI

SUMMARY AND CONCLUSION

The present research program was conducted to manage purple blotch complex of onion for seed production caused by *Alternaria porri* and *Stemphylium vesicarium*. The experiment was conducted in the farm of Sher-e-Bangla Agricultural University, Dhaka during the period from November'2021 to March, 2020 and testing of seed health status and prevalance of seed borne pathogens associated with the harvested onion seeds collected from the multiple treatment experimental plots were studied in August 2020 in the seed pathology laboratory, Sher-e-Bangla Agricultural University, Dhaka.

Onion variety Taherpuri was used in the experiment. The experiment was laid out in a RCBD (one factor) with three replications. There were eight treatments, Viz. T_0 = Control (No field spraying); T_1 = Field spraying with Alamanda leaf extract 1:2 (W/V); T_2 = Field spraying with Neem leaf extract 1:2 (W/V); T_3 = Field spraying with Score 250 EC @ 0.2%; T_4 = Field spraying with Rovral 50 WP @ 0.2%; T_5 = Field spraying with Dithane M-45 @ 0.45%; T_6 = Field spraying with Tricost 1 % WP- @ 0.3% (*Trichoderma harzianum*); T_7 = Field spraying with of Micronutrients ($ZnSO_4$ + Borax) .

The observation was made on the effect of the treatments on percent leaf infection, percent disease index (leaf), percent seed stalk infection, percent stalk area disease, yield contributing characters, seed yield of onion, percent seed germination, percent seed borne pathogenic infection.

The % leaf infection was observed four times at an interval of 15 days from 35 DAP. The lowest percent leaf infection (3.66 %) was found in case of foliar spray of Rovral 50 WP at the first observation (35 DAP) and the highest leaf infection (30.5 %) was found in control treatment. For each treatment % leaf infections were increased gradually. At the last observation at 80 DAP, the highest % leaf infection (49.5 %) was recorded in control treatment and the lowest (17.59 %)

was found in the foliar spray of Rovral 50 WP similar trend of results were found at 50 DAP, 65 DAP.

The lowest percent disease index (0.41 %) in leaf was observed in case of foliar spray of Rovral 50 WP which was followed by foliar spray of Score 25 EC (0.95 %), Dithane M-45 (1.24 %), Neem leaf extract (1.45 %), Tricost (*Trichoderma hargianum*) (2.13 %), while the highest PDI was observed under control (3.95 %) at 35 DAP. At 50 DAP and 65 DAP, the highest PDI was observed in control treatment preceded by micronutrients, Allamanda leaf extract. Finally the highest PDI (50.80 %) was found in control treatment at last observation 80 DAP and lowest PDI (3.64 %) was recorded in the foliar spray Rovral 50 WP, which was statistically same as foliar spray of Score 250 EC (7.86 %). The result showed that the PDI was increased with increasing age of onion. But in every observation the lowest PDI recorded in Rovral 50 WP treated plots and the highest PDI was found in control treatments.

The % seed stalk infection was observed three times at an interval of 15 days from 80 DAP. The lowest percent leaf infection (5.53 %) was found in case of foliar spray of Rovral 50 WP at the first observation (80 DAP) and the highest seed stalk infection (32.80 %) was found in control treatment. For each treatment % seed stalk infections were increased gradually. At the last observation at 110 DAP, the highest % seed infection (75.80 %) was recorded in control treatment and the lowest (16.21 %) was found in the foliar spray of Rovral 50 WP similar trend of results were found at 95 DAP.

The lowest percent seed stalk area disease (0.61 %) was observed in case of foliar spray of Rovral 50 WP which was followed by foliar spray of Score 25 EC (1.35 %), Dithane M-45 (1.37 %), Neem leaf extract (1.61 %), Tricost (*Trichoderma harzianum*) (1.79 %), while the highest SAD was observed under control (3.11 %) at 80 DAP. At 95 DAP, the highest SAD was observed in control treatment preceded by micronutrients, Allamanda leaf extract. Finally the highest SAD (22.05 %) was found in control treatment at last observation 110 DAP and lowest SAD (2.05 %) was recorded in the foliar spray Rovral 50 WP, which was

statistically same as foliar spray of Score 250 EC (3.58 %). The result showed that the SAD was increased with increasing age of onion. But in every observation the lowest SAD recorded in Rovral 50 WP treated plots and the highest SAD was found in control treatments.

The effects of treatments on stalk height, no. of umbel, thousand seed weight, seed yield was found positive and significant. The highest stalk height (76.03 cm) was found from the plot where foliar spraying was applied with Rovral 50 WP, which was statistically identical with Score 250 EC (74.85 cm). The highest number of ssed stalk per hill (2.33) was found from the plot of foliar spraying with Rovral 50 WP @ 0.2%. The highest number of umbel per plot (135.35) was also found from the plot of foliar spraying with Rovral 50 WP @ 0.2%. Similarly the highest weight of thousand seed (3.472 gm) was found from the plot where foliar spraying was applied with Rovral 50 WP, which was statistically identical with Score 250 EC (3.418 gm).

The highest seed yield (707.45 kg/ha) was obtained from the plot where Rovral 50 WP @ 0.2% was applied followed by Score 250 EC (675.50 kg/ha), which was statistically similar with Dithane M-45 (656.75 kg/ha).The lowest seed yield (320.75 kg/ha) was obtained from the plot of control (T_0).

The highest performance (93.5 %) in respect of seed germination was observed in Rovral 50 WP treated harvested onion seeds followed by Score 250 EC (90.8 %), Dithane M-45 (88.5 %), Neem leaf extract (85.5 %), Tricost (*Trichoderma harzianum*) (81.5 %). The lowest seed germination was recorded in control which was preceded by Micronutrients, Allamanda leaf extract.

The lowest number of seed borne pathogenic infections (34) were recorded on the harvested seed of T_4 (Rovral 50 WP) treated plot, which was 8.5 % of total pathogenic infection followed by Score 250 EC (48), Dithane M-45 (54), Neem leaf extract (74), Tricost (*Trichoderma harzianum*) (86), which was 12.0 %, 13.5 %, 18.5 %, 21.5 % of total seed borne pathogenic infection . The highest number of seed borne pathogenic infections (160) were recorded on the harvested seed of T_0 (control) plot, which was 40.0 % of total seed borne pathogenic infection

preceded by Micronutrients (106), Allamanda leaf extract (88), which was 26.5 %, 22.5 % of total seed borne pathogenic infection. Out of 10 species of seed borne pathogens were identified. *Aspergillus flavus, Aspergillus niger, Botrytis cinerea, Penicillium sp.* were predominant. The lowest seed infection by *Alternaria porri* and *Stemphylium vesicarium* on harvested onion seeds from the experimental plot respectively were 0.25 % and 0.75 % in case of Rovral 50 WP followed by Score 250 EC treated plot. It indicates that the purple blotch complex pathogens were potentially control by foliar spraying of Rovral 50 WP and Score 250 EC.

From the findings of the present investigation it may be conclude that Rovral 50 WP had promising effect in reducing the incidence and severity of purple blotch complex of onion increasing seed yield. Score 250 EC and Dithane M-45 also showed better performance in suppressing the disease. Neem leaf extract and Tricost (*Trichoderma harzianum*) also showed moderately good performance in suppressing the disease. Micronutrients and Allamanda leaf extract were not proved to be effective against the disease. Thus, onion seed grower may be suggested to apply Rovral 50WP @ 0.2% with seven days interval in controlling purple blotch complex of onion for increasing seed yield and quality seed production of onion. However the several treatments experiments need to be carried out in different Agro- Ecological Zones (AEZ) for at least 3 consecutive years to justify the findings of the present experiment.

CHAPTER VII

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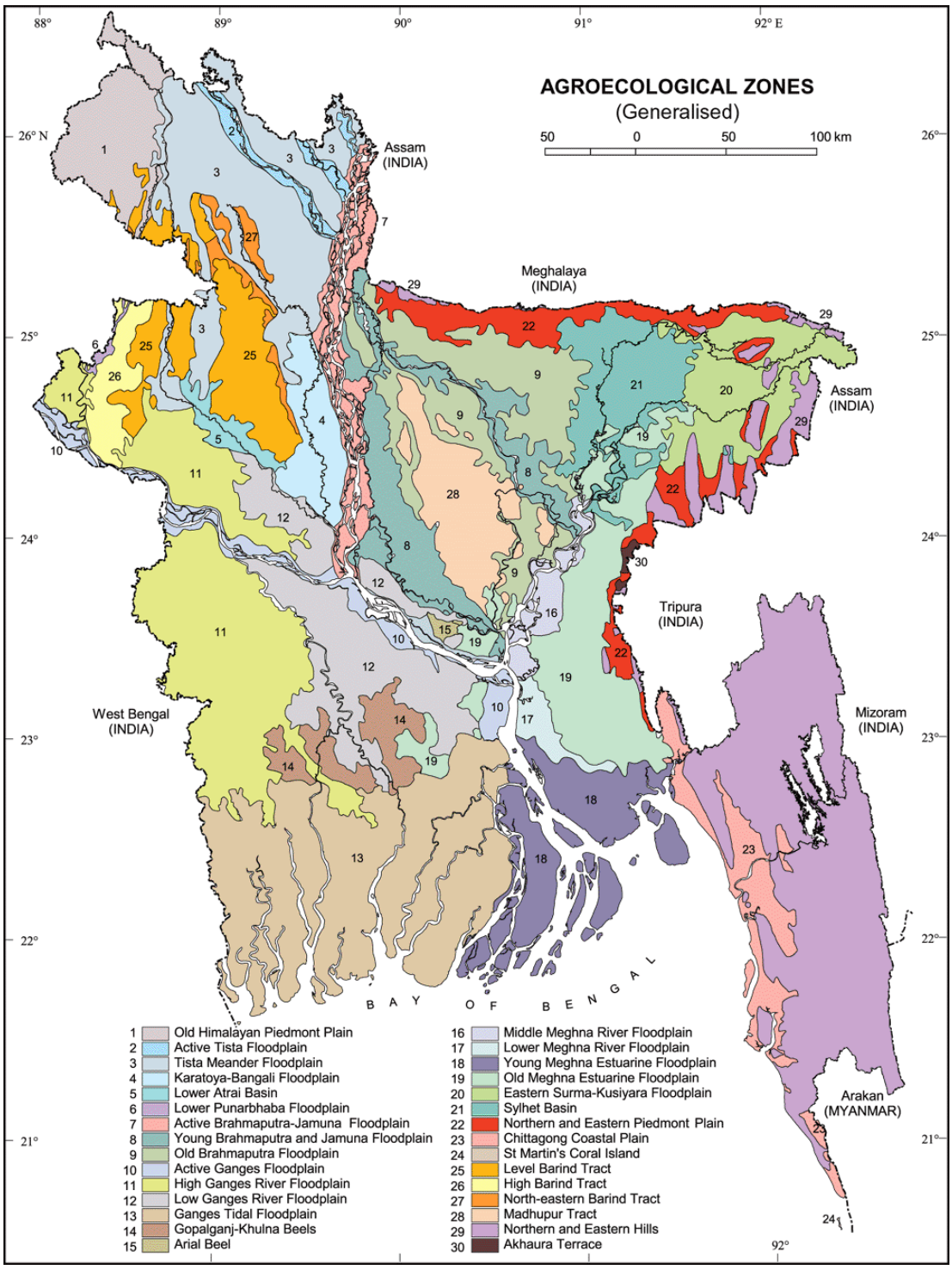
CHAPTER VIII

APPENDICES

Appendix I: Experimental location in the map of Agro-Ecological Zones of Bangladesh



Appendix-II: Particulars of the Agro-ecological Zone of the Experimental Site.

Agro-ecological region	: Madhupur Tract (AEZ-28)
Land Type	: Medium high land
General soil type	: Non- Calcareous Dark gray floodplain soil
Soil series	: Tejgaon
Topography	: Up land
Location	: SAU Farm, Dhaka
Field level	: Above flood level
Drainage	: Fairly good
Firmness (consistency)	: Compact to friable when dry.

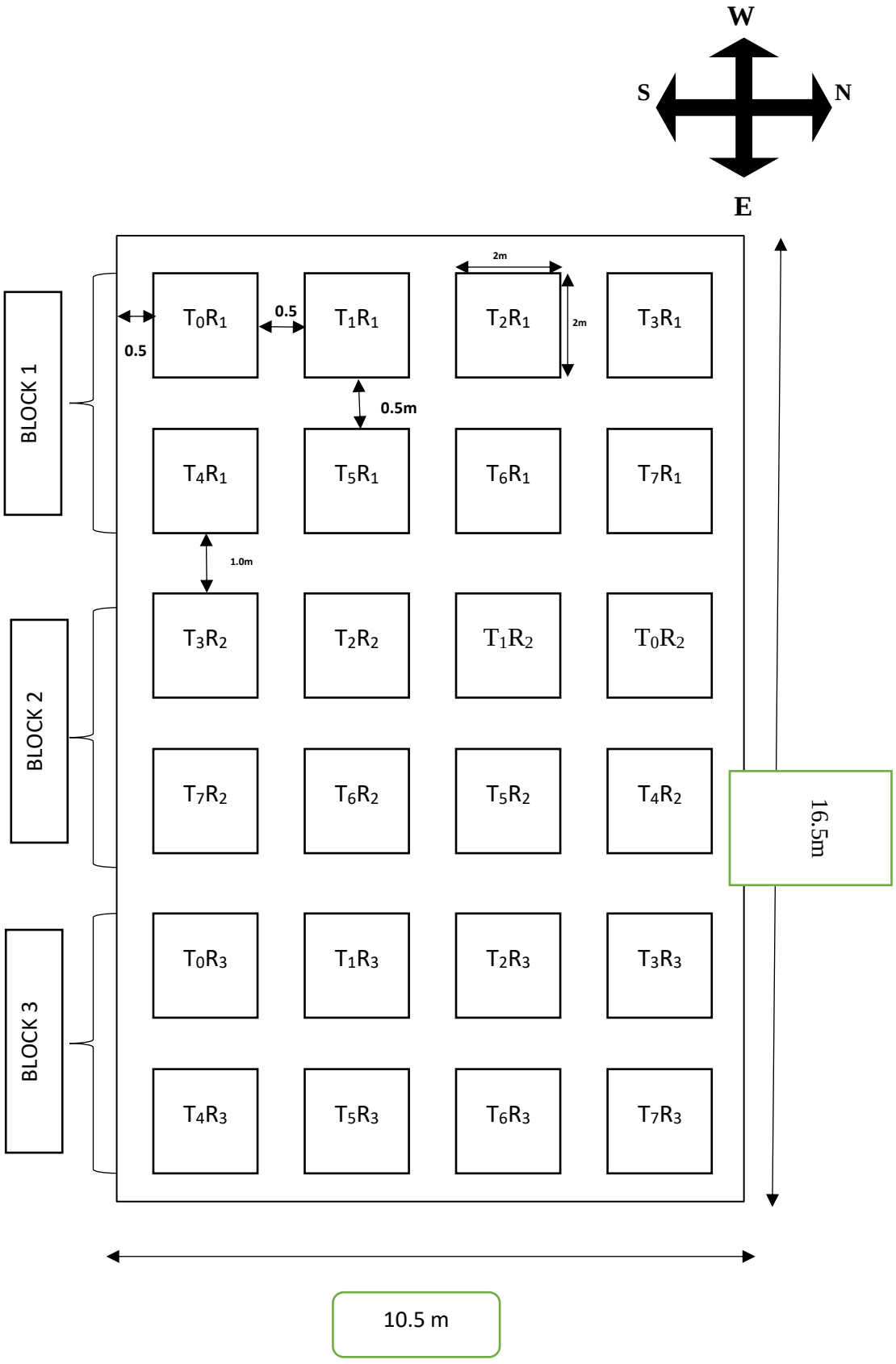
Appendix III: Monthly mean weather of the experimental site

Monthly mean of daily maximum, minimum and average temperature, relative humidity, total rainfall and sunshine hours during November/2019 to March/2020 are given bellow:

Year	Month	Air temperature(0c)			Relative Humidity (%)	Rainfall (mm)	Wind Speed (km/hr)
		Max.	Min.	Average			
2019	November	25.50	6.70	16.10	54.80	0.0	0.6
	December	23.80	11.70	17.75	46.20	0.0	1.3
2020	January	22.75	14.26	18.51	37.90	0.0	1.2
	February	27.20	21.00	24.10	52.44	20.4	1.4
	March	34.70	24.60	29.65	65.40	165.0	1.3

Source: Bangladesh Meteorological Department (Climate Division), Agargoan, Sher- e-Bangla Nagar, Dhaka-1207

Appendix IV: Layout of the field experiment: (RCBD)



Appendix-V: Some abbreviations and symbols used in the body of the Thesis

Abbreviations	Full word
%	Percent
@	At the rate of
AEZ	Agro-Ecological Zone
Agric.	Agriculture
Agril.	Agricultural
Agron.	Agronomy
ANOVA	Analysis of variance
BARI	Bangladesh Agricultural Research Institute
BBS	Bangladesh Bureau of Statistics
BD	Bangladesh
BSMRAU	Bangladesh Sheikh Mujibur Rahman Agricultural University
CEC	Cation Exchange Capacity
cm	Centi-meter
CV%	Percentage of coefficient of variation
DAI	Days After Incubation
DAP	Days After Planting
DF	Degrees of Freedom
SS	Sum of Squares
MS	Mean square
EC	Emulsifiable concentration
P	Probability
et al.	and others
etc.	Etcetera
FAO	Food and Agricultural Organization
g	Gram
hr.	Hours
Kg/ha	kiligrams per hectare
kg	Kilogram
m	Meter
m2	Square meter
MOA	Ministry of Agriculture
MSE	Mean square of the error
No.	Number
NUV	Near Ultra Violet
PDI	Percent disease index
PDA	Potato dextrose agar
SAD	StalkArea Diseased
ppm	Parts per million

RCBD	Randomized complete block design
Rep.	Replication
Res.	Research
SAU	Sher-e-Bangla Agricultural University
Sc.	Science
SE	Standard Error
Univ.	University
var.	Variety
WP	Wetable Powder
J.	Journal

Appendix-VI: ANOVA table of the experiment

Table .01. Effect of treatments on Percent leaf infection at different DAP

Source of variance	35 DAP					50 DAP					65 DAP					80 DAP				
	DF	SS	MS	F	P	DF	SS	MS	F	P	DF	SS	MS	F	P	DF	SS	MS	F	P
Replication	2	5.60	2.80			2	42.25	21.125			2	0.36	0.178			2	70.42	35.212		
Treatment	7	1633.69	233.385	20.77	0	7	2137.98	305.426	29.17	0	7	2109.35	301.336	45.20	0	7	2153.49	307.641	18.46	0
Error	14	157.28	11.234			14	146.59	10.471			14	93.33	6.666			14	233.30	16.664		
Total	23	1796.57				23	2326.82				23	2203.04				23	2457.21			
CV %	19.78					14.58					10.61					12.25				

Table .02. Effect of treatments on Percent disease index at different DAP

Source of variance	35 DAP					50 DAP					65 DAP					80 DAP				
	DF	SS	MS	F	P	DF	SS	MS	F	P	DF	SS	MS	F	P	DF	SS	MS	F	P
Replication	2	5.2913	2.64566			2	12.041	6.0203			2	202.03	101.017			2	446.27	223.133		
Treatment	7	29.8743	4.26775	26.52	0	7	186.667	26.6667	43.92	0	7	2903.83	414.833	41.11	0	7	4937.14	705.306	73.02	0
Error	14	2.2533	0.16095			14	8.499	0.6071			14	141.29	10.092			14	135.23	9.660		
Total	23	37.4189				23	207.207				23	3247.15				23	5518.64			
CV %	20.46					21.68					22.40					14.14				

Table .03. Effect of treatments on Percent stalk infection at different DAP

Source of variance	80 DAP					95 DAP					110 DAP				
	DF	SS	MS	F	P	DF	SS	MS	F	P	DF	SS	MS	F	P
Replication	2	204.20	102.102			2	137.36	68.679			2	689.59	344.79		
Treatment	7	1712.51	244.644	139.82	0	7	2459.93	351.418	23.05	0	7	7500.11	1071.44	129.57	0
Error	14	24.50	1.750			14	213.42	15.244			14	115.77	8.27		
Total	23	1941.21				23	2810.71				23	8305.47			
CV %	7.66					14.46					8.32				

Table .04. Effect of treatments on Percent stalk area disease at different DAP

Source of variance	80 DAP					95 DAP					110 DAP				
	DF	SS	MS	F	P	DF	SS	MS	F	P	DF	SS	MS	F	P
Replication	2	2.8003	1.40015			2	38.069	19.0345			2	58.83	29.414		
Treatment	7	12.1295	1.73279	16.42	0	7	351.318	50.1884	55.09	0	7	1085.84	155.120	73.68	0
Error	14	1.4774	0.10553			14	12.756	0.9111			14	29.48	2.105		
Total	23	16.4072				23	402.143				23	1174.14			
CV %	18.13					16.75					14.23				

Table. 5. Effect of treatments on stalk height

Source of variance	Degree of freedom	Sum of square	Mean square	F value	Probability
Replication	2	44.556	22.2778		
Treatment	7	380.506	54.3580	58.76	0.0000
Error	14	12.950	0.9250		
Total	23	438.012			
CV %	1.34				

Table. 6: Effect of treatments on number of seed stalk /hill

Source of variance	Degree of freedom	Sum of square	Mean square	F value	Probability
Replication	2	0.59290	0.29645		
Treatment	7	1.08645	0.15521	25.78	0.0000
Error	14	0.08430	0.00602		
Total	23	1.76365			
CV %	4.04				

Table. 7: Effect of treatments on number of umbel / plot

Source of variance	Degree of freedom	Sum of square	Mean square	F value	Probability
Replication	2	457.10	228.552		
Treatment	7	1913.37	273.338	39.23	0.0000
Error	14	97.55	6.968		
Total	23	2468.02			
CV %	2.15				

Table. 8: Effect of treatments on weight of thousand seed (gm)

Source of variance	Degree of freedom	Sum of square	Mean square	F value	Probability
Replication	2	0.18233	0.09116		
Treatment	7	0.70180	0.10026	40.97	0.0000
Error	14	0.03425	0.00245		
Total	23	0.91838			
CV %	1.52				

Table. 9: Effect of treatments on Seed yield (kg/ha)

Source of variance	Degree of freedom	Sum of square	Mean square	F value	Probability
Replication	2	20053	10026.7		
Treatment	7	377919	53988.4	315.45	0.0000
Error	14	2396	171.1		
Total	23	400368			
CV %	2.37				