ECO-FRIENDLY APPROACHES FOR THE MANAGEMENT OF FRUIT ROT OF EGGPLANT CAUSED BY *Phomopsis vexans* FOR SEED PRODUCTION

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This is to certify that the thesis entitled, **"ECO-FRIENDLY APPROACHES FOR THE MANAGEMENT OF FRUIT ROT OF EGGPLANT CAUSED BY *Phomopsis vexans* 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 PATHOLGY** *embodies the results of a piece of bona fide research work carried out by* **ASIF NOOR** *bearing Registration No.* **13-05518** *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 source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated: 15 September, 2020

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LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATION/SYMBOL	ELABORATION
%	Percentage
°C	Degree Centigrade
AEZ	Agro-Ecological Zone
BARI	Bangladesh Agricultural Research Institute
CFU	Colony Forming Unit
cm	Centimeter
CMI	Commonwealth Mycological Institute
CRD	Completely Randomized Design
CV	Coefficient of Variance
DAI	Days After Inoculation
DAP	Days After Planting
EC	Emulsifiable Concentrate
Ed.	Edited
Eds.	Edition
et al.	And others
etc.	Et cetera (and so forth)
FAD	Fruit Area Diseased
g	Gram
ha^{-1}	Per hectare
ISTA	International Seed Testing Association
Kg	Kilogram
LSD	Least Significant Difference
ml	Milliliter
mm	Millimeter
MP	Muriate of Potash
MT	Metric Ton
PDA	Potato Dextrose Agar
PDI	Percent Disease Index
psi	Pound per square inch
RCBD	Randomize Complete Block Design
SAU	Sher-e-Bangla Agricultural University
t	Ton
T	Treatment
TSP	Triple Super Phosphate
v	Volume
viz.	Videlicet (namely)
w	Weight
WDG	Water Dispersible Granule

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The Author

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

ECO-FRIENDLY APPROACHES FOR THE MANAGEMENT OF FRUIT ROT OF EGGPLANT CAUSED BY *Phomopsis vexans* FOR SEED PRODUCTION

ABSTRACT

Phomopsis fruit rot is a major constraint of quality seed production of eggplant in Bangladesh. The *in vitro* experiments were conducted in the M.S. Laboratory of the Department of Plant Pathology and the field experiment was conducted in the Central Farm, Sher-e-Bangla Agricultural University, Dhaka-1207 during October 2018 to May 2019. A set of experimentation strategically designed to select the highest disease mitigating treatments for employing in ecofriendly management approaches against *Phomopsis vexans* for seed production of eggplant. The isolated organism causing fruit rot of eggplant was identified as *Phomopsis vexans* based on its key characteristics (CMI Description no. 338). For the management of the disease, a bioagent (*Trichoderma harzianum)*, three fungicides viz. Companion, Autostin 50WDG and Tilt 250EC, four plant extracts viz. Allamanda leaf extract, Garlic clove extract, Turmeric rhizome extract and Onion bulb extract, and Krishibid Organic Fertilizer were evaluated in the experiment. The foliar spray of *Trichoderma harzianum* ("Biotech Care Trichoderma Suspension") was found most effective in controlling the incidence and severity of fruit rot caused by *Phomopsis vexans*, increasing the fruit yield by 142.05% and seed yield by 146.84% compared to control. Seed germination of harvested seeds increased by 88.41% over control. Soil amendment with Krishibid Organic Fertilizer combined with Autostin 50WDG spraying was also found effective in increasing the fruit yield by 137.39%, seed yield by 142.54% and seed germination by 93.56% compared to control. Among the plant extracts, Garlic bulb extract and Turmeric rhizome extract were found most effective in increasing the fruit yield by 112.16%, 96.93%; seed yield by 112.15%, 100.05%, respectively over control.

CHAPTER I

INTRODUCTION

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Eggplant (*Solanum melongena* L.), also known as Brinjal or Aubergine is an important solanaceous crop of tropics and sub-tropics in the world. The eggplant is of much importance in the warm areas of Far East, being grown extensively in Bangladesh, India, Pakistan, China and the Philippines. Asia possesses the largest eggplant production which comprises 87% of the world production and more than 90% of the world production area (Chowdhary and Gaur, 2009). The crop is ranked 4th as vegetables in the world having global production of 51.3 million tons in about 1.8-million-hectare area of land in the world in 2016 **(**FAO 2018). In Bangladesh, it is one of the most common, popular and principal vegetable crops grown throughout the country. Its position in terms of production is 1st in winter vegetable crops in Bangladesh (BBS, 2017). The total cultivable area of eggplant is 78,458 acres with total annual production of 3,40,150 metric tons (BBS, 2017).

In Bangladesh, eggplant is being vastly cultivated in most of the districts and consumed as a cooked vegetable in various ways. Eggplant (*Solanum melongena* L.) has multifarious use as a dish item in Bangladesh (Rashid, 1976). It is nutritious, being low in calories, fat, sodium and is a nonstarchy fruit that is cooked as a vegetable. It is described as the 'King of vegetables' due to its versatility uses as food (Choudhary and Gaur, 2009). Eggplant is also known to have ayurvedic medicinal properties and is good for diabetic patients. It has also been recommended as an excellent remedy for those suffering from liver complaints (Shukla and Naik, 1993).

Eggplant can be grown throughout the year as perennial crop in the tropical climate but in sub-tropics, it is grown as summer vegetables. It is a small perennial herb. The fruit is pendent and is fleshy berry borne singly or in clusters. The shape of fruit varies from ovoid, oblong, obovoid, or long cylindrical. The

seeds are borne on the fleshy placenta and the placentae with the seeds completely fill the locular cavity.

This economically important vegetable crop can be cultivated year-round especially during the lean period when there is scarcity of seasonal vegetables. In comparison to other vegetables, the market price of eggplant remains high throughout the season which becomes even higher in Ramadan. Thus, it provides continuous source of income which brings solvency to the eggplant growers as well as contributing to our national economy.

The production and quality of eggplant is highly affected due to its susceptibility to a wide range of pests and pathogens. In our country, eggplant is known to suffer from 12 diseases. Among them Phomopsis fruit rot caused by *Phomopsis vexans* is regarded as one of major diseases of eggplant (Meah *et al*., 1998) which causes yield loss up 30-50% (Das, 1998). Fruit rot and leaf blight, caused by *Phomopsis vexans*, is a major disease reducing yield and market value of crop by 20-30% (Beura *et al*., 2008; Pandey, 2010). Phomopsis blight ranks second, next to bacterial wilt of eggplant and varies in severity depending on area, soil type and weather (Meah *et al.*, 2003).

Harter (1914) first reported the occurrence of disease from India. In Bangladesh, the disease was first reported by Fakir (1983). The pathogen may infect all above ground parts of eggplant in all stages of its growth and development. When the fruits are infected, circular spots with concentric rings are formed and gradually fruits become mummified and rotten (Meah *et al.*, 2003). The fruits may get infected at all stages of their development and are rendered unfit for human consumption. This disease causes serious loss to brinjal production in terms of fruit yield by affecting seed germination, seedling mortality, killing the plants, rotting of fruits and spoiling the fruit quality (Beura, 2008).

The causal organism of the disease *Phomopsis vexans*, is believed to originate from South Asia (Prance and Nesbitt 2005) and also reported to infect some wild *Solanum* species (Datar and Ashtaputre, 1988). It is readily transmitted through seeds internally as well as externally (Porter, 1943; Vishunavat and Kumar,

1993). Seed is the infection source of *P. vexans* and may serve as a substrate for pathogen survival (Pan *et al*., 1995). The pathogen remains on the seed coat and the cotyledons of eggplant seeds causing various degrees of seed discoloration and minute black pycnidial bodies which is distinctly observed on the surface of the dry seed (Karuna *et al*., 1994). *Phomopsis vexans* is both externally and internally seed borne and remains viable for about 14 months in soil with plant debris and in the seed from infected fruits (Sekara *et al.*, 2007). *Phomopsis vexans* is a pycnidial fungus with an apparent sexual form in the genus *Diaporthe*, easily seedborne and producing large numbers of conidia. It causes disease in *Solanum melongena*, its only significant host, causing poor seed germination and damping off of seedlings, leaf and stem lesions and fruit rot, both in the field and after harvest (Chalkley, 2010).

Seed is the most important input for crop production. Quality and healthy seed is crucial for the sustainable crop production. Healthy or pathogen free seeds are regarded as the vital factor for optimum plant population as well as good harvest. Seed health can be affected by direct infection by pathogens or through contamination of seeds by pathogenic proposals as contaminants in, on or with the seeds or as concomitant contamination (Rashid *et al*., 2000). Infection of seed by the pathogens and the presence of propagules of pathogens in a seed lot is vitally important because infected seeds/seed lot may fail to germinate, cause infection to seedlings and growing plants. So, healthy seed is considered as an important factor for successful crop production. The lack of quality seeds and the prevalence of seed borne organisms are the main constraints in maintaining the crop production. Fakir (1998) estimated more than 400 seed borne diseases in 72 crops inflicting an estimated yield loss amounting to around Tk. 1000 million, i.e. 200 million US dollars annually.

Seeds of vegetables are more vulnerable to attack by pathogens and quickly deteriorate during storage. The germination potentiality of seeds cannot be assessed easily just from their external appearances. For a good crop, good seed

germination is essential which indicates that the seed should be pure, viable and healthy.

Seed production of eggplant is difficult due to the long duration of fruit ripening. For seed production, the plants are kept in the field for much longer period for proper maturity. During this extended period, the fruits become more susceptible to pathogenic attack and thus seed production is severely hampered (Islam, 2005).

To control this disease for commercial production, a number of synthetic pesticides are being used indiscriminately at large scale. In 2017, uses of pesticides in Bangladesh were 37258.00 MT, of which fungicides were 16800.00 MT (Faruq, 2018). This causes serious environment pollution and human health hazards. To control such fungal disease, many chemical fungicides particularly the carbendazim (0.2 %) are commonly used in brinjal (Habib *et al*., 2007). However, indiscriminate use of chemical pesticides in a long run lead to residual toxicity, induced resistance to pathogen, environmental pollution and side effects on human and animal health (Avinash and Hosmani, 2012).

Being a vegetable crop, exclusive dependence of chemical methods of management of diseases is not always advisable (Campbell, 1989). Improper use of pesticides has led to residue accumulation on eggplants (Chowdhury *et al.*, 2013). Consequently, importing countries have imposed restrictions on vegetables export especially eggplants from Bangladesh (Rahman, 2016).

The negative impact of excessive and indiscriminate use of harmful pesticides has brought changes in attitude towards uses of pesticides in agriculture. Efforts have been made to develop alternate approaches to synthetic chemicals for the management of plant diseases. Many plant extracts and bioagents are reported to be antimicrobial against plant pathogenic fungi (Harman, 1989; Cook, 1993; Lawson *et al*., 1998; Bowers *et al.*, 2000; Hassan, 2000; Hossain, 2004). Plants contain thousands of constituents and are valuable sources of new and biologically active molecules possessing antimicrobial property. Many plants and plant products have been reported to have antimicrobial properties against

plant pathogenic fungi (Shetty *et al.*, 1984; Grayer and Harborme, 1994; Lawson and Kennedy 1998; Bowers and Locke, 2000).

Biological control of pathogens is an environmentally sound method and avoids other threats posed by the use of chemicals such as health hazards and development of resistance in pathogens (Kumar *et al*., 2017). The antagonistic activity of *Trichoderma* sp. was demonstrated more than eight decades ago (Weindling, 1934) and today it is the most extensively used biocontrol agent (Mukherjee *et al.*, 2012).

Proper management of the pathogen using ecofriendly components can substantially improve the quality of eggplant and significant increase of fruit yield. In this context, the present investigation was undertaken to screen out and evaluate the potential management options which will be effective against *Phomopsis vexans* to develop a compatible, effective, economic and environmental friendly practice for the management of this disease.

Objectives

1. To isolate and identify the causal organism of Phomopsis fruit rot of eggplant; and
2. To evaluate the selected ecofriendly components for the management of Phomopsis fruit rot of eggplant for seed production.

CHAPTER II

REVIEW OF LITERATURE

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REVIEW OF LITERATURE

Fruit rot of eggplant caused by *Phomopsis vexans* has been treated as one of the major constraints to eggplant cultivation. The pathogen affects all the aerial parts of the plant especially the fruits which makes them inedible as well as limiting its potential yield.

2.1 History and Distribution

The origin of Eggplant is considered to be India. Vavilov (1928) mentioned its center of origin as in the Indo-Burma region. Although originated in India, it has a secondary center of variation in China. In China, eggplant has been known for the last 1500 years (Chen and Li, 2009).

Phomopsis vexans has been reported from many areas in the warmer parts of most continents (Smith *et al.*, 1988). The area of origin of the host *Solanum melongena* is southern Asia (Prance and Nesbitt, 2005) where it is also reported to infect some wild *Solanum* species (Datar and Ashtaputre, 1988).

Spegazzini (1881) described a fungus occurring on leaves of *Solanum melongena* in Italy as *Phyllosticta hortorum*. Halsted (1892) reported the same fungus on leaves and fruits of eggplants in New Jersey, USA, as *Phoma solani*. In 1905, Smith observed septate conidia in the USA and proposed the name *Ascochyta hortorum* instead of *P. hortorum*. Harter (1914) studied morphological features and reported that *Phoma solani* and *Phyllosticta hortorum* were the same species. He also concluded that the genus to which the fungus belonged was *Phomopsis* instead of *Phoma*, *Phyllosticta* or *Ascochyta*. Harter observed and described the beta conidia (stylospores) as characteristic of the genus. He proposed the name *Phomopsis vexans* for the fungus.

In India, fruit rot of brinjal caused by *Phomopsis vexans* was first reported in Gujarat by Harter (1914). A description of *Phomopsis vexans (Diaporthe vexans),* its transmission, geographical distribution and host on *Solanum*

melongena L. *was* given by Punithalingam and Holliday (1972). In Bangladesh, the occurrence of the disease was first reported by Fakir (1983).

Chalkley (2010) reported that the fungus has been widely distributed in areas of most continents, but only a few of those are in Europe and Africa.

Seed transmission may explain the broad historical distribution of *Phomopsis vexans* but limitation of its host range to a non-staple vegetable crop can allow for its avoidance and eradication by cultural methods. As a result, it does not appear often on lists of restricted pathogens, even though it may cause yield losses of more than 50 % (Chalkley, 2010).

2.2 Symptomatology

Phomopsis blight and fruit rot caused by the fungus *Phomopsis vexans* is a serious disease which can infect all above ground parts of the plant (Thompson, 1957). Its infestation as seedling blight, leaf spot and fruit rot has been reported by Rangaswamy (1979). The most destructive phase of the disease is the fruit rot (Singh, 1992)

The symptoms of Phomopsis fruit rot was first described by Harter (1914). Initially, the symptoms appear as minute sunken greyish spots with a brownish halo. Later, they enlarge and coalesce to produce concentric rings of yellow and brown zones. These spots gradually increase in size covering most of the rotten fruit surface on which conidiomata develop concentrically. Pycnidia develop readily in lesions on young stems but rarely on older ones. Pycnidia on fruits are larger than those on the other plant parts. Fruit rot due to *Phomopsis* is more likely to begin at the top from infection of the calyx (Edgerton and Moreland, 1921).

Walker (1952) reported the blight of young seedling as the first phase of phomopsis blight. The stem girdled slightly above the soil surface. The seedling topple over and die. Dark brown lesion is formed on the stem which turns gray in the center as the pycnidia develops. On the leaves, round or oval brown spots

develop and becomes irregular in shape as they enlarge up to 3cm in diameter. The center containing pycnidia becomes gray. Infected fruits on the plants turns soft and watery at first but later as the pycnidia develops abundantly over the surface, the fruit becomes black and mummified.

Pawar and Patel (1957) stated the symptoms range from poor germination and seedling blight to fruit rot. Post emergence damping off of seedlings results from infection of the stem just above the soil surface. The symptoms on leaves are more prominent during the early stages of plant growth. The lesions first are small more or less circular and buff to olive later becoming cinnamon buff with an irregular blackish margin. Affected leaves drop prematurely and the blighted areas become covered with numerous black pycnidia. The whole fruit may become mummified if the infection enters the fruits through calyx.

Punithalingam and Holliday (1972) described the symptoms as irregular conspicuous leaf spots which later coalesce. Lower leaves may be affected first. Plants become stunted and girdling cankers cause death. Spots on fruits are pale, sunken, conspicuous and may affect the whole fruit; fruit may drop or remain attached, becoming mummified after a soft decay. Pycnidia are abundant.

According to Ashrafuzzaman (1986), damping off takes place at seedling stage due to this disease. Leaf may be attacked at any time. Usually, the first symptoms appear on the lower leaves. Spots are clear, circular and grayish. On aged spots, numerous pycnidia are formed. Cankers observed at the base of the stem, bark is cracked and woody portion opened. Light colored, sunken spots are observed on fruit. Black pycnidia are formed in spots and the fruits become mummified.

Kumar *et al.* (1986) reported that fruit rot of eggplant caused by *Phomopsis vexans* appeared as minute, circular, water soaked, sunken, grayish spots with brownish halo which later enlarged to produce concentric rings and brownish zones. Spots increased in size becoming larger rotten areas on which pycnidia developed.

Singh (1992) stated that the disease is present in one or other form of seedling stage of the plants to its maturity. It appears as damping off in seed bed. After transplanting, the leaves coming in contact with the soil may get infected and show clearly defined circular, gray to brown spots with light colored center. The old spot show numerous black pycnidia.

Fruit spots are similar to those on leaves but are much larger leaving diseased fruit unmarketable. Symptoms first appear as pale sunken circular to oval areas on the fruit surface. These later turn brown and enlarge up to two to three inches in diameter, often two or more lesions merge to cover much of the fruit surface. Affected fruits become soft and watery at first. Decay may penetrate rapidly throughout the fruit causing a light brown discoloration of the flesh. Under dry conditions fruit shrivel and become mummified. Black pycnidia arranged in a concentric, target like pattern can usually be seen in the center of fruit lesions (Kemble *et al.*, 1998).

Generally, spots first appear on seedlings shortly after they emerge. Leaf spots can occur at any time during the season though older leaves are most susceptible. Spots are circular with a distinct narrow brown margin about one inch in diameter. Lesions are typically gray to brown developing a light-colored center as they age. Black, pimple like structures develop in the center of old lesions; these are pycnidia which are the fungal reproductive structures where spores are produced. Diseased leaves may turn yellow and drop prematurely. Lesions can also form on stems and branches (Islam, 2005).

Pernezny *et al.* (2009) stated that round or oval spots develop on the leaf and stems on older plant. Infected fruit exhibit spots which appear as pale sunken areas which may cover the entire fruit if not treated. Abundant pycnidia are present in the fruit spots.

Schwartz *et al.* (2009) mentioned that fruit become shriveled, dry and form black mummies if conditions become dry.

2.3 The Pathogen

2.3.1 Morphology of *Phomopsis vexans*

Edgerton and Moreland (1921) described the hyphae of *Phomopsis vexans* as hyaline, septate and 2.5-4 µm in diameter. Pycnidia are subepidermal, erumpent, dark, thick-walled and flattened to globose shaped, varying in size, often 100-300 µm in diameter with or without beak. Phialides are hyaline, simple or branched, sometimes septate, 10-16 µm long and arising from the innermost layer of cells lining the cavity. Alpha conidia are hyaline, aseptate, sub-cylindrical and 5-8 × 2-3 µm in size. Beta conidia are filiform, curved, hyaline, septate, 18-32 × 0.5-2.0 µm in size and non-germinating.

According to Grazt (1942), perithecia of *Phomopsis vexans* culture are usually in clusters, 130-350 µm diameter and beaked. Beaks are sinuous, carbonaceous, irregular and 80-500 µm long. Asci are clavate, sessile, 24-44 × 5-12 µm, eight-spored. Ascospores are biseriate, hyaline, narrowly ellipsoid to bluntly fusoid, one-septate, constricted at the septum and 9-12× 3-4.5 µm in size.

Pycnidia of *Phomopsis vexans* on stems, leaves and fruits are solitary or gregarious, initially immersed, black, globose to irregular, up to 350µm wide having opening by 20-50 wide ostioles. Conidiophores (phialides) are hyaline, simple or branched, sometimes septate,10-16µm long, arising from the innermost layers of cells lining the cavity. Conidia are fusoid to ellipsoidal, hyaline, filiform, curved, rarely erect and 20-30 × 0.5-1µm in size (Punithalingum and Holliday, 1972).

Sugha *et al.* (2002) reported that alpha and beta are two forms of same conidium. *Phomopsis vexans* produces only one type of conidia in its Pycnidia, which are hyaline, one celled, sub-cylindrical and 5-9 × 2-2.8 µ in size during summer months, which gradually changed into the beta form. *Phomopsis vexans* produces alpha conidia at 25°C and beta conidia at 16°C.

Anwar *et al.* (2017) studied the morphological characteristics of Phomopsis vexans and reported that the pathogen produced white circular colony *in vitro* with profuse mycelium, which later turned to grayish white with compact mycelium. Pycnidia (177.5×152.0 μm) appear in concentric rings having hyaline, guttulated, elliptical, subcylindrical, one celled alpha conidia (6.2×2.4μm) and hyaline, filliform, curved beta conidia (15.7×1.3 μm).

2.3.2 Physiological aspects of *Phomopsis vexans*

A temeperature ranged between 15-30°C is suitable for the growth of pathogen. The best growth was found at 25°C (Harda *et al*., 1973; Ahmad, 1987). Divinagracia (1969) found 25°C as the best temperature to obtain maximum mycelial growth of *Phomopsis vexans*. Mycelial growth was inhibited at 35°C.

Islam *et al.* (2009) investigated the effect of temperature, light and pH on the mycelial growth and sporulation behavior of *Phomopsis vexans* and reported that the pathogen grew best at 25°C, under 12/12h cycle light and pH 5.5 of culture was optimum for the growth and sporulation of the pathogen.

Jakatimath *et al.* (2017) tested nine different media to find out the suitable media for the growth and sporulation of the pathogen. The study revealed the Potato Dextrose Agar (PDA), oat meal agar and Sabouraud's agar were suitable for the mycelial growth and sporulation of *Phomopsis vexans.*

2.4 Yield loss due to Phomopsis fruit rot

Under normal weather conditions, fruit rot caused by *Phomopsis vexans* lead 12-25 % yield loss due to flower drop and fruit rot (Kannan *et al.,* 1998). It reduces yield and marketable value of the crop from 20-50 % (Panwar *et al.,* 1970; Jain and Bhatnagar, 1980; Kaur *et al.* 1985; Khan, 1999; Thippeswamy *et al.*, 2005; Akhtar *et al.,* 2008; Beura *et al.,* 2008; Pandey, 2010; Jayaramaiah *et al.,* 2013). Das (1998) reported that seedling rot and fruit rot caused by *Phomopsis vexans* can lead to yield losses of 15-50%.

Rangaswamy and Mahadevan (2002) estimated loss of fruit yield ranging from 67.2-100.0 % especially when the crop infected at younger stage.

Such a huge loss in fruit yield due to *Phomopsis* fruit rot was attributed to decreased fruit number (34.8 %) and fruit weight (17.0 %) as reported by Kidasha (2010) and due to poor seed germination and plant stand due to *Phomopsis* infection (Thippeswamy *et al.*, 2005). Loss in fruit yield was as high as 70 % due to *Phomopsis vexans* (Cristina, 2002).

2.5 The incidence and severity of Phomopsis fruit rot

The disease incidence of phomopsis fruit rot was up to 100 % and disease severity was 9.8 % in Dohazari cultivar. Due to phomopsis blight and fruit rot of eggplant, a loss equivalent to Taka 808 million (US$ 134 million) per annum was also recorded by Meah *et al.* (2002).

Islam (2005) conducted an epidemiological survey on the incidence and severity of phomopsis blight and fruit rot of eggplant in 18 major growing areas of Bangladesh and reported that the prevalence was higher in the southern part of Bangladesh with moderate weather than the other part of Bangladesh. Sandy loam soil with moisture in the range of 50-60%, moderate air temperature (20-28°C) with high atmospheric moisture (65-75% RH) significantly influenced the prevalence of the disease.

2.6 Pathogenicity

Lou *et al*. (2006) reported that a spore suspension of 10^6 spores/ml of *Phomopsis vexans* collected from PDA cultures was used to spray inoculate potted *Ilex crenata* Thunb. var. *convexa* plants that were kept for 48 h under a polyethylene sheet cover and grown at 22-25°C in a greenhouse. After 5 to 10 days when inoculated trees showed symptoms resembling those seen in nature. The same fungus used as inoculum was re-isolated from the margins of necrotic tissues of inoculated plants but not from controls sprayed with sterile water.

Selfed seedlings of nine field tolerant lines along with two field susceptible lines were grown in plastic trays and inoculated with a spore concentration of $5x10^5$ spores/ml after 21 days and at seven days thereafter and proved Koch's postulate (Hazra *et al*., 2006).

2.7 Seed borne nature of the pathogen and its impact

Phomopsis vexans is a seed borne fungi at significant levels (Edgerton and Moreland, 1921; Toole *et al*., 1941). It is readily transmitted in and on the seed (Porter, 1943; Vishunavat and Kumar, 1993).

Seed infection adversely affects the seed quality, causing seed discoloration, reduced seed weight and density, poor germinability and reduced viability (Toole *et al*., 1941; Porter, 1943). Panwar (1957) stated that *Phomopsis vexans*, the causal organism of fruit rot of brinjal may remain viable for about 14 months in soil debris and in the seeds from infected fruits which causes poor germination.

Seed quality is adversely affected in an advanced stage of the disease and infected seed becomes discolored with poor germinability and reduced seed viability (Toole *et al.*, 1941; Porter, 1943; Vishunavat and Kumar, 1993). Pycnidia are produced in the seed coat, between the seed coat and endosperm, and in the endosperm tissue (Vishunavat and Kumar, 1994). Pre emergence and post emergence damping off of seedlings occurred due to seed infection and approximately one third of the plants were lost at each stage (Kaushel and Kumar, 1995).

Pan *et al*. (1995) studied the seed borne nature of *Phomopsis vexans* and reported that the pathogen was present in seed coat and on the cotyledons of the seeds.

Khan (1999) analyzed 22 seed samples collected from 8 growing areas of Bangladesh and reported that the infection of seeds by *Phomopsis vexans* ranged from 0.5 to 7 %.

2.8 Effect of Chemicals against *Phomopsis vexans*

Mohanty *et al.* (1994) studied the efficacy of several fungicide against the pathogen and carbendazim showed the best result which completely inhibited the cultural growth of *Phomopsis vexans*.

Meah *et al.* (1998) reported Bavistin as the most effective fungicide for the control of fruit rot of eggplant caused by *Phomopsis vexans.*

Jadeja (2003) found carbendazim (0.05%) effective in reducing disease incidence, dried branches and dried plants infected with *Phomopsis vexans* in brinjal. Increased yield of 19.4 q/ha was found with sprayings of 0.1% carbendazim.

Copper oxychloride (0.3 %), mancozeb (0.25 %), zineb (0.25 %), captan (0.25 %), thiophanate methyl (0.25 %), carbendazim (0.1 %) and tebuconazole (0.05 %) sprayed at 10 day intervals from disease initiation, were effective against *Phomopsis* blight with the greatest reduction in disease incidence using carbendazim (80.10 %) followed by tebuconazole (76.10 %) (Manna *et al.*, 2004).

Islam (2005) reported that Bavistin (50% carbendazim) even at 50 ppm concentration, completely inhibited mycelial growth of *Phomopsis vexans* in the laboratory and effectively controlled phomopsis blight and fruit rot of eggplant in the net house and in the field at 0.1% concentration.

Thippeswamy *et al*. (2006) evaluated the efficacy of carbendazim, carboxin, mancozeb, captaf and Dithane-Z78. Fungicides such as mancozeb, carbendazim and captaf were found superior for the inhibition of *Phomopsis vexans* and also increased seed germination in brinjal.

Akhtar (2007) investigated the efficacy of carboxin, carbendazim and mancozeb at 50, 100, 250, 500 and 1000 ppm against five isolates including Pv 10, Pv 11, Pv 16, Pv 25 and Pv 36. Complete growth inhibition of all the isolates was recorded at 1000 ppm of carboxin. Complete growth inhibition of isolates Pv 16,

Pv 25 and Pv 36 and some minor growth in isolates Pv 10 and Pv 11 were recorded at 500 ppm concentration. Complete inhibition at 1000, 500, 250 and 100 ppm carbendazim concentrations were recorded in all isolates except Pv 10 at 100 ppm.

Beura *et al.* (2008) reported carbendazim as the best control for *Phomopsis vexans* which also maximized the yield.

Sharma and Razdan (2012) reported that Carbendazim 12% + Mancozeb 63% @ 0.2 inhibited the mycelial growth completely.

Jakatimath *et al.* (2017) studied the efficacy of several fungicides against *Phomopsis vexans* and reported that carbendazim, tebuconazole and hexaconazole at 0.25, 0.5, 0.75 and 1.00 per cent concentration showed high inhibition of 100 per cent of mycelial growth of the pathogen. The least mycelial inhibition was observed in case of captaf (63.33%).

Ekka *et al.* (2017) reported that Saaf (Carbendazim 12% + Mancozeb 63% WP) completely inhibited the mycelial growth and sporulation at 0.25 and 0.20 percent.

2.9 Effect of Plant extracts against *Phomopsis vexans*

Mohanty *et al.* (1995) studied the allelopathic effect of five aqueous leaf extracts in controlling *Phomopsis vexans*, the causal agent of phomopsis fruit rot of brinjal. Fungal growth was inhibited to a maximum by leaf extracts of *Allamanda cathertica* (93.75%) followed by *Aegle marmelos* (85.38%). Leaf extracts of *Catheranus roseus*, *Polyalthia longifolia* and *Azadizachta indica* were equally effective, but that of *Octimum sanctum* was the least effective causing 52.23% growth inhibtion.

Khaleduzzaman (1996) investigated the effect of four plant extracts in controlling seed borne fungi. Garlic bulb extract was found best in reducing seed-borne prevalence and increasing germination percentage of seed followed by ginger, neem and bishkatali extracts.

Panda *et al.* (1996) studied the efficacy of leaf extracts from *Polyalthia longifolia*, *Aegle mermelos*, *Azadizachta indica*, *Catheramus roseus*, *Octimum sanctum* and *Allamanda cathertica* against *Phomopsis vexans*. Leaf extracts of *Allamanda cathertica* was found the most effective.

Khan (1999) tested the efficacy of leaf extract from Allamanda, Bael and Neem for the management of phomopsis blight and fruit rot of eggplant in field condition. Allamanda leaf extract was found most effective among the three plant extracts.

Meah (2003) found garlic bulb extract (1:1) and allamanda leaf extract most efficient in controlling *Phomopsis vexans* which reduced severity of leaf blight and fruit rot by 71-75% in the laboratory, nursery house and in the field.

Hawlader (2003) reported that eggplant seed treated with allamanda leaf extracts (1:1) showed higher germination rate and nursery diseases were also considerably decreased.

Jadeja (2003) reported that 10% extract of turmeric rhizome, onion bulb and garlic clove inhibited the growth of *Phomopsis vexans* causing stem and branch blight in eggplant. The inhibition of mycelial growth and pycnidia formation was highest in rhizome of turmeric followed by ginger extracts and garlic clove extracts.

Garlic bulb and allamanda leaves extract caused 76-100% inhibition of mycelial growth of *Phomopsis vexans* (Islam,2004).

Islam (2005) investigated the efficacy of 11 botanicals for the management of phomopsis blight and fruit rot of eggplant. Allamanda leaf extract and garlic bulb inhibited mycelial growth and spore germination of *Phomopsis vexans* in both laboratory test and in the field condition significantly.

Masuduzzaman *et al.* (2008) found that compounds extracted from *Allamanda cathartica* prevented growth of *Phomopsis vexans* in culture at specified concentrations.

Rahman *et al.* (2009) reported that plant extracts might be a substantial alternative in controlling plant diseases. Leaf extract obtained from the recognized medicinal plant *Allamonda cathertica*, was found effective in controlling *Phomopsis vexans* (100%).

Das *et al.* (2018) studied *in vitro* efficacy of some antifungal plant extracts and reported that the aqueous extract of 15% concentration of *Allium sativum* effectively inhibited (100%) the mycelial growth of *Phomopsis vexans*, followed by seed extract of *Ricinus cummunis* (77.1%).

2.10 Effect of *Trichoderma* spp. against *Phomopsis vexans*

Biological control of plant diseases involving the use of antagonistic microorganisms offers an excellent alternative to chemical control. A vast number of microorganisms present in rhizosphere have been considered as important in sustainable agriculture because of their biocontrol potentials as well as plant growth promotional activities (Jagadish, 2006).

The antagonistic activity of *Trichoderma* was demonstrated more than eight decades ago (Weindling, 1934) and today it is the most extensively used biocontrol agent (Mukherjee *et al.*, 2012).

Jadeja (2003) studied the antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride* against *Phomopsis vexans* and reported that both antagonists had shown promising performance in inhibiting the growth of the pathogen and also pycnidia formation.

Islam (2005) investigated the antagonistic effect of 14 bioagents and reported that *Trichoderma harzianum* CP, *Trichoderma harzianum*$_{22}$ and *Trichoderma* sp EP-1 were found effective against *Phomopsis vexans* in *in vitro* and in field condition.

Srinivas *et al*. (2005) evaluated the effectiveness of *Trichoderma harzianum* against *Phomopsis vexans* in eggplant cultivar and reported that formulation of *Trichoderma harzianum* was effective in reducing the *Phomopsis vexans*

infection and increasing the seed germination, vigor index in comparison with control.

Quan *et al*. (2007) found *Trichoderma* spp. as strong antagonists against *Phomopsis brevistylospora* causing rot on post-harvest rockmelon.

Ghosh (2017) applied three soil borne fungi *Trichoderma viride*, *Trichoderma harzianum* and *Beauveria bassiana* for the management of Phomopsis fruit rot of brinjal. The result showed that *T. viride* gave maximum crop protection followed by *T. harzianum* and *B. bassiana.* Application of *Trichoderma viride* (10^7 spore/ml) during seedling treatment and four consecutive spraying of this suspension at the interval of 15 fifteen days after the initiation of fruits was recommended.

CHAPTER III

MATERIALS AND METHODS

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3.1. *In vitro* Experiment

3.1.1. Experimental site

The *in vitro* experiments were conducted at M.S. Laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka.

3.1.2. Experimental period

The experiments were conducted in winter to late winter season from December, 2018 to June, 2019.

3.1.3. Experimental design

The experiments were laid out in a Completely Randomized Design (CRD) with three replications.

3.1.4. Collection of diseased specimens

Eggplant (*Solanum melongena* L.) fruits showing initial stages of the symptoms were collected from Kawran Bazaar Vegetable Market, Dhaka. The samples were immediately placed in polythene bag in airtight condition. The samples were preserved in the laboratory for isolation of pathogen.

3.1.5. Sterilization of materials and equipment

Glass petri dishes were sterilized in hot air oven at 160°C for 30 minutes. PDA media and distilled water were sterilized in an autoclave following the method (Hazra, 1988) at 121°C under 15 pound per square inch (psi) for 20 minutes. 0.1 % sodium hypochlorite (NaOCl) was used for surface sterilization of eggplant fruits. Inoculation chamber was sterilized and prepared by spraying 70% ethanol in the inner surface of the Laminar Air Flow (LAF) cabinet. Equipment like needles, forceps, blades etc. were sterilized by using 70% ethanol. During culture transfer, they were sterilized in flame to avoid contamination. All the materials

and equipment except pathogen culture were further sterilized under UV light inside the Laminar Air Flow (LAF) cabinet.

3.1.6. Preparation of culture media

The standard Potato Dextrose Agar (PDA) media was used in *in vitro* experiment.

Table 1. Composition of Potato Dextrose Agar (PDA) media

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

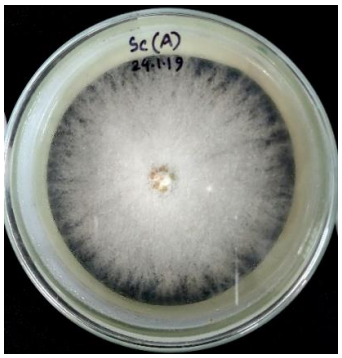
Cleaned and peeled potato tubers were sliced into pieces. Then the pieces were boiled in distilled water to collect the extract by sieving with a fine piece of cloth. Dextrose and agar were dissolved in the potato extract and the volume was made up to 1000 ml by adding distilled water. After preparation, 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°C under 15 pound per square inch (psi) 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 medium was poured into each petri dishes (9 cm diameter) inside Laminar Air Flow (LAF) with proper cautions and then allowed to solidify.

3.1.7. Isolation of *Phomopsis vexans* by Tissue planting method

The sample showing typical Phomopsis fruit rot symptom was selected and washed in running tap water to remove dust particles and then air dried. The infected fruit surface was sterilized with cotton swab soaked in 70% ethanol solution and rewashed with distilled water. The infected tissue (fruit) was cut into small pieces (2-3 mm) with a margin of healthy tissue around it and placed in PDA media in sterilized petri dishes. The dishes were incubated at 25±2°C to allow the pathogen to grow. When the growth of the fungus was visible, the mycelia of the organism was transferred into several fresh PDA plates by hyphal tip culture method to prepare pure culture of the pathogen and preserved at room temperature for further study (Plate 1).

A. Infected eggplant fruit showing typical Phomopsis fruit rot symptom

B. Growth of the pathogen after tissue planting on PDA media



C. Pure culture of the pathogen

Plate 1. Isolation of the fungus by Tissue planting method

3.1.8. Maintenance of culture of *Phomopsis vexans*

The pure culture of the pathogen was subsequently transferred to fresh PDA plates and stored in refrigerator at 4°C temperature for further use.

3.1.9. Pathogenicity test

The pathogenicity of the fungal culture was confirmed by following the procedure of Koch's postulates. Fruits of healthy, disease-free plant were artificially inoculated by spraying with spore suspension of the pathogen. The fruits containing inocula were covered with polythene bags to ensure high humidity for disease development. After ten days of inoculation, the symptoms were observed and the fungus was reisolated from the fruit portion showing typical disease symptoms and compared with the original isolate.

3.1.10. Preparation of spore suspension of *Phomopsis vexans*

Spore suspension of *Phomopsis vexans* was prepared using 30-35 days old culture. After the formation of pycnidia, 5 ml/plate sterile water was added and the spore masses was scrapped away with sterile scalpel. The conidial suspension was made with additional 45 ml water and blended in an electric blender for 2 minutes and filtered through sterile cheesecloth. The suspension having spore density of 5×10^6 spore/ml was determined by using Haemocytometer at 10X magnification.

3.1.11. Bioassay

3.1.11.1. Cup method

Three discs (5 mm) of the medium were scooped from three places from a PDA plate maintaining equal distance from the center by a sterilized disc cutter. One-millimeter solution of fungicide/plant extract/bioagent was put into each hole. In case of control, only sterile water was poured into the cup. The plates were then stored overnight in refrigerator for diffusion of the solutions in the media around the cup. 5 mm block of 7 days old culture of the pathogen was cut using sterilized block cutter and placed at the center of the plate. The linear growth of the fungus was recorded in every 24 hrs until the control plates were fulfilled.

3.1.11.2. Measurement of radial growth

The radial growth (cm) of *Phomopsis vexans* in petri dishes was measured after 7 days of incubation. The radial growth of the mycelium of the pathogen in each plate was measured by taking average of the two diameters taken right angles for each colony. Inhibition of radial growth was calculated based on colony diameter on control plate using the following formula (Vincent, 1947).

$$I = \frac{C - T}{C} \times 100$$

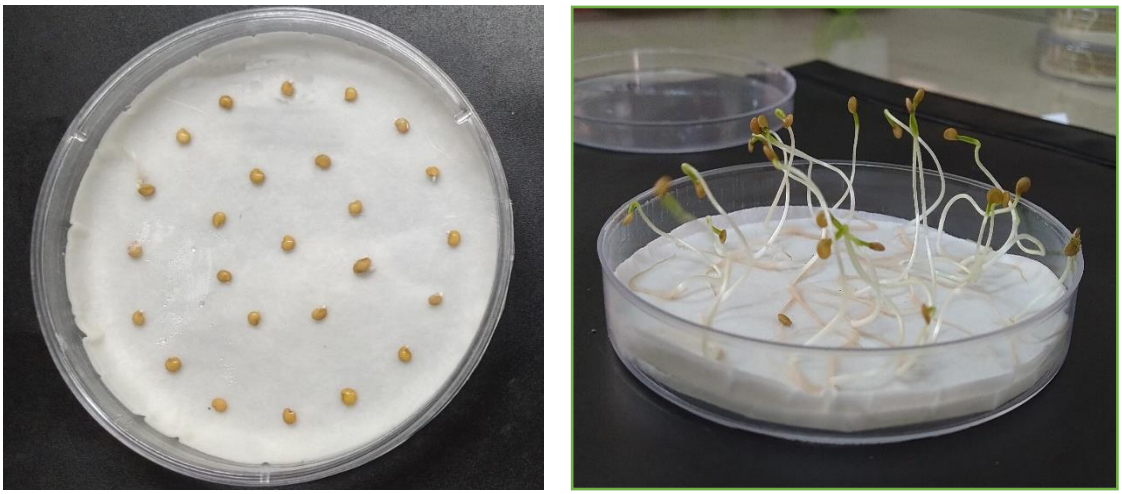
Where, I = Percent Inhibition of mycelial growth over control

C= Radial growth of pathogen in control

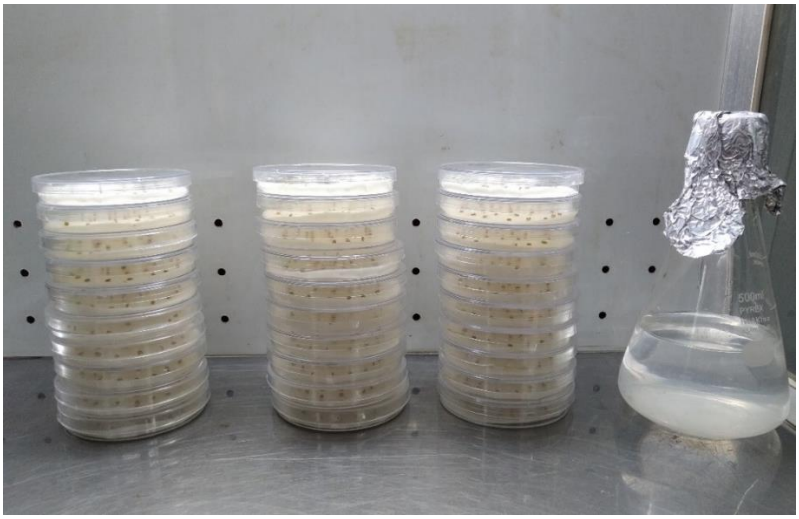
T= Radial growth of pathogen in treatment applied

3.1.12. Seed health test

The blotter incubation method (ISTA, 2001) was used to evaluate the seed health of the harvested seeds. Three pieces of blotter paper were soaked in sterile water and then placed in 9 cm petri dish. 25 seeds were placed per petri dish and incubated at 25°C at 12h light and darkness cycle. The blotter papers were kept moist by adding sterile water by using a dropper as per requirement. After 10 days, germination percentage and prevalence of pathogens were recorded (Plate 2).



Seed health testing in blotter method



Incubation at room temperature

Plate 2. The blotter incubation method (ISTA, 2001)

3.2. Field Experiment

3.2.1. Experimental site

The experiment was conducted in the experimental field, Central Farm, allotted for the students of Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207.

3.2.2. Experimental period

The experiment was conducted during October, 2018 to May, 2019.

3.2.3. Variety used

Brinjal variety 'BARI Bt Begun 2' was used for the experiment. The seeds were collected from BADC (Bangladesh Agricultural Development Corporation), Dhaka.

3.2.4. Experimental design

The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications.

3.2.5. Land Preparation

A piece of medium high land having well drainage system was selected. The soil texture of the experimental site was loam to clay loam belonging to AEZ-28: Madhupur Tract (**Appendix 1**). The land was ploughed thoroughly with a power tiller followed by laddering was done to obtain good tilth condition. The clods of the land were crushed into small pieces. Weeds, stubbles and crop residues were removed from the land.

3.2.6. Layout of the experiment

The experimental field was divided into three blocks, each representing a replication. Unit plot size was 4.5 sq.m. (4.5m x 1m). Plot to plot distance was maintained 0.5 m and block to block distance was 1 m. Soil in between two plots were taken over to raise the plot in order to facilitate free drainage of water (**Appendix 2**).

3.2.7. Raising of seedlings

Seedlings were raised in seedbed with proper care and management. Seedbed was prepared by mixing soil, sand and well decomposed cowdung in proportion of 2:1:1. Shade was provided to save the young and delicate seedlings from heavy showering and scorching sunlight. Seedlings were observed regularly and watering was done as per requirement up to transplanting in the main field (Plate 3).

Eggplant seeds sown in seedbed

Germinating seed in seedbed

Seedlings ready to transplant

Plate 3. Raising of seedlings in seedbed

3.2.8. Transplanting of seedlings to the main field

Healthy and disease-free seedlings were selected and transplanted into the experimental field followed by watering. Transplanting was done in the afternoon with proper cautions to minimize transplant shock.

3.2.9. Intercultural operations

3.2.9.1. Shading

Shade was given using newspaper to protect the transplanted eggplant seedlings from high intensity of sunlight.

3.2.9.2. Gap filling

Gap filling was done to maintain optimum plant population.

3.2.9.3. Irrigation

Irrigation was given as per requirement at 10-15 days interval.

3.2.9.4. Weeding

Weeding was done to ensure proper growth of the plant. During the experimental period, first weeding was done 20 Days After Planting (DAP), followed by 40 DAP, 55 DAP and 70 DAP.

3.2.9.5. Application of fertilizers and manures

The following dose of fertilizers and manures were applied as per recommendation of Handbook of Agricultural technology, BARI (BARI, 2017).

Table 2. Dose of fertilizers and manures applied in the field experiment

Fertilizers / Manures	Dose /ha
Urea	300 kg
TSP	150 kg
MP	200 kg
Cow dung	10 tons

The 1/3rd urea, half of MP and whole amount of other fertilizers were applied as basal dose and rest 2/3rd urea and half of MP was applied at 30 DAP and at fruit setting followed by an irrigation.

30 DAP

50 DAP

70 DAP

Fruiting stage

Harvesting stage of eggplant

Plate 4. Different growth stages of eggplant for seed production

3.2.10. Treatments

T_1 = *Trichoderma harzianum* ("Biotech Care Trichoderma suspension")

T_2 = Allamanda leaf extract (1:2)

T_3 = Garlic clove extract (1:2)

T_4 =Turmeric rhizome extract (5%)

T_5 = Onion bulb extract (1:2)

T_6 = Companion @ 0.2%

T_7 = Autostin 50WDG @0.2%

T_8 = Tilt 250EC @0.05%

T_9 = Krishibid Organic Fertilizer (as soil amendment) combined with Autostin 50WDG spraying

T_{10} = Control

3.2.11. Preparation of plant extracts

Table 3. Plant extracts used in the experiment

Local name	Scientific name	Plant parts used	Concentration
Allamanda	*Allamanda cathertica*	Leaf	1:2 (w:v)
Garlic	*Allium sativum*	Clove	1:2 (w:v)
Onion	*Allium cepa*	Bulb	1:2 (w:v)
Turmeric	*Curcuma longa*	Rhizome	5 %

For the preparation of Allamanda leaf extract, Garlic clove extract, Onion bulb extract and Turmeric rhizome 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 and crushed in a mortar and pestle. For getting the extract, the crushed 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, Garlic clove extract, Onion bulb extract and 1:20 ratio (5%) in case of Turmeric rhizome extract (Plate 5).

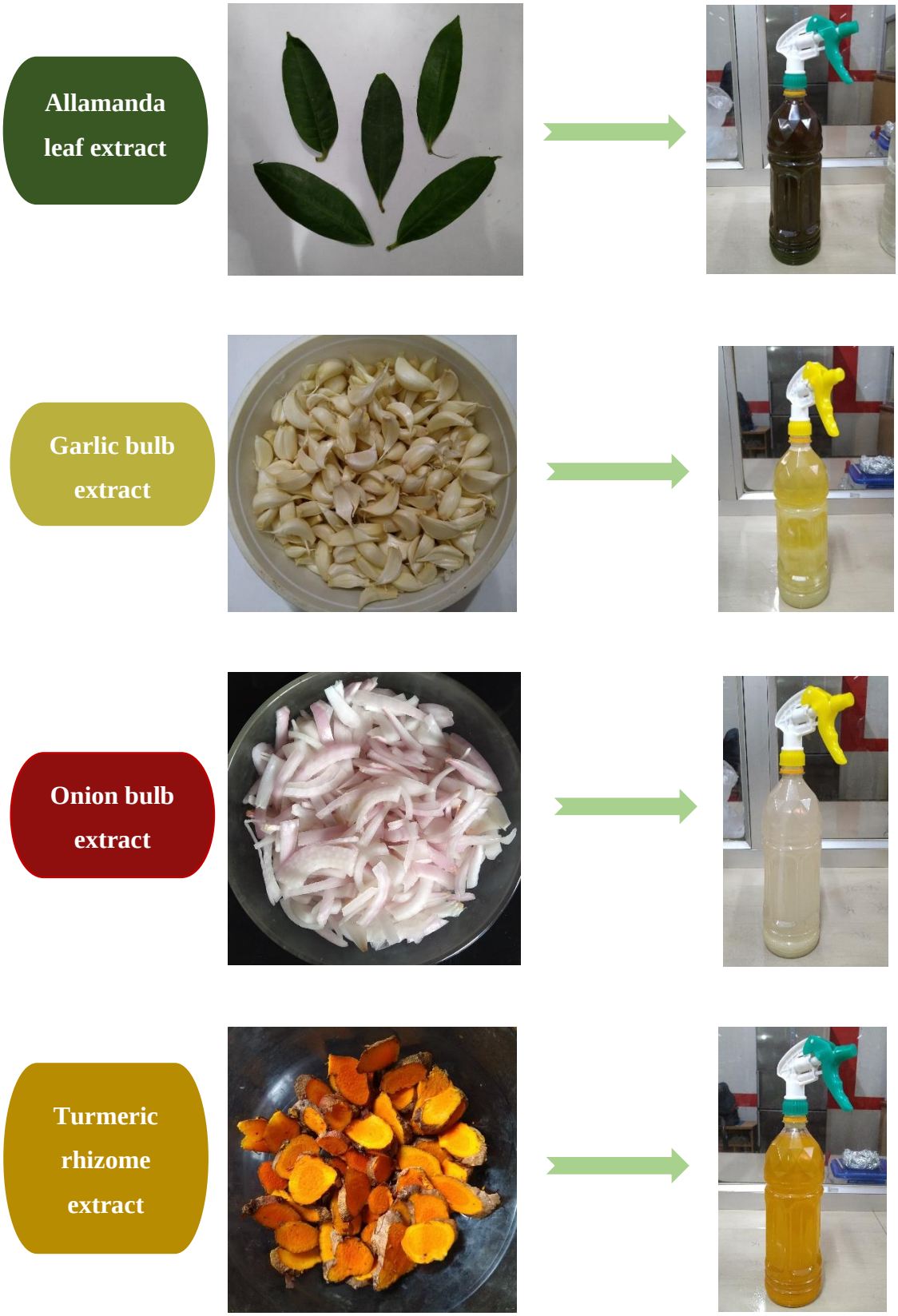


Plate 5. Preparation of Plant extracts

3.2.12. Preparation of Fungicide solutions

Table 4. Fungicides used in the experiment

Trade name	Common name	Conc. Used
Companion	Mancozeb (63%) + Carbendazim (12%)	0.2 %
Tilt 250EC	Propiconazole	0.05%
Autostin 50WDG	Carbendazim	0.2%

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

Companion **Tilt 250EC** **Autostin 50WDG**

Plate 6. Fungicides used in the experiments

3.2.13. Preparation of *Trichoderma harzianum* spore suspension

3 ml formulated *Trichoderma harzianum* suspension (Biotech Care Trichoderma Suspension) was mixed with one liter sterile water to prepare the spore suspension.

Plate 7. "Biotech Care Trichoderma Suspension" used in the experiments

3.2.14. Application of bioagent, plant extracts, fungicides and Krishibid Organic Fertilizer (as soil amendment)

Among the treatments, Krishibid Organic Fertilizer was mixed with soils of the rootzone of the crop during transplanting (Plate 8). Spore suspension of *Trichoderma harzianum*, plant extract and fungicide solutions were applied at the fruiting stage of the crop. The pre-inoculation sprays were applied one day before inoculation which was followed by two subsequent sprays with 7 days interval @ 50ml/plant. The spraying was done by a hand sprayer to cover the whole surface of the fruit. Precautionary measures were taken to prevent drifting of spray materials to the neighboring plants using a polythene barrier (Plate 9).

Plate 8. Application of Krishibid Organic Fertilizer during transplanting of eggplant seedlings

Plate 9. Precautionary measure taken by using polythene barrier to prevent spray drift

3.2.15. Inoculation of eggplant by *Phomopsis vexans*

Spore suspension of *Phomopsis vexans* containing 5×10^6 spore/ml was sprayed 20 days after fruit formation. After inoculation, the plants were covered with polythene bags for 24 hrs to ensure favorable microclimate for the successful infection by the pathogen (Plate 10).

Plate 10. Plants covered with polythene bags for successful inoculation

3.2.16. Seed extraction

The harvested ripen fruits are stored at the room temperature for 5 days which allowed to fruits to become soften and the seeds to mature fully. Then the fruits are cut in order to collect the pulps using a spoon. The pulps were dipped into water to separate the seeds from the pulp. Seeds were collected and dried to reduce the moisture content to 8% and then stored for further study.

3.2.17. Data collection

After inoculation and spraying of fungicides, plant extracts and spore suspension of bioagent, data were taken on the following parameters:

1. % Fruit infection
2. % FAD (Fruit area diseased)
3. Fruit weight and Seed weight of individual plot

3.2.18. Calculation of PDI (Percent Disease Index)

Using the following scale (Islam, 2005), disease severity was calculated:

Table 5. Standard area diagram for measurement of disease severity of phomopsis fruit rot of eggplant

% FAD	Scale
0-1%	1
1.1-10%	2
10.1-25%	3
25.1-50%	4
>50%	5

Disease severity or Percent Disease Index (PDI) was calculated by using the following formula developed by McKinney (1923):

$$\textbf{PDI} = \frac{\text{Sum of total disease rating} \times 100}{\text{Total no. of observations} \times \text{The highest grade in the scale}}$$

3.2.19. Analysis of data

All collected data were tabulated and analyzed following standard procedure (Gomez and Gomez, 1984). Treatment means were compared by LSD (Least Significant Difference) value. Statistix 10 computer package was used for performing the statistical analysis.

CHAPTER IV

RESULTS AND DISCUSSION

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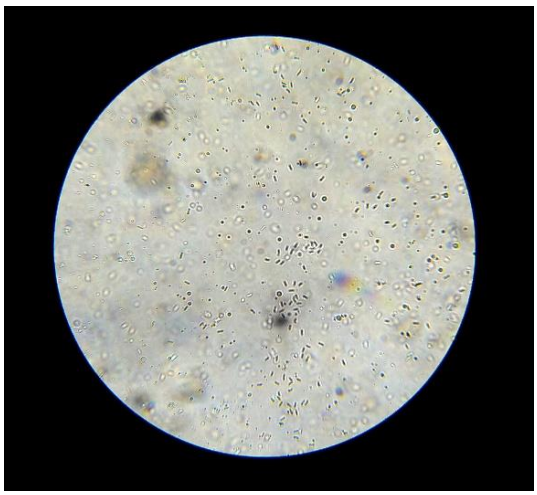
4.1. Isolation and identification of the pathogen

The fungus causing fruit rot of eggplant was isolated and studied in the laboratory. The pure culture of the fungus was obtained and identified as *Phomopsis vexans*.

In the PDA media, the pathogen produced whitish mycelia which later turned to grayish white. In around 30 days, globose to irregular shaped, black pycnidia appeared having hyaline, elliptical, one celled alpha conidia (Plate 11).

The pathogen was resembled with the literature described by Punithalingum and Holliday (1972), Islam (2005), Anwar *et al.* (2017) and CMI description No. 338. Islam (2005) also got beta conidia of the fungi along with alpha conidia. *Phomopsis vexans* produced alpha conidia at 25°C and beta conidia at 16°C (Sugha *et al.*, 2002).

Pure culture of *Phomopsis vexans* with pycnidia



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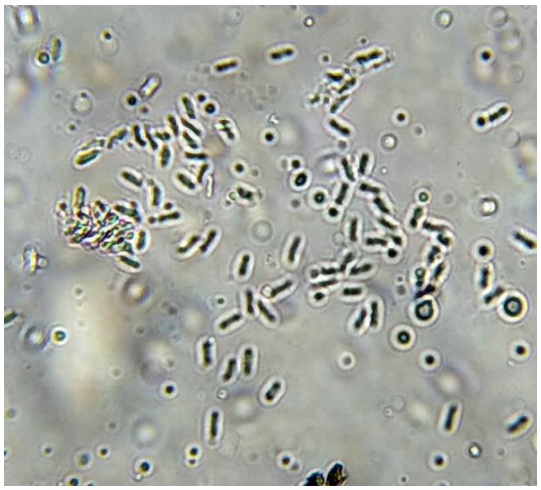


40X

Alpha conidia of *Phomopsis vexans* under compound microscope

Plate 11. Pure culture and sporulation (alpha conidia) of *Phomopsis vexans*

4.2. *In vitro* evaluation of different treatments against radial mycelial growth of *Phomopsis vexans*

The bioagent, fungicides and plant extracts evaluated in the experiment exhibited effectiveness against *Phomopsis vexans*. All the treatments applied in the experiment inhibited the mycelial growth of the pathogen significantly (Table 6).

The lowest mycelial growth was recorded in case of *Trichoderma harzianum* spore suspension (4.333 mm) and Autostin 50WDG (4.567 mm) having 95.04% and 94.77% inhibition of mycelial growth over control, respectively. Companion (7.167 mm) also inhibited the mycelial growth significantly. The highest mycelial growth was observed in the control (87.3 mm). Among the plant extracts, Garlic clove extract was found most effective in reduction of mycelial growth (91.52%) followed by Allamanda leaf extract (88.05%) and Turmeric Rhizome extract (87.97%). Both Onion bulb extract (78.92%) and Tilt 250 EC (77.66%) reduced the mycelial growth of the pathogen which was comparatively lower than the other treatments but significantly higher compared to control.

The present result is supported by the findings of other researchers. Jadeja (2003) reported that the antagonist *Trichoderma harzianum* had shown promising performance in inhibiting the growth of *Phomopsis vexans*. He also reported that Garlic clove extract and Turmeric rhizome extract highly suppressed the mycelial growth of the pathogen.

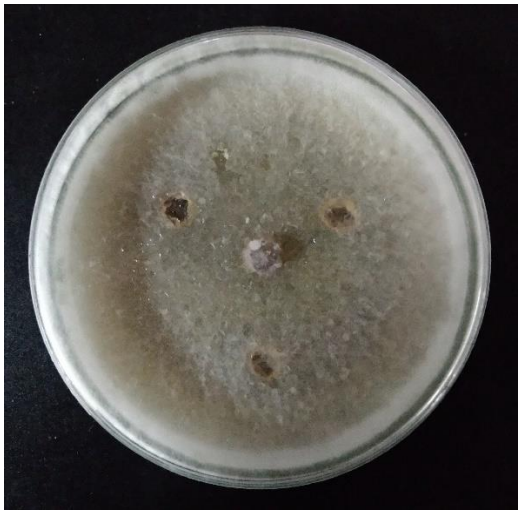
The antibiotic substance allyl thiosulphate present in the garlic (Cavallito *et al.*, 1944) might be the cause of suppressive nature of Garlic bulb extract against the pathogen. Garlic bulb extract caused 76-100% inhibition of mycelial growth of the pathogen (Islam,2004).

Islam (2005) also found *Trichoderma harzianum*, Bavistin (50% carbendazim) and Garlic bulb extract highly effective in inhibiting the mycelial growth of *Phomopsis vexans* in *in vitro.*

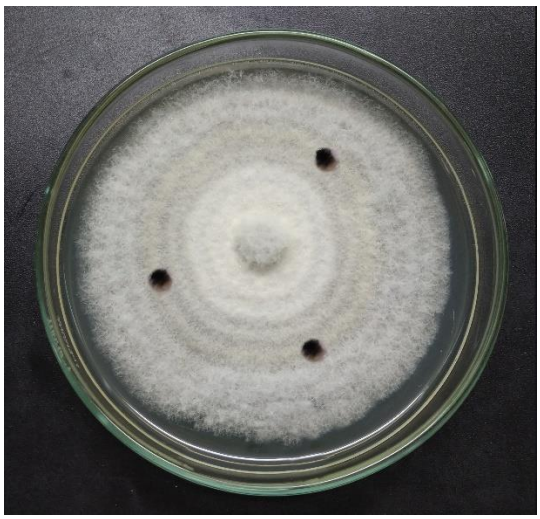
Sharma and Razdan (2012) reported that Carbendazim 12% + Mancozeb 63% @ 0.2% inhibited the mycelial growth of *Phomopsis vexans*. Das (2018) reported that the aqueous extract (15% concentration) of *Allium sativum* effectively inhibited the mycelial growth of *Phomopsis vexans.*

Table 6. *In vitro* evaluation of different treatments against radial mycelial growth of *Phomopsis vexans*

Treatment	**Radial mycelial growth (mm)**	**% inhibition of mycelial growth over control**
Trichoderma harzianum spore suspension	4.333 f	95.04
Allamanda leaf extract	10.433 d	88.05
Garlic clove extract	7.4 e	91.52
Turmeric rhizome extract	10.5 d	87.97
Onion bulb extract	18.4 c	78.92
Companion	7.167 e	91.79
Autostin 50WDG	4.567 f	94.77
Tilt 250EC	19.5 b	77.66
Control	87.3 a	-
LSD (0.05)	**0.2917**	
CV (%)	**0.90**	



Inhibition of mycelial growth of *Phomopsis vexans* by *Trichoderma harzianum*



Control Plate

Plate 12. *In vitro* evaluation of *Trichoderma harzianum* against *Phomopsis vexans*

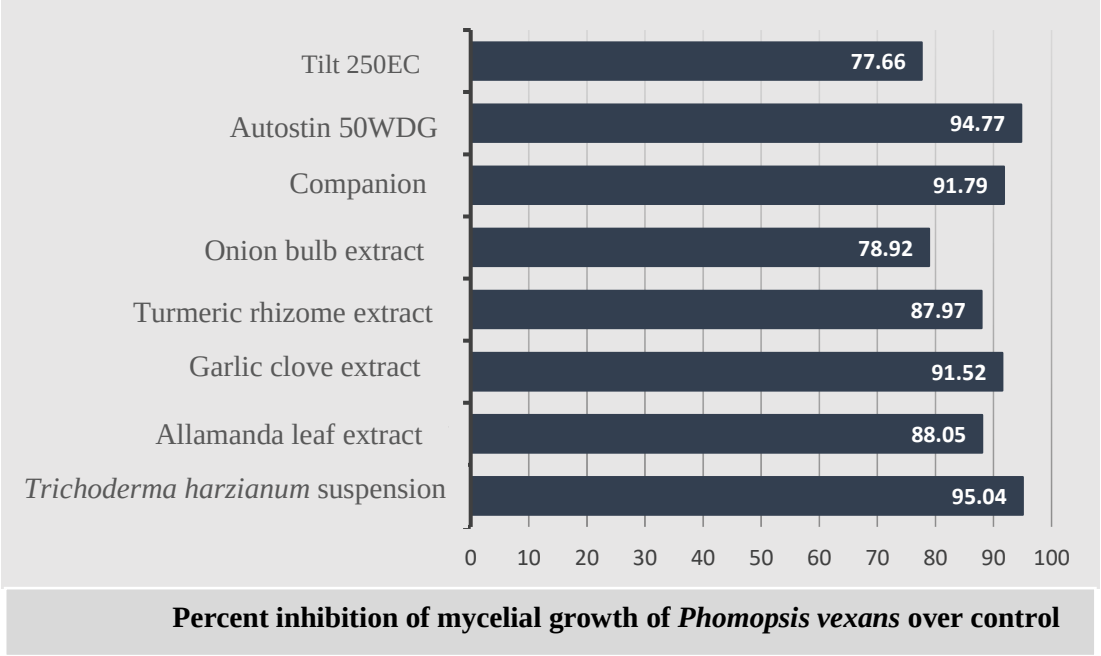


Figure 1. *In vitro* evaluation of bioagent, fungicides and plant extracts against mycelial growth of *Phomopsis vexans*

4.3 Symptomatology

The characteristic symptoms of phomopsis fruit rot appeared ten days after inoculation with *Phomopsis vexans*. On the infected fruit surface, the symptom first appeared as pale, sunken, round to oval light brownish spots. Initially, the affected fruit became soft and watery. These spots gradually increased in size and coalesced to form larger rotten area and covered much of the fruit surface. As the disease progressed, concentric ring with brownish halo appeared. At the later stage, black, pimple like pycnidia appeared on the fruit surface. Severely infected fruits became shriveled, dried and deformed (Plate 13).

Similar symptoms were reported by the other investigators. Kemble *et al.* (1998) reported that symptoms first appeared as pale sunken circular to oval areas on the fruit surface. These later turned brown and enlarged up to two to three inches in diameter, often two or more lesions merge to cover much of the fruit surface. Islam (2005) reported that lesions are typically gray to brown developing a light-colored center as they age. Pycnidia are black, pimple like structures develop in the center of old lesions. Pernezny *et al.* (2009) stated that infected fruit exhibit spots which appear as pale sunken areas which may cover the entire fruit if not treated.

Plate 13. Infected fruit showing Phomopsis fruit rot symptom after inoculation with *Phomopsis vexans*

4.4 Evaluation of different treatments on disease incidence of fruit rot of eggplant caused by *Phomopsis vexans*

The treatments applied for the management of phomopsis fruit rot of eggplant in the field condition significantly reduced the disease incidence on fruit (Table 7).

Spraying of *Trichoderma harzianum* spore suspension was found most effective with the lowest disease incidence (6.95%) which was statistically similar to Autostin 50WDG (8.25%). Krishibid Organic Fertilizer combined with Autostin 50WDG spraying also reduced the disease incidence (10.85%), significantly. Among the plant extracts, Garlic clove extract was found highly effective in reducing disease incidence (11.32%) followed by Turmeric rhizome extract (12.54%). The highest disease incidence (30.65%) was observed in the control plot. The second highest disease incidence (18.15%) was recorded in the plot treated with Tilt 250EC followed by Onion bulb extract (17.38%) which was statistically similar to Allamanda leaf extract (14.69%).

The highest reduction of disease incidence (77.32%) was recorded in *Trichoderma harzianum* spore suspension treated plot, followed by Autostin 50WDG (73.08%) and Krishibid Organic Fertilizer combined with Autostin 50WDG spraying (64.60%). Garlic clove extract gave the highest reduction of disease incidence over control (63.06%) among the plant extracts, followed by Turmeric Rhizome extract (59.09%) and Allamanda leaf extract (52.07%). The plots treated with Tilt 250EC and Onion bulb extract gave the lowest reduction of disease incidence over control which were 40.78% and 43.30%, respectively.

The findings were in agreement with the findings of other investigators. Ghosh (2017) applied *Trichoderma harzianum* for the management of Phomopsis fruit rot of eggplant. The result showed spraying of *Trichoderma harzianum* suspension at the interval of 15 fifteen days after the initiation of fruits was highly effective. Meah (2003) reported that spraying of spore suspension of *Trichoderma harzianum* was effective in controlling phomopsis blight and fruit rot of eggplant in the field.

Islam and Sitansu (1992) reported that in a field trial against *Phomopsis vexans*, Bavistin 50WP (0.1%) provided nearly complete control of leaf blight and fruit rot of eggplant with 3 sprays at 15 days interval. Islam (2005) reported that Bavistin (50% carbendazim) effectively controlled phomopsis fruit rot in the field condition. He also reported that garlic bulb extract inhibited mycelial growth and spore germination of *Phomopsis vexans* in the field condition.

Table 7. Evaluation of different treatments on disease incidence of fruit rot of eggplant caused by *Phomopsis vexans*

Treatment	**Disease Incidence (%)**	**% reduction of disease incidence over control**
T_1 = *Trichoderma harzianum* spore suspension	6.95 f	77.32
T_2= Allamanda leaf extract	14.69 bc	52.07
T_3= Garlic clove extract	11.32 c-e	63.06
T_4= Turmeric rhizome extract	12.54 cd	59.09
T_5= Onion bulb extract	17.38 b	43.30
T_6= Companion	13.37 cd	56.38
T_7= Autostin 50WDG	8.25 ef	73.08
T_8= Tilt 250EC	18.15 b	40.78
T_9= Krishibid organic fertilizer (as soil amendment) combined with Autostin 50WDG spraying	10.85 de	64.60
T_{10}= Control	30.65 a	-
LSD (0.05)	**3.6595**	
CV (%)	**14.80**	

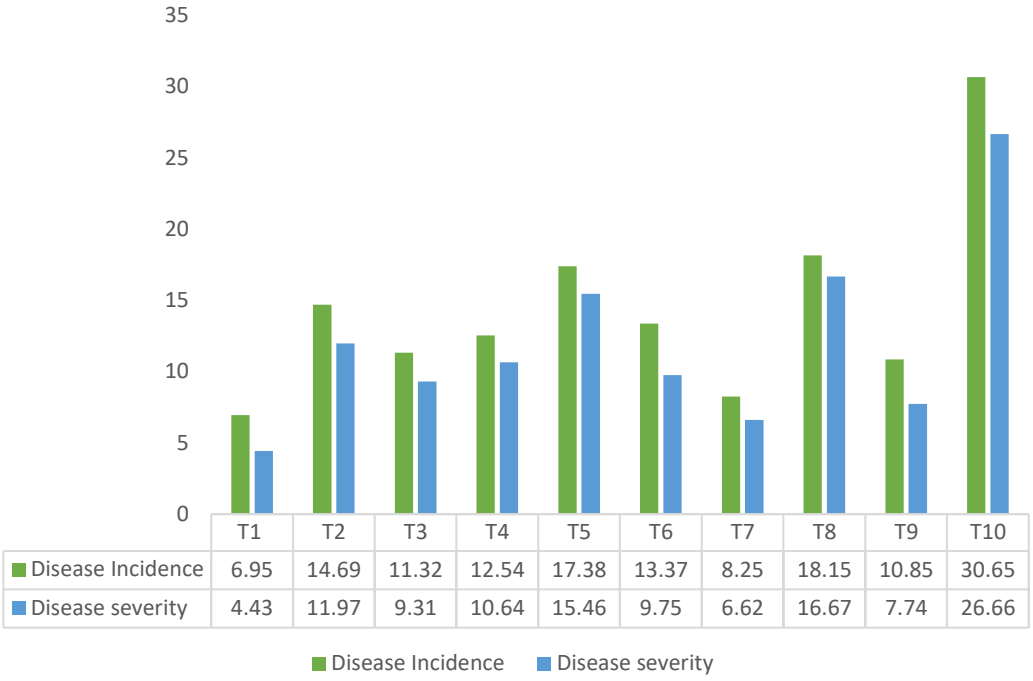
4.5 Evaluation of different treatments on disease severity (PDI) of fruit rot of eggplant caused by *Phomopsis vexans*

All the treatments applied in the experiment have remarkable effect in decreasing the disease severity of eggplant fruit (Table 8). The lowest disease severity (4.43%) was recorded in the field treated with *Trichoderma harzianum* spore suspension which was statistically similar to Autostin 50WDG (6.62%). *Trichoderma harzianum* spore suspension reduced the disease severity by 83.38%, followed by Autostin 50WDG (75.17%). Krishibid organic fertilizer combined with Autostin 50WDG spraying was also found effective in reducing the disease severity (70.97%) significantly over control. The fungicide Companion have also found promising in reducing the disease severity (63.43%) of fruit rot of eggplant over control. Among the plant extracts, Garlic clove extract gave the lowest disease severity of 9.31% which was 65.08% reduction of disease severity over control. Turmeric rhizome extract also reduced the disease severity 60.09%) significantly which was statistically similar to Allamanda leaf extract (55.10%). Both Tilt 250EC and Onion bulb extract have showed moderate efficacy in reducing the disease severity with a reduction of 37.47% and 42.01%, respectively.

The findings were keeping with the findings of Meah *et al.* (1998) who stated that Bavistin 50WP @ 0.2% applied both before and after inoculation of *Phomopsis vexans* on the surface rough fruit showed effective performance in reducing percent fruit infection and percent fruit area diseased producing the least sized lesion. The mechanism of disease reduction by *Trichoderma* strains was explained as the competition for space and nutrition with the pathogenic fungi (Alabouvette *et al.,* 1992) and the lylic activity of extra cellular enzymes of *T. harzianum* also aided to antagonize the pathogen (Elad *et al.*, 1982). Meah (2003) found garlic bulb extract (1:1) efficient in controlling *Phomopsis vexans* which reduced severity of leaf blight and fruit rot by 71-75% in the field. Islam (2005) reported that *Trichoderma harzianum*, Bavistin (50% carbendazim) and Garlic bulb extract effectively controlled phomopsis fruit rot in the field.

Table 8. Evaluation of different treatments on disease severity (PDI) of fruit rot of eggplant caused by *Phomopsis vexans*

Treatment	Percent Disease Index (PDI)	% reduction of PDI over control
T_1= *Trichoderma harzianum* spore suspension	4.43 g	83.38
T_2= Allamanda leaf extract	11.97 c	55.10
T_3= Garlic clove extract	9.31 de	65.08
T_4= Turmeric rhizome extract	10.64 cd	60.09
T_5= Onion bulb extract	15.46 b	42.01
T_6= Companion	9.75 c-e	63.43
T_7= Autostin 50WDG	6.62 fg	75.17
T_8= Tilt 250EC	16.67 b	37.47
T_9= Krishibid organic fertilizer (as soil amendment) combined with Autostin 50WDG spraying	7.74 ef	70.97
T_{10}= Control	26.66 a	-
LSD (0.05)	**2.2505**	
CV (%)	**11.00**	



T_1 = *Trichoderma harzianum* spore suspension	**T_5= Onion bulb extract**	**T_9= Krishibid organic fertilizer (as soil amendment) combined with Autostin 50WDG spraying**
T_2= Allamanda leaf extract	**T_6= Companion**	**T_{10}= Control**
T_3= Garlic clove extract	**T_7= Autostin 50WDG**	
T_4= Turmeric rhizome extract	**T_8= Tilt 250EC**	

Figure 2. Effect of different treatments on disease incidence and disease severity (PDI) of fruit rot of eggplant caused by *Phomopsis vexans*

4.6. Effect of different treatments on fruit yield t ha^{-1} of eggplant

Application of different treatment significantly influenced the fruit yield of eggplant (Table 9). The highest yield (21.30 t ha^{-1}) was recorded in the plot sprayed with *Trichoderma harzianum* spore suspension and Krishibid organic fertilizer combined with Autostin 50WDG spraying (20.89 t ha^{-1}) which were statistically similar to Autostin 50WDG (19.98 t ha^{-1}). Among the plant extracts, Garlic clove extract (18.67 t ha^{-1}) was the most effective in increasing fruit yield. Both Companion (17.78 t ha^{-1}) and Turmeric rhizome extract (17.33 t ha^{-1}) were equally effective in increasing fruit yield over control. The lowest yield (8.8 t ha^{-1}) was recorded in the control plot followed by Tilt 250EC (13.30 t ha^{-1}) and Onion bulb extract (15.11 t ha^{-1}).

The highest increase of fruit yield (142.05%) over control was recorded in the plot treated with *Trichoderma harzianum* treated plot followed by Autostin 50WDG (127.05%) and Krishibid Organic Fertilizer combined with Autostin 50WDG spraying (137.39%). Among the plant extracts, Garlic Clove extract treated plot resulted the highest increase of fruit yield (112.16%) over control, followed by Turmeric rhizome extract (96.93%). The lowest increase of fruit yield (60.00%) was recorded in the plot treated with Tilt 250EC.

Similar findings were recorded by Beura *et al.* (2008) who reported carbendazim as the best control for *Phomopsis vexans* which also maximized the yield.

Table 9. Effect of different treatments on fruit yield of eggplant

Treatment	**Fruit Yield (t ha^{-1})**	**% fruit yield increased over control**
T_1 = *Trichoderma harzianum* spore suspension	21.3 a	142.05
T_2= Allamanda leaf extract	16.04 b-d	82.27
T_3= Garlic clove extract	18.67 a-c	112.16
T_4= Turmeric rhizome extract	17.33 a-d	96.93
T_5= Onion bulb extract	15.11 cd	71.70
T_6= Companion	17.78 a-d	102.05
T_7= Autostin 50WDG	19.98 ab	127.05
T_8= Tilt 250EC	13.30 d	51.14
T_9= Krishibid Organic Fertilizer (as soil amendment) combined with Autostin 50WDG spraying	20.89 a	137.39
T_{10}= Control	8.8 e	-
LSD (0.05)	**4.4862**	
CV (%)	**15.46**	

4.7. Correlation and Regression study between disease incidence and severity of Phomopsis fruit rot and fruit yield of eggplant

Correlation and regression study were done to determine the relationship between disease incidence and severity of phomopsis fruit rot and fruit yield (t ha^{-1}) of eggplant. Significant and negative correlation was observed between disease incidence of phomopsis fruit rot and fruit yield (t ha^{-1}).

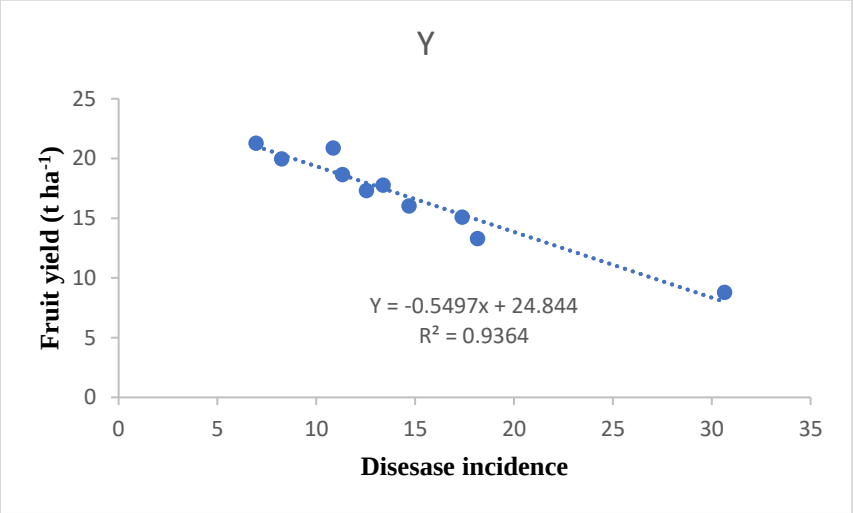


Figure 3. Relationship between disease incidence of Phomopsis fruit rot and fruit yield (t ha^{-1})

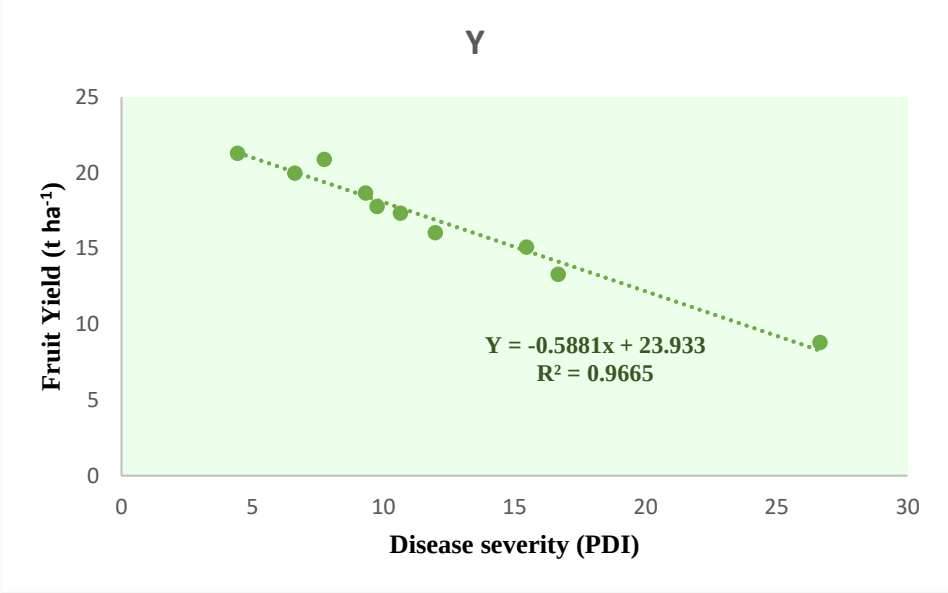


Figure 4. Relationship between and disease severity (PDI) of Phomopsis fruit rot and fruit yield (t ha^{-1})

4.8. Effect of different treatments on seed yield (kg ha^{-1}) of eggplant

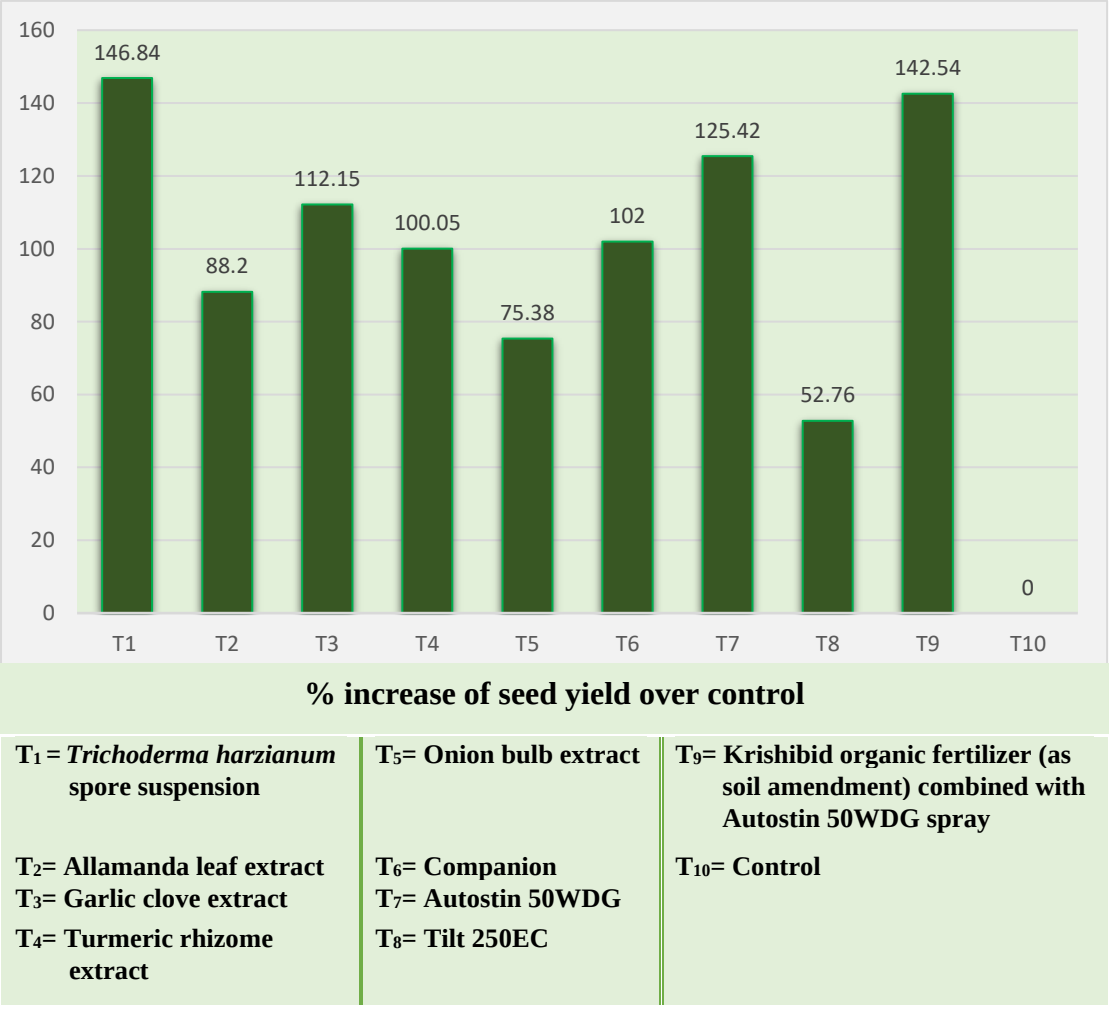
Seed yield of eggplant was highly influenced by the application of different treatments (Table 10). The highest seed yield (92.44 kg ha^{-1}) was obtained from the plot treated with *Trichoderma harzianum* spore suspension which was statistically similar to Krishibid Organic Fertilizer combined with Autostin 50WDG spraying (90.83 kg ha^{-1}) and Autostin 50 WDG (84.42 kg ha^{-1}). The lowest seed yield (37.45 kg ha^{-1}) was recorded in control plot. Among the plant extracts, Garlic clove extract treated plot resulted the highest yield (79.45 kg ha^{-1}) followed by Turmeric rhizome extract (74.92 kg ha^{-1}) and Allamanda leaf extract (70.48 kg ha^{-1}). Among the other treatments, the lowest seed yield (57.21 kg ha^{-1}) was observed in the plot treated with Tilt 250EC which was also significantly higher than the control plot.

The plot treated with *Trichoderma harzianum* spore suspension resulted highest increase of seed yield (146.84%) over control followed by soil amendment with Krishibid Organic Fertilizer combined with Autostin 50WDG spraying (142.54%). The lowest increase of seed yield (52.76%) was recorded in the plot treated with Tilt 250EC. Among the plant extracts, the highest increase of seed yield (112.15%) was recorded followed by Turmeric rhizome extract (100.05%).

The findings of the present investigation were in agreements with the findings of Meah and Howlader (2003). Howlader (2003) reported that spraying of spore suspension of *Trichoderma harzianum* yielded good result against the fruit rot of eggplant in the field. Islam (2005) stated that *Trichoderma harzianum*, Bavistin (50% carbendazim) and garlic bulb extract were found effective for the management of phomopsis fruit rot.

Table 10. Effect of different treatments on seed yield (kg ha^{-1}) of eggplant

Treatments	Seed Yield (kg ha^{-1})	% increase of seed yield over control
T_1 = *Trichoderma harzianum* spore suspension	92.44 a	146.84
T_2= Allamanda leaf extract	70.48 c-e	88.20
T_3= Garlic clove extract	79.45 a-c	112.15
T_4= Turmeric rhizome extract	74.92 b-d	100.05
T_5= Onion bulb extract	65.68 de	75.38
T_6= Companion	75.65 b-d	102.00
T_7= Autostin 50WDG	84.42 ab	125.42
T_8= Tilt 250EC	57.21 e	52.76
T_9= Krishibid organic fertilizer (as soil amendment) combined with Autostin 50WDG spraying	90.83 a	142.54
T_{10}= Control	37.45 f	-
LSD (0.05)	**13.494**	
CV (%)	**10.80**	



T_1 = *Trichoderma harzianum* spore suspension	**T_5= Onion bulb extract**	**T_9= Krishibid organic fertilizer (as soil amendment) combined with Autostin 50WDG spray**
T_2= Allamanda leaf extract	**T_6= Companion**	**T_{10}= Control**
T_3= Garlic clove extract	**T_7= Autostin 50WDG**	
T_4= Turmeric rhizome extract	**T_8= Tilt 250EC**	

Figure 5. Effect of different treatments on percent increase of seed yield over control

4.9. Correlation and Regression study between incidence and severity of Phomopsis fruit rot and seed yield (Kg ha^{-1}) of eggplant

Correlation and regression study were done to determine the relationship between disease incidence and severity of Phomopsis fruit rot and seed yield (kg ha^{-1}) of eggplant. The study revealed that the disease incidence and severity (PDI) were significantly and negatively correlated with seed yield (kg ha^{-1}) of eggplant.

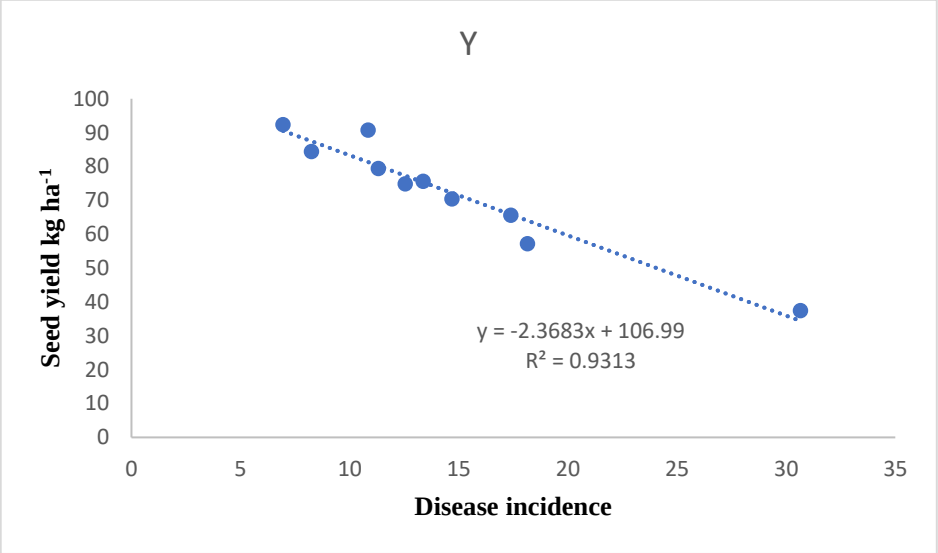


Figure 6. Relationship between disease incidence of Phomopsis fruit rot and seed yield (kg ha^{-1})

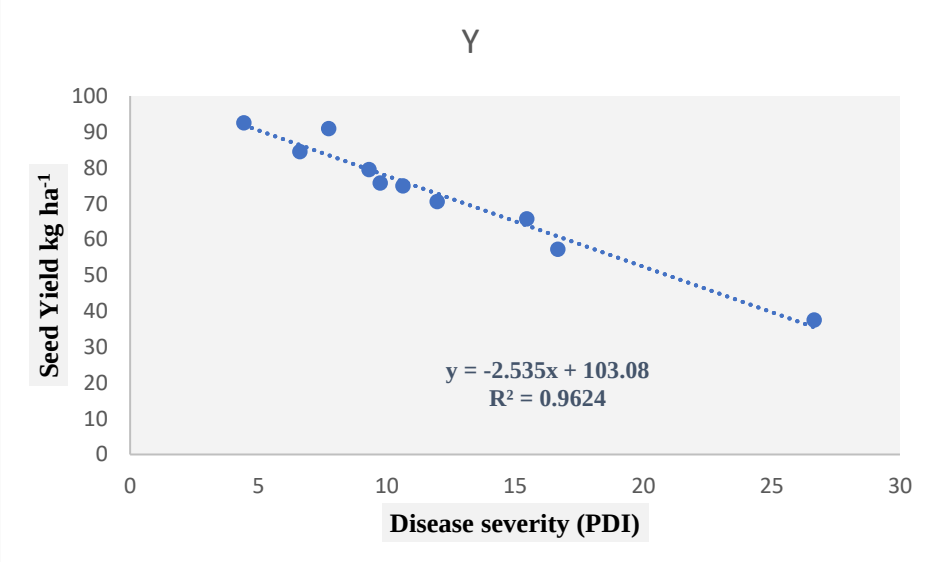


Figure 7. Relationship between disease severity (PDI) of Phomopsis fruit rot and seed yield (kg ha^{-1})

4.10. Effect of different treatments on seed health of eggplant

Application of different treatments significantly affected the seed health of the eggplant seeds (Table 11).

The highest increase of seed germination (93.56%) of harvested seed was recorded in case of soil amendment with Krishibid Organic Fertilizer combined with Autostin 50WDG spraying followed by foliar spray of *Trichoderma harzianum* spore suspension (88.41%). The lowest increase of seed germination (60.00%) was recorded in the plot treated with Tilt 250EC. Among the plant extracts, Garlic clove extract and Turmeric rhizome extract gave the highest increase of seed germination (80.67 %) over control.

Soil amendment with Krishibid Organic Fertilizer combined with Autostin 50WDG spraying resulted the lowest incidence of *Phomopsis vexans* (1.33%) in harvested seeds followed by foliar spray of *Trichoderma harzianum* spore suspension (2.67%). The highest incidence of the pathogen (38.33%) was recorded in control plot. Among the plant extracts, Garlic clove extract was found most effective with the lowest incidence (6.33%) of *Phomopsis vexans* followed by Turmeric rhizome extract (8.33%).

The findings were in agreement with the findings of other researchers. Srinivas *et al.* (2005) reported that the formulation of *Trichoderma harzianum* was effective in reducing the *Phomopsis vexans* infection and increasing seed germination in comparison with control. Meah *et al.* (2003) found that *T. harzianum* significantly controlled the nursery diseases caused by *Phomopsis vexans* and increased seed germination by 49% over control.

Khaleduzzaman (1996) investigated the effect of four plant extracts in controlling seed borne fungi and reported that Garlic bulb extract was found best in reducing seed-borne prevalence and increasing germination percentage of seed.

Table 11. Effect of different treatments on seed health of eggplant

Treatment	Seed germination %	% incidence of *Phomopsis vexans* in seed	% increase of seed germination over control
T_1 = *Trichoderma harzianum* spore suspension	92.47 ab	2.67 g	88.41
T_2= Allamanda leaf extract	84.87 d	11.00 cd	72.92
T_3= Garlic clove extract	88.67 c	6.33 f	80.67
T_4= Turmeric rhizome extract	88.67 c	8.33 e	80.67
T_5= Onion bulb extract	83.60 d	11.67 c	70.33
T_6= Companion	88.67 c	9.33 de	80.67
T_7= Autostin 50WDG	91.20 bc	2.67 g	85.82
T_8= Tilt 250EC	78.53 e	19.00 b	60.00
T_9= Krishibid organic fertilizer (as soil amendment) combined with Autostin 50WDG spraying	95.00 a	1.33 g	93.56
T_{10}= Control	49.08 f	38.33 a	-
LSD (0.05)	**2.9094**	**1.9169**	
CV (%)	**2.03**	**10.17**	

4.11. Correlation and Regression study between % incidence of *Phomopsis vexans* in seed and seed germination %

Correlation and regression study were done to determine the relationship between incidence of *Phomopsis vexans* in seed and seed germination %. Significant and negative correlation was observed between incidence of *Phomopsis vexans* in seed and seed germination % of the harvested seeds. The regression equation was Y = -1.1879x + 97.221, where x denotes % incidence of *Phomopsis vexans* and Y denotes seed germination %. Co-relation co-efficient $R^2 = 0.9726$, which was statistically significant.

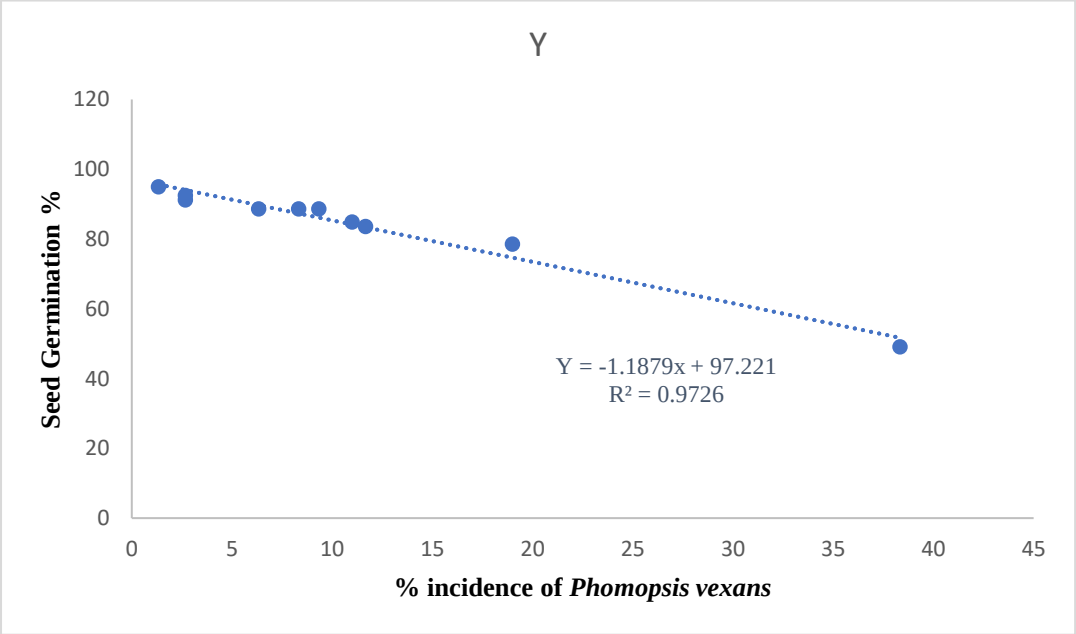


Figure 8. Relationship between % incidence of *Phomopsis vexans* in seed and % seed germination

CHAPTER V

SUMMARY AND CONCLUSION

CHAPTER V

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Fruit rot of eggplant caused by *Phomopsis vexans* is one of the most destructive diseases of eggplant. It is regarded as the main barrier of quality seed production of eggplant in Bangladesh due to the seed borne nature of *Phomopsis vexans*. The most recommended management practice of this disease is the application of systemic fungicides. But the indiscriminate use of chemical fungicide is hazardous to human health and poses adverse effect on ecosystems. Isolation, identification and the development of alternative eco-friendly options other than chemical fungicides against *Phomopsis vexans* were the main purposes of the present study. The experiments were conducted during winter to late winter season from October 2018 to June 2019 at M.S. laboratory of Department of Plant Pathology and in the experimental field of Central Farm allotted for the students of Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka 1207. The pathogen isolated from diseased fruit sample was identified as *Phomopsis vexans* based on its key characteristics (CMI Description no. 338) and the pathogenicity of the fungus was confirmed by Koch's Postulates. A bioagent *Trichoderma harzianum*, three fungicides viz. Companion, Autostin 50WDG and Tilt 250EC, four plant extracts viz. Allamanda leaf extract, Garlic clove extract, Turmeric rhizome extract and Onion bulb extract, and Krishibid Organic Fertilizer (as soil amendment) combined with spraying of Autostin 50WDG were evaluated against *Phomopsis vexans* for the management of Phomopsis fruit rot of eggplant.

The bioagent, *Trichoderma harzianum* was proved to be the most effective in both laboratory and field experiments. In laboratory, the highest inhibition of mycelial growth of *Phomopsis vexans* was recorded (95.04%). In the field experiment, the highest increase of fruit yield (142.05%) and seed yield (146.84%) was recorded over control. The increase of seed germination of

harvested seeds was significantly higher (88.41%) compared to control in case of application of *Trichoderma harzianum* as foliar spray.

Soil amendment with Krishibid Organic Fertilizer combined with spraying of Autostin 50WDG was also found highly effective in increasing the fruit yield (137.39%) and seed yield (142.54%) compared to control. The highest increase of seed germination (93.56%) over control was also recorded.

Among the plant extracts, Garlic clove extract and Turmeric rhizome extract were found effective against *Phomopsis vexans* in reducing disease incidence and disease severity (PDI). The plot treated with Garlic clove extract gave the highest increase of fruit yield (112.16%) over control followed by Turmeric rhizome extract (96.93%). The increase of seed yield over control was also highest (112.15%) in case of foliar spray of Garlic clove extract followed by Turmeric rhizome extract (100.05%).

Based on the efficacy of bioagent, fungicides, plant extracts and soil amendment explored in the experiment against *Phomopsis vexans* in controlling Phomopsis fruit rot of eggplant, it may be suggested that foliar spray of *Trichoderma harzianum* ("Biotech Care Trichoderma Suspension"), soil amendment with Krishibid Organic Fertilizer combined with spraying of Autostin 50WDG, Garlic clove extract and Turmeric rhizome extract could be effective as eco-friendly approach for the management of the disease for seed production.

CHAPTER VI

REFERENCES

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REFERENCES

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CHAPTER VII

APPENDICES

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APPENDICES

Appendix 1: Soil Properties of the experimental field

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
Elevation	:	8.45
Location	:	SAU Farm, Dhaka.
Field level	:	Above flood level.
Drainage	:	Fairly good.
Firmness (consistency)	:	Compact to friable when dry.

Particle size distribution of Soil

Sand : 34%

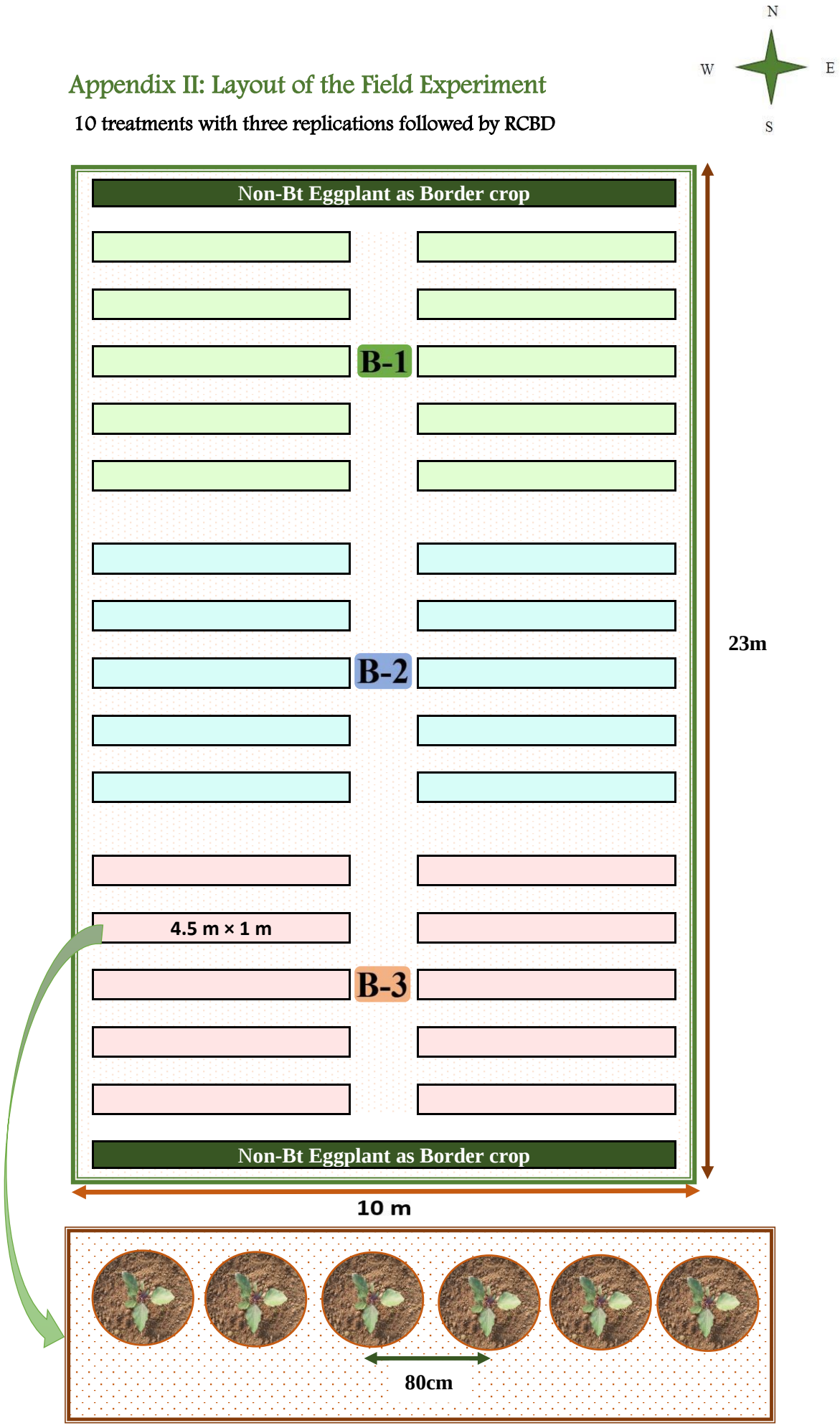
Silt : 46%

Clay : 20%

Source: Soil Resource Development Institute (SRDI)

Appendix II: Layout of the Field Experiment

10 treatments with three replications followed by RCBD



Appendix 3. View of the experimental field

Experimental field

Individual block

Individual plot