EVALUATION OF COMPATIBLE BIO-AGENTS AND SELECTED BOTANICALS AGAINST RHIZOME ROT DISEASE OF GINGER

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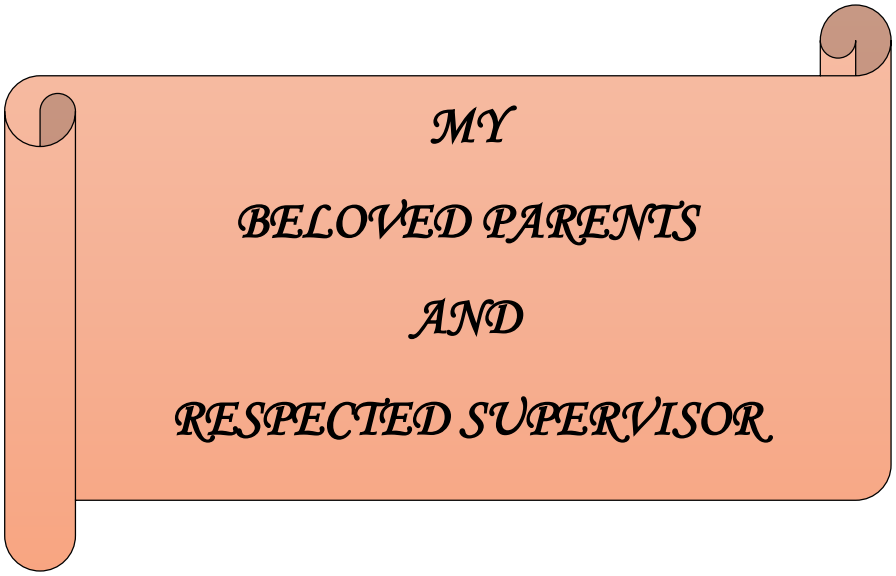

CERTIFICATE

This is to certify that the thesis entitled ***'EVALUATION OF COMPATIBLE BIO-AGENTS AND SELECTED BOTANICALS AGAINST RHIZOME ROT DISEASE OF GINGER'****. Submitted to the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of Master of Science in Plant Pathology, embodies the results of a piece of bona fide research work carried out by Registration No.18-09162 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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.

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ABSTRACT

A pot and lab experiments were conducted in Bio-agents laboratory and the net house of The Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh during the period from July,2019 to June, 2020. The study was carried out to evaluate the efficacy of selected compatible bio-agent viz. *Verticillium lecanii* (T_1), *Beauveria bassiana* (T_2), *Metarhizium anisopliae* (T_3), *Trichoderma viride* (T_4) and botanicals viz.Tea wastage (T_5), Neem leaves extract (T_6), Allamanda leaves extract (T_7) against the pathogen of rhizome rot disease responsible for ginger decline. The selected bio-agents were applied as a bio-fortified materials. In the pot bio-agents were bio-fortified with the selected substrates; cow dung, mustard oil cake, poultry manure, dust and wheat grain. Data on disease incidence and disease severity was recorded at 50, 70 and 90 Days After Planting (DAP). Among the selected bio-agents the lowest disease incidence and severity was found in T_3 (*Metarhizium anisopliae*) and the highest in T_1 (*Verticillium lecanii*). Among the selected botanicals the lowest disease incidence and severity was found in T_5 (Tea wastage) and the highest was found in T_7 (Allamanda leaf extract). No disease was found in T_6 (Neem extract) treatment up to harvesting. The selected treatments were also gave the promising performance in inhibition of radial mycelial growth of *Fusarium oxysporum* over untreated control. From the *in-vitro* management study it was found that *Trichoderma viride* (T_4) and *Metarhizium anisopliae* (T_3) gave the better performance in inhibition of radial mycelial growth. Among the botanicals, neem extract gave the promising performance in inhibition of radial mycelial growth. However, from the present study it may be concluded that *Metarhizium anisopliae*, *Trichoderma viride* and Neem leaf extract can be used for management of rhizome rot disease of ginger.

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ABBREVIATIONS AND ACRONYMS

% : per cent

cm : centimeter

gm : gram

i.e. : that is

l : liter

mm : millimeter

LF : Liquid Formation

PSA : Potato Sucrose Agar

CRD : Completely Randomized Design

LSD : Least Significant Difference

DI : Disease Incidence

DS : Disease Severity

DAP : Days After Planting

Et el. : and others

Viz., : Namely

BBS : Bangladesh Bureau of Statistics

FAO : Food and Agriculture Organization

INTRODUCTION

Ginger (*Zingiber officinale*) is one of the earliest species belongs to the family Zingiberaccae and an important oriental spice crop in the world. Ginger is a spice crop whose rhizome, ginger root or ginger, is widely used as a spice and a folk medicine. It is a herbaceous perennial which grows annual pseudo stems (false stems made of the rolled bases of leaves) about a meter tall bearing narrow leaf blades. The inflorescences bear pale yellow with purple flowers and arise directly from the rhizome on separate shoots (Sutarno *et al.*, 1999). It has special significance as spice for tropical countries where it is produced and consumed in large quantities (Rahim. 1992). It has been cultivated and used in Asia from very ancient time and the useful parts of this crop are the rhizomes (Purseglove *et al*., 1988). In Bangladesh ginger is mainly used as spice. It is cultivated all over the country where Rangpur, Nilphamari, Tangail, Khulna, Pabna, Jessore, Rangamati, Bandharban, Khagachori, Chittagong, and Chittagong Hill tracts are the most suitable places for its commercial cultivation. Ginger is grown by the small farmers as their cash crop in different parts of Bangladesh. The annual production of ginger is 80234 metric tons & the total area of ginger cultivation is about 23747 acres and the yield per acre is about 3379 kg (BBS 2020) in the country which is not sufficient for our national demand. According to FAO (2020), the annual production of ginger is 4,080,927 metric tons and the total area of ginger cultivation is about 393762 acres all over the world. Thus, the deficit amount has to import from abroad to meet up the national demand. In western countries, ginger is widely used for culinary purpose in ginger-bread, biscuits, cakes, pudding, soups and pickles. It is a frequent constituent of curry powder. It is one of the most widely used spices in Chinese cookery. It is also used in medicine as a carminative and aromatic stimulant to the gastrointestinal tract, externally as an aphrodisiac and internally as a counter irritant. Ginger has a very long history of use in various forms of traditional and alternative medicine. It’s

been used to aid digestion, reduce nausea, and help fight the flue and common cold, to name a few of its purposes. The unique fragrance and flavor of ginger come from its natural oils, the most important of which is gingerol. Gingerol has powerful anti-inflamatory and antioxidant effects, according to research. For instance, it may help reduce oxidative stress, which is the result of having an excess amount of free radicals in the body.

Ginger is affected by various diseases, such as, Rhizome rot, Bacterial wilt, Soft rot, blight etc. Among all of these, rhizome rot is most damaging one (Chattopadhya,1997). The spice trade generally considers Bangladesh's ginger to be of the best quality and as a result, it commands a premium price on the world market. However, production has steadily declined overtime mainly due to rhizome rot disease in the major production areas. This has led many growers to abandon ginger cultivation. Over the last five years the Plant Protection Unit of the Ministry of Agriculture has conducted research in the identification of the cause and control of this disease. The main pathogens associated with this disease are the fungus *Fusarium* spp. and the root knot nematode *Meloidogyne* sp. Occasionally, the fungi *Rhizoctonia solani*, and *Pythium* sp., along with the bacterium *Pseudomonas* sp. have been isolated from diseased rhizomes. The infected plant appeared the two types of symptoms; above ground symptoms and below ground symptoms. The above-ground symptoms are; plants from infected rhizomes are stunted and become yellow, lower leaves dry out and turn brown then eventually the aboveground shoots dry out completely. Infected plant collapses very slow (up to several weeks). The below ground symptoms are; diseased rhizomes show a brown discoloration, are normally shriveled in appearance and eventually decay leaving the outer shell intact with only fibrous internal tissue remaining. The disease is spread unintentionally by the use of infected rhizomes from the previous crop, although these rhizomes may appear normal and healthy. Hence, selecting clean material

based on appearance may not be sufficient to control the disease. The disease is important because it causes economic losses to growers resulting in increased prices of products to the consumers. Rhizome rot of ginger is a serious constraint for the cultivation of ginger in Bangladesh.

The management of ginger decline is very difficult; however, it can be managed up to satisfactory level through certain chemical and botanical practices. Rhizome rot of ginger can be controlled by the application of fungicides. Many researchers worked on the chemical control of the disease and they found very promising effect of different chemicals against the disease (Stirling *et al.,* 2006; Usman, 2006; Meena and Mathur, 2005; Singh and Gomez, 2001). Systemic and contact fungicides like Bavistin 50WP, Ridomil Gold MZ72, Captan, Dithane M-¬45, Copper Oxychloride and Bordeaux mixture etc. were reported effective against the disease (Sagar, 2006). However, chemicals treatment increase the cost of production and continuous use of the chemicals results in accumulation harmful chemical residues in soil as well as plant products causing serious environmental pollution, deleterious effect to non-target beneficial soil microorganism. Biological control of plant pathogens is an eye catching alternative to decrease heavy dependence of modern agriculture on costly chemical fungicides, which not only cause environmental pollution but also lead to the development of resistant strains. In search of eco-friendly approach several researchers investigated on organic products, bio-agents, plant extract for the management of the disease (Dohroo *et al.,* 1994; Ram *et al.,* 2002; Anandaraj and Sharma, 2003; Ambia, 2006). Now a days *Trichoderma sp* is frequently used as a bio-agent against soil borne fungal pathogens (Ahmmed and Hossain, 2006). Therefore, biocontrol of plant pathogens by antagonistic fungus *Trichoderma* is considered as one of the best alternatives to chemical due to the advantages such as cost-effective, eco-friendly, enhanced penetration and composting (Saba, 2012). Number of experiments have been undertaken to control seedling diseases (caused

by *R.solani* and *S.rolfsii*) of crops using *Trichoderma* both in-vitro and in-vivo (Begum and Bhuiyan, 2007; Islam and Bhuiyan, 2006; Rahman, 2004).

However, scanty of researches have been performed in Bangladesh on use of *Trichoderma* fortified compost to reduce the seedling diseases and its growth promotion. Therefore, it is necessary to explore the potentiality of *Trichoderma* fortified compost at field condition to control soilborne pathogens and also in increasing yield potentiality of Ginger. Bio-fortified of other selected botanicals are also effective to control soilborne pathogen and also in increasing yield potentiality of Ginger. Soil amendment using cow dung, poultry wastes, saw dust, mustard oil cake, wheat grain are now being considered as environment friendly approach that make the soil suppressive improving the antagonistic activities of the soil microorganisms. Rhizome rot of ginger is a prevalent problem to the farmers with the resultant effect of reduce yield much below than the expectation. There are no proper management practices available in the literature to control rhizome rot diseases. In Bangladesh condition, no systematic research work has been done on the control of this disease. But the problem needs to give urgent attention. Considering the above circumstances, the present investigation has been undertaken to identify the suitable management component(s) for controlling rhizome rot of ginger.

The aim of this proposed study is to evaluate the compatible Bio-agents and selected botanical extracts against the pathogen of rhizome rot disease that responsible for ginger decline. In this proposed study, the species of selected compatible bio-agents viz. *Trichoderma viride, Verticillium lecanii, Metarhizium anisopliae* and *Beauveria bassiana* and selected botanical extracts viz. neem leaves extract, allamanda leaves extract and tea wastage were used to study its compatibility under *in vitro* conditions.

Objectives:

In view of the above facts, the research work was carried out to achieve the following specific objectives:

- To isolate, identify and characterize the causal organism of rhizome rot disease of Ginger.
- To evaluate the selected bio-agents and botanical extracts against the pathogens of rhizome rot disease of Ginger.
- To study the compatibility of selected bio-agents and botanicals in controlling the rhizome rot disease of Ginger.

REVIEW OF LITERATURE

In the cultivation of ginger, a number of diseases for the growers are a very crucial. Among the diseases rhizome rot of ginger is a very important one. A few studies on the related to control of rhizome rot of ginger have been carried out in the country as well as of the world The works so far done in Bangladesh is not adequate and conclusive. Nevertheless, some of the important and informative works and research findings so far been done at home and abroad on this aspect have been reviewed in this chapter.

2.1. Ginger

A recent list of mechanisms are *viz.*,mycoparasitism, antibiosis, competition for nutrients or space, tolerance to stress through enhanced root and plant development, solubilisation and sequestration of inorganic nutrients, induced resistance and inactivation of the pathogens enzymes (Lewis *et el.,* 2001).

Ichitani (1980) worked on the control of rhizome rot of ginger by cultivating successively and protectively for immature rhizome production in plastic house. He reported that *Pythium zingiberum* was not consistently isolated from rotted ginger tissues and rhizome rot disease did not develop when disease free rhizomes were sown in soil fumigated with methyl bromide. He found that rhizome rot incidence was reduced when seed treated with echlomezol and methyl bromide.

Sharma and Dohroo (1991) described the post harvest management of rhizome rot *(Fusarium oxysporum f sp. zingiberi trujillo*) of ginger through chemical and antagonist. They also described that *Trichoderma* and *Gliocladium virens* inhibited growth of *Fusarium oxysporum f sp. zingiberi* in vitro by 73 and 68 percent, respectively.

Kim *et el.,* (1996) reported that average 18.1 % rhizome rot of ginger is recorded in Korea Republic and the disease starts early July, spreads rapidly in rainy season.

2.2. Bio-agents

Different biological control agents (BCAs) can be used for the control of plant diseases. These include fungi, bacteria and actinomycetes. The most important BCAs belong to the genus *Trichoderma* species, *Bacillus* species, *Pseudomonas* species and streptomycetes. Biological control of plant pathogens is an eye catching alternative to decrease heavy dependence of modern agriculture on costly chemical fungicides, which not only cause environmental pollution but also lead to the development of resistant strains (Harman *et.el.,* 2004).

Apart from bio control ability, the BCAs possess other traits such as rhizosphere competence, tolerance of fungicides, saprophytic competitive ability, ability to tolerate high and low temperatures, adaptability to different edaphic conditions, good searching ability, host specificity, high reproduction rate, short life cycle, adaptability, well adapted to different stages of life cycle of target host, able to maintain itself after reducing host population (Okigbo and Ikediugwu, 2000) have showed that *Trichoderma viride* displaced the naturally occurring mycoflora on the surface of the turmeric.

Trichoderma species are known to suppress infection of root by soil borne pathogens like *Macrophomina phaseolina*, *Rhizoctonia solani, Fusarium* species and *Pythium* species on various crops (Ehteshamul-Haque, *et el.,* 1990).

Species of *Trichoderma* also have growth promoting capabilities that may or may not be integral to biological control (Dubey *et el.*, 2007).

Trichoderma harzianum has shown effective control of root infecting fungi and root-knot nematodes (Spiegel and Chet, 1998).

Trichoderma harzianum isolated from rhizome rot suppressive soils reduced the disease and increased plant growth and yield (Ram *et el.*, 1999).

It has been reported that many *Trichoderma* species has an innate and/or induced resistance to many fungicides but the level of resistance varies with the fungicide (Omar, 2006).

Pseudomonas fluorescens is a Plant Growth Promoting Rhizobacteria (PGPR) as well as a broad spectrum biocontrol agent for soil borne as well as foliar pathogens including nematodes (Omar, 2006). This is an ideal candidate in organic agriculture and plays significant role in integrated disease management of turmeric. In view of this, investigation was conducted to test the possibility of combining *Trichoderma* and *Pseudomonas* species with fungicides under laboratory condition. The long term goal is to develop an effective IDM package for managing soil borne plant disease as well as to prevent the resistance development in pathogens to fungicides. Integrating chemical resistant *Trichoderma* and *Pseudomonas* species has an importance in the framework of integrated disease management. Disease prevention can be increased by using such tolerant species that keeps pathogens under sufficient pressure so that they cannot thrive. Keeping the above in view, the present work was designed to observe the compatibility of different fungicides with the BCA that is., *Trichoderma viride* (AUT1) and *Pseudomonas fluorescens* (AUP1) *in vitro*.

To develop an effective disease management programme, the compatibility of potential bio agents with fungicides is essential. Combinations of fungicides and

compatible bio agents in an IDM strategy protects the seeds and seedlings from soil borne and seed borne inoculum (Dubey and Patil, 2001).

Integration of compatible bio agents with fungicides may enhance the effectiveness of disease control and provide better management of soil borne diseases (Papavizas and Lewis, 1981).

The combination of BCAs with fungicides would provide similar disease suppression as achieved with higher fungicide use (Monte, 2001). Combining antagonists with synthetic chemicals eliminates the chance of resistance development and reduces the fungicide application. It is therefore, proposed to identify the compatibility of the potential bio agents with commonly used fungicides for the eco-friendly management of the tea diseases. As fungicides should have inhibitory effect on the pathogen but should not have deleterious effect on the antagonists, an understanding of the effect of fungicides on the pathogen and the antagonists would provide information for the selection of fungicides and fungicide resistant antagonists, through compatibility studies *in vitro.*

In addition, this strategy may display even better control of resistant strains of fungal pathogens and May help the commercial growers to reduce the amount of fungicide use, thus lowering the amount of chemical residue in the marketed products. Combined applications of BCAs followed by small quantities of fungicides may help the antagonists and the relative cost of the formulations (Thoudam and Dutta, 2014).

The combined use of BCAs and chemical pesticides has attracted much attention in order to obtain synergistic or additive effects in the control of soilborne diseases (Locke *et el.*, 1985).
Ram *et el.*, (1999) reported from their experiment that rhizome rot of ginger is caused by either *Pythium* or *Fusarium spp.* or both. The bio-control agent

Trichoderma harzianum, isolated from rhizome rot suppressive soils, reduced the disease and increased plant stand and yield of ginger. In order to further enhance the efficiency of disease suppression, a bacterial BCA *Pseudomonas spp*. was evaluated alone and in combination with *T. harzianum* and also with fungicidal rhizome treatment Combination of both BCAs resulted in better germination and plant stand, reduced disease and increased yield. Soil application of BCA was more effective compared with seed treatments. Bavistin + Ridomil MZ increased the efficiency of disease control as compared with their individual treatments Soil application of *T. harzianum* and rhizome treatment with Pseudomonas spp. and fungicides was the most effective among all the treatments tested.

Shanmugam and Varma (1999) conducted an experiment and native microorganisms were isolated from the rhizosphere of healthy ginger plants among rhizome rot affected plants in diseased fields during October 1994 and screened *in virto* for their antagonistic effects against the rhizome rot pathogen *Pythium aphanidermatum* by dual culture and cell free culture filtrate studies. *Aspergillus niger, A. fumigatus, A. flavus* and *Trichoderma vinde*. were found to be potential antagonists.

2.3. Fungicide with BCA

Among the fungicides tested (Copper oxychloride, Mancozeb and Bordeaux mixture). Mancozeb was compatible with all four antagonists. Resident isolates of biocontrol agents (BCAs) *Trichoderma harzianum, T. aureoviride* and *Gliocladium virens* and a non-resident isolate of *T. viride* were evaluated by Ram *et el*. (2000) for suppression of ginger rhizome rot, a rhizome and seed borne disease caused by *Fusarium solani* or *Pythium myriotylum* or both. Rhizomes pelletted with BCA were planted in two sets of pots, one with sterilized but pathogen infested soil, and another with unsterilized, rhizome rot infested field soil. All the four BCAs could establish in ginger rhizosphere and rhizomeplane

and significantly increased in population density and reduced that of *Fusarium spp.*, correlated well with reduction of the disease and significant increase in the yield. The trend of efficacy of each BCA observed in the unsterilized rhizome rot infested field soil was confirmed in sterilized, pathogen-infested soil.

Dohroo *et el.,* (1994) reported that the incidence of rhizome rot of *Z officinale* was minimum in soil treated with Pmus needle and neem cake pow'der. The *Meloidogyne* population was reduced to 74% but no *Pratylenchus* population was recorded in soil following any of the treatments such as neem cake powder, sawdust, Pinus needles or Quercus leaves. *Trichoderma* and *Gliocladium* populations were maximum in neem cake and pinus needle treatments.

Several antagonists were tested by Pandey *et el*., (1992) for the biological control of rhizome rot of ginger caused by *Fusarium oxysporum.* An extract of *Agave americana* was found to be very effective in controlling the disease under laboratory and field conditions, followed closely by culture filtrates/extracts of *Bacillus suhtilis, Cannabis sativa*, *Lyonia ovalifolia* and *Aspergillus niger*. The respective percent reductions of infection over controls were 75.9, 54.7 and 52.0.

A rise in peroxidase activity was recorded by Dohroo (1989) in ginger rhizomes infected by *Pythium pleroticum* and *Fusarium equiseti* 3 days after infection followed by a sharp decline. The decline was nearly double in rhizomes infected by *F. equiseti* compared with that in rhizomes infected by *P. pleroticum*. Polyphenol oxidase activity could not be detected in healthy or inoculated rhizomes.

Chauhan and Patel (1990) reported that rhizome rot disease is associated with a *Pythium spp* and *Fusarium solani*, either together or separately. Pathogenicity of both organisms was confirmed experimentally. This is the first report of *F. solani* causing soft rot of ginger and also of combined infection with *Pythium*, resulting in rapid drying of the shoot, followed by rhizome rot. All the metalaxyl

formulations tested were effective against *Pythium spp*. and Bordeaux mixture gave the best inhibition of *F solani*.

Systemic and contact fungicides like Bavistin 50WP, Ridomil Gold MZ72, Captan, Dithane M-45, Copper Oxychloride and Bordeaux mixture etc. were reported effective against the disease (Sagar 2006).

Rhizome rot of ginger can be controlled by the application of fungicides. Many researchers worked on the chemical control of the disease and they found very promising effect of different chemicals against the disease (Stirling *et el.,* 2006).

Sharma *et el.,* (1978) assessed systemic and contact fungicides in the control of rhizome rot of ginger caused by *Pythium aphanidermatum*. They found that the yield of rhizome was increased when they used fungicides. They reported that the Dithane Z-78 (Zineb) was the best fungicides in controlling rhizome rot of ginger, followed by Captan and Difolatan (Captafol).

Dohroo and Sharma (1983) evaluated fungicides for the control of rhizome rot of ginger caused by *Pythium pleroticum* and *Fusarium equiseti* in storage and obtained good control 6 with Antracol (propineb. 0.25%), Fycop and Blitox-50 (both copper oxychloride, 0.3%) as 30 min rhizome dips.

Rathaiah (1987) tested soft rot (*Pythium muriotylum*) of ginger by Ridomil and in combination with Captafol, Captan or Mancozeb. He stated that dipping or w etting of seed pieces 1 day before planting and soil drenching with a mixture of Ridomil + Captafol 3 months after planting controlled the disease and significantly increased the yield of ginger.

Ramchandran *et el.,* (1989) evaluated 5 systematic fungicides for efficacy against rhizome rot of ginger. The fungicides wrer tested as soil and seed treatments and they found metalaxyl formulations, namely Ridomil 5G granules and apron 35 WS gave the best control of the disease.

Raj *et el*., (1989) observed the chemical control of rhizome rot of ginger by seed and soil treatments .They found that soil treatment with 4% formaldehyde combined with treatment of the rhizome planting material with Toprinm-70

(thiophanate-methyl) at 0.1% gave the best control of this disease caused by *Fusarium oxysporum*. They also noticed that rhizome treated with 0.1% Bavistin (carbendazim) or 0.3% Dithane M-45 (mancozeb) in combination with soil treated with the formaldehyde gave satisfactory control of this disease.

Das *et el.,* (1990) stated the efficacy of fungicides for seed treatment against pre-emergence rhizome rot of ginger They reported the lowest incidence of this disease caused by *Pythium spp*. and highest percentage of germination was yielded by seed treatment with Captan 7 (0.2%) for 30 min, while Captafol (0.2%) and Dithane M-45 (manco/eb) at 0.3% were also effective.

Choe *et el.*, (1996) evaluate the effects of chemicals on the growth of *Pythium zingiberum* causing rhizome rot of ginger and inhabitation of the disease development. They isolated 52 fungal isolates which was obtained from ginger rhizomes with rotting symptoms from fields in Wanju (chonbuk) and Seosan (chungnam), Korea Republic, in 1993. They identified the pathogen as *Pythium zingiberum*. These appeared pathogenic to the plant in a pot test, although there were some variations in virulence among the isolates. Responses of the isolates to fungicides including metalaxyl (MT), metalaxyl + copper oxychloride (me), echlomezol (Em) and propamocarb hydrochloride (Pc) varied depending on the isolates tested. They found mycelial growth was almost completely inhibited by MC and MT at a concentration of 50 mg/litre.

Park *et el*., (1998) reported from their experiment conducted in 1994 that *Zingiber officinale* plant were infected by rhizome rot in seosan, Taean and Iksan, Korea Republic, from September to October. The pathogens associated with rhizome rot were isolated and identified as *Pythium spp., Fusarium spp.,* and bacteria. A total of 68 isolates of *P. zingiberum* w'ere tested for their tolerance to metalaxvl. Nine isolates were tolerant and showed mycelial growth on PDA containing 100 ppm of metalaxvl. At 500-1000 ppm, metalaxvl tolerant isolates grew their mvcelia and formed oospores, while metalaxvl susceptible isolates could not growr at > 10 ppm. Metalaxvl tolerant isolates were completely

inhibited by metalaxvl with carbendzim and with copper oxychloride at 1000 ppm.

Suppression of *Pythium aphanidermatum* and rhizome rot of ginger by *Aspergillus niger*, *A. terreus, Penicillium spp*. and *Absidia cylindrospora* was reported by Balakrishnan et el. (1997). The former 3 fungi inhibited *P. aphanidermatum* by up to 100% by producing fungitoxic non-volatile metabolities. *A. cylindrospora* expressed mild inhabitation (7.03%). *A. cylindrospora and P. aphanidermatum* also exhibited mutual overgrowth in dual culture. A. niger showed good protection against rhizome rot. The severity of rhizome rot infection was low when infested soil was treated with *A terreus*, *Penicillium* species and *A. cylindrospora* The highest yield was recorded with *A niger.*

The efficacy of 0.2% Dithane M-45, 0.3% Ridomil MZ, 0.1% Bavistan, 0.2% Saaf, 0.2% Shield, 0.3% Blitox-50 and 0.25 % Dithane M-45 + 0.05% Bavistin in controlling rhizome rot of ginger caused by Pythium aphanidermatum under storage and field conditions was determined in an experiment carried out in Bihar, India by Singh et el. (2004). Application of 0.3% Ridomil MZ resulted in the lowest incidence of the disease. In field conditions, application of Ridomil MZ resulted in the highest seed germination (96.50%) and yield (250.25 q/ha) and lowest disease incidence (5.0%).

Meena and Mathur (2003) conducted an experiment with three biological control agents i.e *Trichoderma viride, Gliocladium virens* and *Pseudomonas fluorescens* and an effective fungicidal mixture of Ridomil MZ and Bavistin 50 WP were used for treating seed rhizome and soil, individually and in combinations, for the suppression of rhizome rot of ginger Crop and disease parameters, such as crop stand, rhizome yield, rotting percentage and pathogen suppression in the rhizosphere, were determined. Pelleting of seed rhizome with biological control agents was not found effective. Pelleting either with the fungicidal mixture or BCAs combined with soil application of BCAs were effective in suppressing the disease and increasing the yield. In the rhizosphere pot study, integrated

approach resulted in reduction of 10 inoculum density of *R. solani* and increased in the BCAs population. Rhizome seed treatment with fungicidal mixture, followed by soil application of *G. virens* was the most effective treatment and superior to all other treatments.

Jacob *et el.*, (2002) earned out a preliminary trail in Kerala, India to manage the rhizome rot of ginger with combined applications of fungicides. The treatments comprised 4 fungicides (triademefon at 1 g/litre, benomyl at 1 g/litre, bitertanol at 1 g/litre and copper oxychloride at 3 g/litre) and an untreated control. Observations on the percentage of infested hills were recorded at 7, 14 and 21 days after treatment (DAT). The infestation was reduced over control in these treatments ranged 25.33 to 31.34

The effect of soil solarization and fungicidal seed and soil treatments of rhizome rot of ginger cv. Jhadole local was studied in Rajasthan, India by Kusum *et al*., (2002). Field plots inoculated with both pathogens were solarized for 20 day's under ambient day temperature of 37 7-45.0 and night temperature of 26.427.5°C. Seed were dipped in 2000 ppm of Captan (2 g/litre), Ridomil MZ (6.25 g/litre), or Chlorothalonil (2 g/litre) for 40 days before sowing. In non-solarized plots, seed treatment increased sprouting. Ridomil MZ seed treatment + Phorate + Ridomil MZ drench w'as most effective among the treatments in reducing disease intensity and in increasing the number of sprouts (215) and yield (1.51 kg). Phorate alone resulted in greater sprouting, lower disease incidence, and higher yields. In solarized plots, higher number of sprouts (247275), lower disease incidence (2.6-4.2%), and higher yields (1.36-1.62 kg) w-ere recorded for Ridomil MZ seed treatment + Ridomil MZ drcnch, Ridomil MZ seed treatment + Phorate + Ridomil MZ drench, Captan seed treatment + Phorate + Captan drench, and Captan seed treatment + captan drench. In the untreated control, disease intensity was lower in solarized plots (16.6%) than in non-solarized plots (20.4%).

2.4. Botanical extracts

Chavan *et el.*, (2018) carried out a study that the botanicals tested significantly inhibited mycelial growth of *P. aphanidermatum,* over untreated control. Average mycelial growth inhibition was ranged from 21.65 (*E. globulus*) to 86.33 (*A. indica*) per cent. However it was significantly highest with *A. indica* (86.33 %), followed by *A. sativum* (82.67 %), *O. sanctum* (76.07 %), *M. citrifoila* (64.93 %), *P. pinnata* (62.35 %), *M. oleifera* (59.96 %), *A. racemosus* (53.93 %), *L. innermis* (48.22 %), *G. maculate* (45.87 %), *L. camera* (36.83 %), *A. cepa* (32.88 %). However, *E. globulus* and *B. spectabilis* were found less effective with significantly least mycelial growth inhibition of 21.65 and 28.52 per cent, respectively.

Hasnat *et el*.,(2014) carried out a study to evaluate the efficacy of Bavistin 50WP (T1), Ridomil gold MZ72 (T2), Dithane M-45 (T3), Sulcox (T4), neem leaf extracts (T5), alamonda leaf extracts (T6), poultry waste (T7), saw dust (T8), *Trichoderma harzianum* (T9) and untreated control for controlling rhizome rot of ginger caused by *Fusarium oxysporum*. In lab experiment, cup method and disc method were used. In cup method, the highest inhibition (86.33%) was found in case of Bavistin 50 WP followed by Ridomil Gold MZ72, Dithane M-45, *Trichoderma harzianum*, alamanda leaf extracts, neem leaf extracts and sulcox. In disc method, Bavistin 50WP showed highest inhibition zone (5.53cm) followed by Ridomil Gold MZ-72. Among the botanicals, the effect of neem leaf extract was found better than the allamanda leaf extract. Allamanda leaf extract was made statistically similar inhibition zone with sulcox fungicide and *Trichoderma harzianum* In in-vivo assay the lowest disease incidence (27.78%) was recorded in case of Ridomil Gold applied plot which was statistically similar with the plots which were applied with poultry waste, Bavistin 50WP, Dithane M- and saw dust at 240 DAP while the highest disease incidence (63.89%) was recorded in untreated control plot.

MATERIALS AND METHODS

An experiment was conducted to evaluate the efficacy of selected bio-agents and botanicals against the pathogen of rhizome rot disease responsible for ginger decline. The selected treatments were evaluated through pot culture and lab conditions. Materials used and methodology followed in this study are included in this chapter.

3.1. Experimental site

The experiment was conducted in Bio-agents Laboratory and in the net house of The Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207.

3.2. Weather condition of the experimental site

The geographical situation of the experimental site was under the subtropical climate, characterized by three distinct seasons, the monsoon or rainy season from November to February and the pre monsoon period or hot season from March to April and monsoon period from May to October (Edris *et al*., 1979). The total annual rainfall of the experimental site was 218 mm and average monthly maximum and minimum temperature were 29 45°C and 13 86°C, respectively. Details of the metrological data of air temperature, relative humidity, rainfalls and sunshine during the period of the experiment was collected from the Bangladesh Meteorological Department (Climate Division), Agargaon, Dhaka..

3.3. Experimental Duration

The experiment was carried out during the period July, 2019 to June, 2020.

3.4. Design and layout of the experiment

The experiment was laid out in completely randomized design (CRD). In total eight treatments with three replications were codified to achieve the desired objectives.

3.5. Treatments of the experiment

Treatments codified for this study are given below-

T_0 – Control

T_1– *Verticillium lecanii*

T_2 – *Beauveria bassiana*

T_3 – *Metarhizium anisopliae*

T_4 – *Trichoderma viride*

T_5 – Tea wastage

T_6 –Neem extract

T_7– Allamanda leaf extract

3.6. *In-vitro* study-1 (Bio-agent multiplication)

3.6.1. Collection of Bio-agents

The species of selected bio-agents were collected from local dealer of ACI Bangladesh Ltd. The details of bio-agents were used in this study are given in Table1.

Table 1: Selected bio-agents used in the study.

Sl. No	Trade Name	Name of Bio-agents
01.	Bio-Catch	*Verticiilium licanii*
02.	Bio-Magic	*Metarhizium anisopliae*
03.	Bio-Power	*Beauveria bassiana*
04.	Tricho-ACI	*Trichoderma viride*

3.6.2. Multiplication of Bio-control agents

The selected bio-agents were cultured in artificial media PSA for multiplication, and appearance of mycelium and spores were studied through microscopy.

3.6.3. Method of inoculation

3.6.3.1. *Verticillium lecanii*

The pour plate technique was adopted for the inoculation of *Verticillium lecani* 1.50 % LF (Bio-Catch) into the solid medium. One gram of liquid formulation of *Verticillium lecani* 1.50 % LF (Bio-Catch) was thoroughly mixed in a flask containing 99 ml of sterile distilled water labelled flask 1:1:10,000 dilution (10^{-4}) and serially diluted upto 10^{-6}. Four replicates were maintained for each dilution factor. Pipetted out 1ml of supernatant solution and transferred into petri dish. The melted agar medium was poured into petri dish containing the suspension of *Verticillium lecani* 1.50 % LF (Bio-Catch). The inoculation was carried out aseptically by using laminar air flow chamber. Allowed the medium to solidity for few minutes. Then labelled each petri dish with inoculation date.

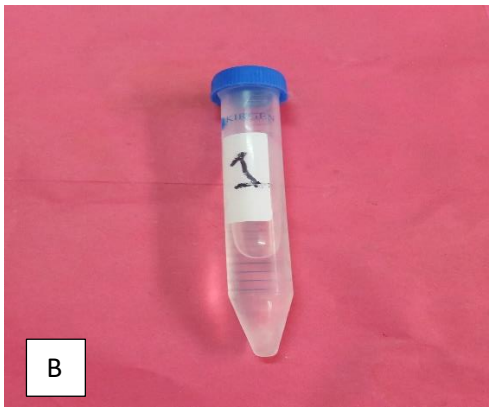
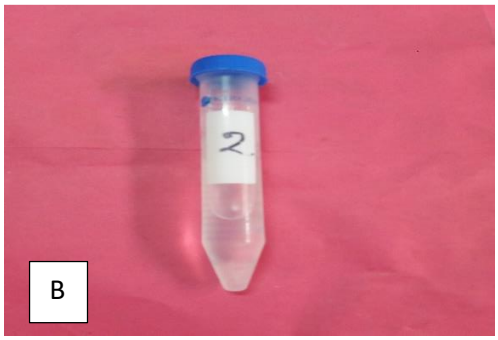


Plate 1- Bio-catch pack (A), Liquid formation (B), Verticillium lecanii grown on PSA plate after pouring supernatant solution of Bio-catch (C).

3.6.3.2. *Materhizium anisopliae*

The pour plate technique was adopted for the inoculation of *Metarhizium anisopliae* 1.15 % WP (Bio Magic) into the solid medium. One gram of powdered formulation of *Metarhizium anisopliae* (Bio Magic) was thoroughly mixed in a flask containing 99 ml of sterile distilled water labelled flask 1:1:10,000 dilution (10^{-4}) and serially diluted up to 10^{-8}. Four replicates were maintained for each dilution factor. Pipetted out 1ml of supernatant solution and transferred into petri dish. The melted agar medium was poured into petri dish containing the suspension of *Metarhizium anisopliae* (Bio Magic). The inoculation was carried out aseptically by using laminar air flow chamber. Allowed the medium to solidity for few minutes. Then labelled the each petri dish with inoculation date.



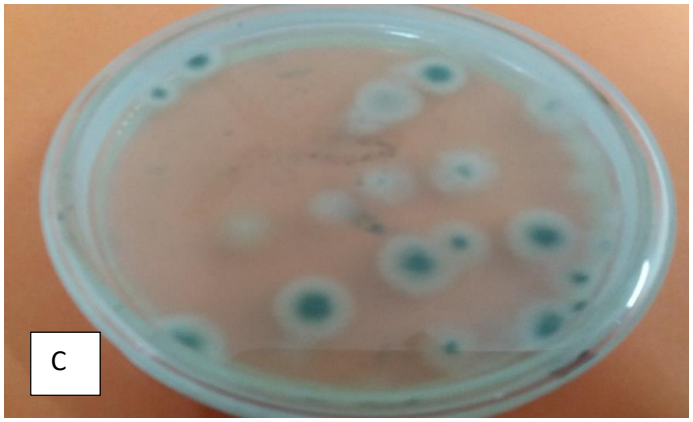
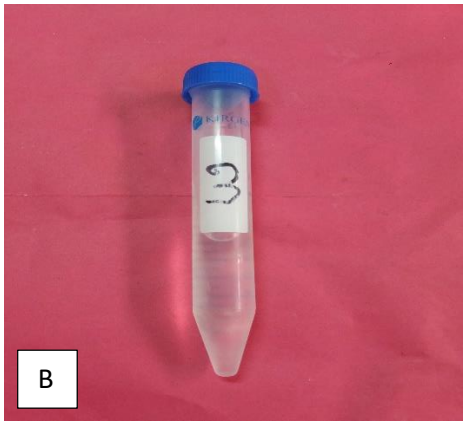


Plate 2- Bio-magic pack (A), Liquid formation (B), Serial dilution/pour (1 X 10^{-10} dilution) of Bio Magic and observed *Metarhizium anisopliae*. (C)

3.6.3.3. *Beauveria bassiana*

The pour plate technique was adopted for the inoculation of *Beauveria bassiana* 1.50 % LF (Bio-Power) into the solid medium. One gram of liquid formulation of *Beauveria bassiana* 1.50 % LF (Bio-Power) was thoroughly mixed in a flask containing 99 ml of sterile distilled water labelled flask 1:1:10,000 dilution (10^{-4}) and serially diluted up to 10^{-8}. Four replicates were maintained for each dilution factor. Pipetted out 1ml of supernatant solution and transferred into petri dish. The melted agar medium was poured into petri dish containing the suspension of *Beauveria bassiana* 1.50 % LF (Bio-Power). The inoculation was carried out aseptically by using laminar air flow chamber. Allowed the medium to solidity for few minutes. Then labelled each petri dish with inoculation date.



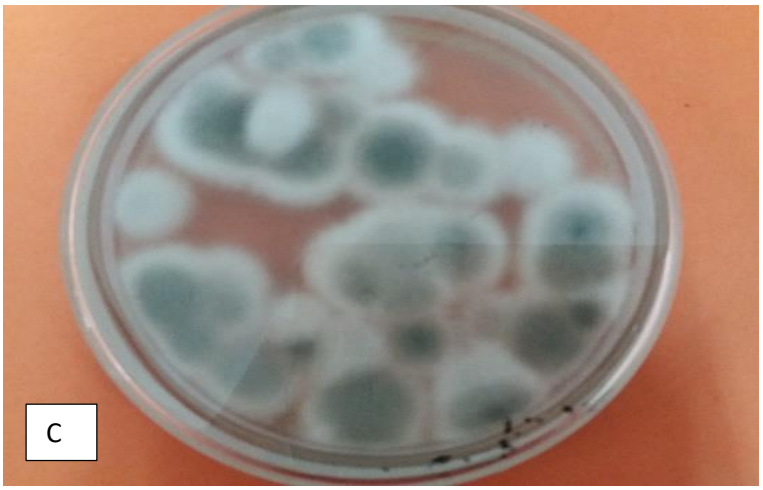


Plate 3-Bio-power pack (A), Liquid formation (B), Serial dilution/pour (1 X $10^{-8)}$ dilution of Bio power and observed *Beauveria bassiana.*(C).

3.6.3.4. *Trichoderma viride*

For the treatment purpose *Trichoderma viride* solution was collected from the available sources and diluted with distilled water. The diluted solution was applied in petridish in cup method.

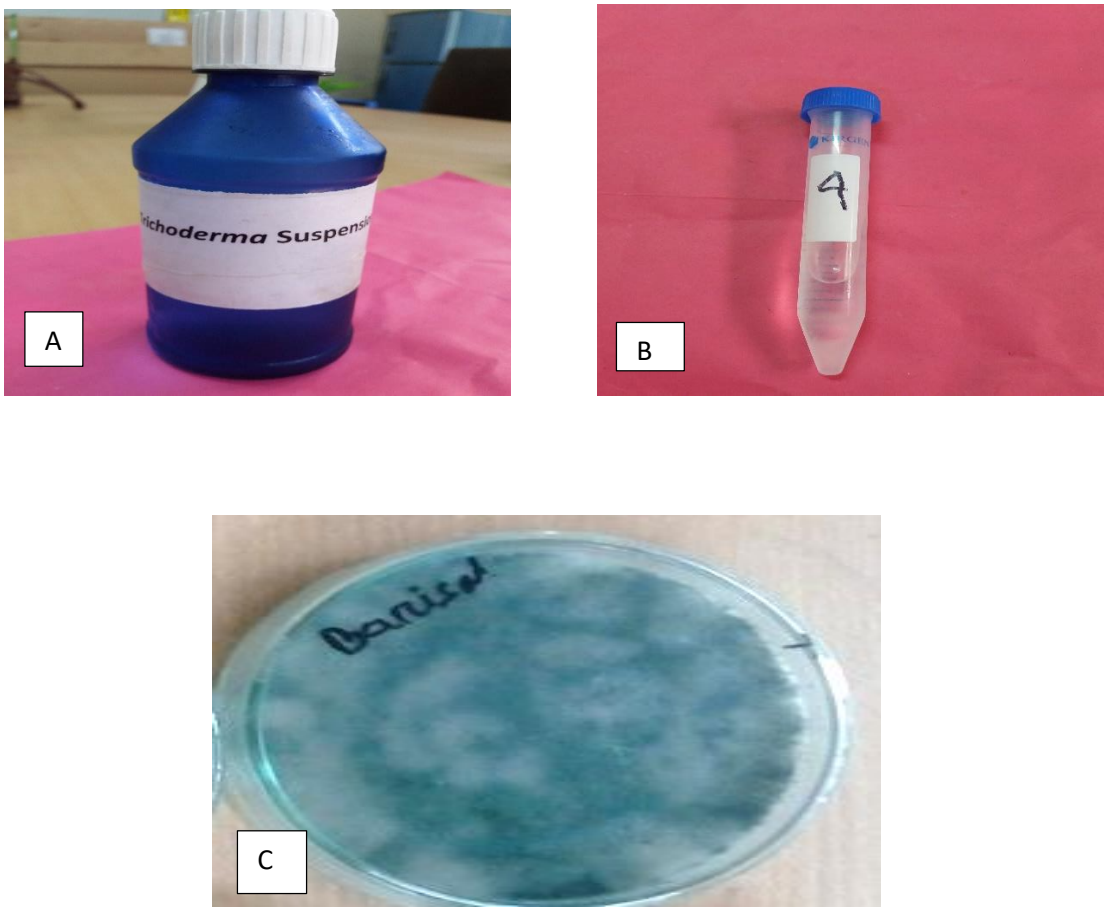


Plate 4- Tricho-ACI suspension (A) Liquid formation (B) Serial dilution/pour($10x10^{-4}$) of Biocontrol agent and observed *Trichoderma viridae*. (C).

3.6.4. Quality Test

Quality test performed through analytical method (pour plate method) for morphological and cultural variability test and counting the viable spores/colony forming units (CFU's) on selective medium using Hema-cytometer

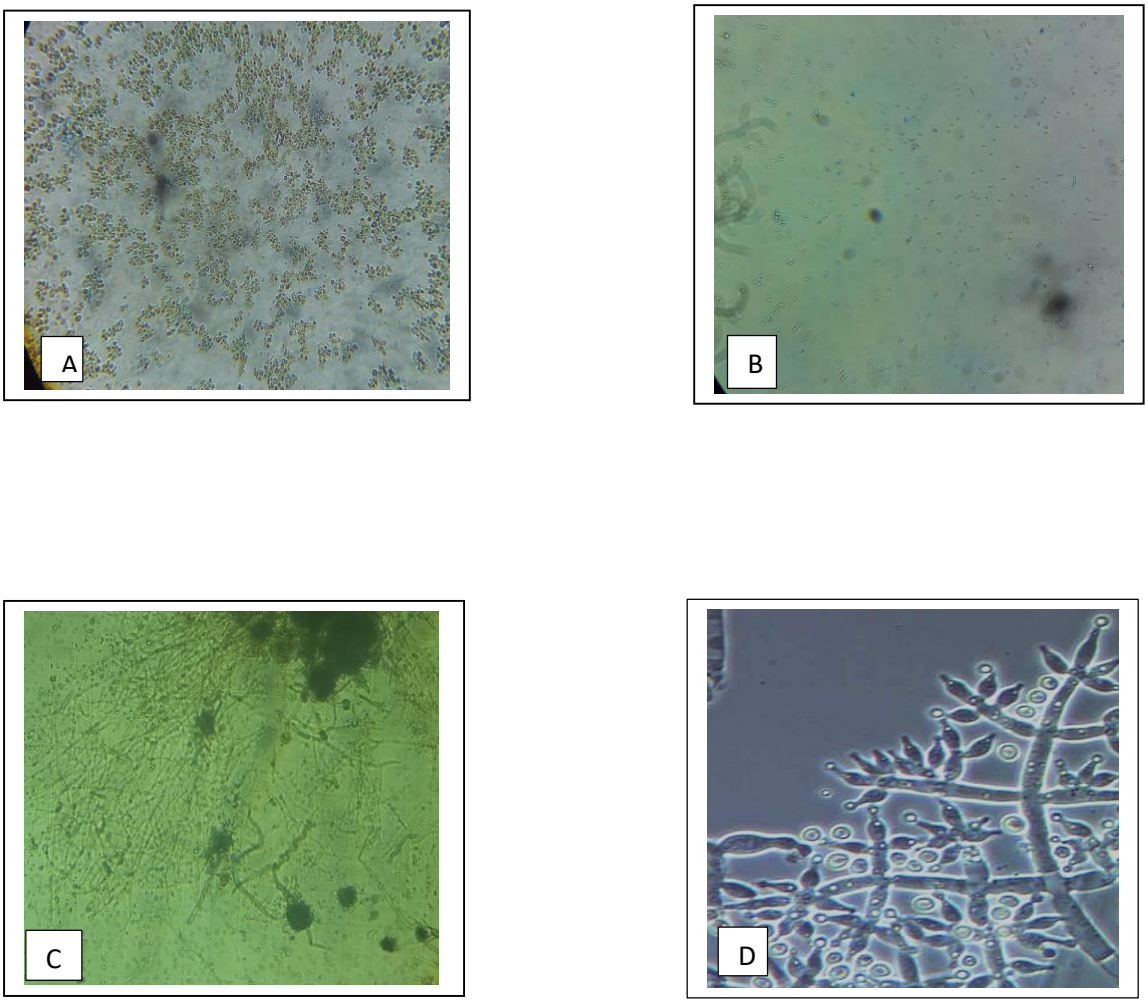


Plate 5- Viable spores count (CFU's) thought hemacytometer and counted 1 X 10^{-4} colony per ml minimum (*Beauveriana bassiana*) (A), Viable spores count (CFU's) thought hemacytometer and counted 1X10^{-10} colony/ml minimum *(Metarhizium anisopilae*)(B) , Viable spores count (CFU's) thought hemacytometer and counted 1 X 10^{-6} colony per ml minimum (*Verticillium lecanii*)(C), Viable spores count (CFU's) thought hemacytometer and counted colony per ml (*Trichoderma viride*)(D).

3.7. *In-vivo* study (Pot culture)

3.7.1. Planting materials

In this research work, the rhizomes of gingers were used as planting materials. The rhizomes were collected from Bangladesh Agricultural Research Instute. The rhizomes of ginger were broken into small pieces bearing 1-2 buds The average weight of individual pieces was 40-50 gm.

3.7.2. Prepared bio fortified compost of selected bio-agents

Five different substrates such as saw dust, poultry manure, mustard oil cake, wheat grain and cow dung were used to make selected bio fortified compost. To do this 8 kg saw dust, 18 kg poultry manure, 8 kg masturd oil cake, 8 kg wheat grain and 18 kg cow dung was collected. Cow dung, poultry manure, saw dust and mustard oil cake was placed in a pit for decomposition. Liquid formation of selected bio-agent *Trichoderma viride*, *Verticillium lecanii*, *Metarhizium anisopliae* and *Beauveria bassiana* were mixed with wheat grains separately (2 kg wheat grain/pit) for decomposition. After decomposition, bio-fortified substrates were transferred into pot and colonized bio-agent was mixed in selected pots.

Ingradients	Amount
Cow dung	18 kg
Poultry Manure	18 kg
Mustard Oil Cake	8 kg
Saw Dust	8 kg
Wheat grain	8 kg

Plate 6- Prepared bio fortified compost of selected bio-agents, Cow dung, poultry manure, saw dust and mustard oil cake was collected(A), This substrates were placed in selected pot(B), Liquid formulation of selected bio-agent(C), Selected bio-agents were mixed with wheat grain separately, E. Bio fortified substrates were transferred into pot(D).

3.7.3. Pot preparation and rhizomes sowing

Rhizomes were sown in pots that were prepared with bio-fortified soil media and normal soil. Rhizomes were sown just next day of rhizomes treatment with Gibberellic acid (GA_3) for enhancing for germination. Pot to pot distance was maintained 20 cm and row to row distance 45 cm. Rhizomes were sown at a depth of 5 to 7 cm.

Plate 7- Rhizomes sowing in prepared pot (A), (B).

3.7.4. Pot culture in net house conditions

Pots were placed in net house for proper plant growing and checking the compatibility of selected bio-control agents and botanicals.

3.8. Collection of botanicals extract

The selected botanicals viz. Neem leaves extract, Allamanda leaves extract and Tea wastage were collected from locally and prepared to study its compatibility with selected bio-control agents under *in vitro* conditions. The details of botanicals extract were used in this proposed study are given in Table2.

Table 2: Selected botanicals extract used in the study.

Sl. No	Name of Botanical extracts	Scientific Name
01.	Neem leaves extract	*Melia azadiracta*
02.	Allamanda leaves extract	*Allamanda cathartica*
03.	Tea wastage	*Camellia sinensis*

3.8.1. Preparation of plant extracts

For extraction of juice, required amount of respective parts of each plant was taken, washed in tap water, crushed in a mortar and pestle. The crushed materials were blended in an electric blender adding equal amount of sterile water for 1:1 solution The blend was filtered through sterile cheese cloth. The supernatant was diluted in equal amount of sterile water for 1:2 solution.

3.8.2. Application of botanicals extract

Extracted botanicals were added directly to the selected pots/plants as a treatment for checking the compatibility of selected bio-agents.

3.8.3. Intercultural operation

When the plantlets started to emerge in the pots it was always kept under careful observation and various intercultural operations were accomplished as and when necessary plants for better growth and development of the ginger plants.

3.8.4. Irrigation

Light over-head irrigation was provided with a watering can to the plots immediately after sowing of rhizome seeds. Surface irrigation was given time to time as needed.

3.8.5. Weeding

Weeding was carefully done for proper growth and development of rhizomes wilt required intervals.

3.8.6. Plant protection

The plants were protected from the attack of insect-pest by spraying insecticide. Insecticide was used as required according to the recommended doses.

3.9. Data recording before harvesting

Data were collected in respect of the plant growth characters. The following parameters were set up for data recording before harvesting on the basis of above ground symptom.

3.9.1. Infected tiller (%)

Percent of infected hill was estimated with counting every tiller from each pot.

3.9.2. Disease incidence (%)

Disease incidence was measured in percentage on the basis of infected plant at 50,70 and 90 days after planting.

3.9.3. Disease severity (%)

Disease severity was measured in percentage on the basis of infected tiller at 50,70 and 90 days after planting.

3.10. Harvesting

Harvesting was done when rhizomes were properly matured that was indicate by yellowing all leaves and dried.

Plate 8- Pictorial view of harvesting of rhizome in net house (A), (B).

3.11. Data recording after harvesting

Data were collected in respect of the yield of ginger. Data were collected in disease incidence in below ground symptom, disease severity in below ground symptom, number of rhizome, weight of rhizome, fresh weight, dry weight and radial growth of mycelium in different days. The following parameters were set up for recording data and for the interpretation of the results.

3.11.1. Disease incidence in below ground symptom

Disease incidence was measured in percentage on the basis of below ground symptom.

3.11.2. Disease severity in below ground symptom

Disease severity was measured in percentage on the basis of below ground symptom.

3.11.3. Isolation and identification of pathogen

The diseased rhizomes were collected in polythene bag and taken to the laboratory of Department of Plant Pathology. Sher-e-Bangla Agricultural University, Dhaka. Then the diseased rhizomes were surface sterilized with Chlorox (1:1000) for one minute Then the rhizomes were washed into sterilized water thrice and placed in a petridish. The petridish containing rhizomes were incubated at 25±1°C for seven days. Then the organism grew freshly on the rhizome and isolated and cultured again on another PSA plate to have pure culture. Finally the pure culture of the pathogen was obtained and identified.

3.11.4. Number of rhizome

The number of rhizome was recorded in each pot at the time of harvest.

Plate 9- Pictorial view of rhizome in ginger plant (A), (B).

3.11.5. Fresh weight of rhizome

The fresh weight of rhizome per pot was recorded in kg at the time of harvest.

3.11.6. Dry weight of rhizome

Dry weight of rhizome per pot was measured in kg some days after harvest.

3.12. *In-vitro* study-2 (Evaluation of bio-control agents and botanicals extract in-vitro)

To study the compatibility of selected bio-agents and botanicals extract, the poisoned food technique was applied.

3.12.1. Poisoned Food Technique Method

The poisoned food technique the purpose of this experiment was to evaluate the efficacy of tested fungicides at different concentrations against *Trichoderma viride* which were available currently on market to control fungal pathogens. The quantity of fungicides needed to get the desired concentration was added to 100

ml sterilized, molten PSA medium in 250 ml conical flask, mixed well and poured in sterilized petri dishes at the rate of 15-20 ml per plate. To avoid contamination, all ten or 7 fungicides were exposed to UV light for a period of 30 min before adding it into the medium. After solidification of the medium, mycelial discs of 8 mm diameter from actively growing fungal antagonist were cut and placed at the centre of each petri dish. Control consisted of PSA medium alone inoculated with the antagonist. Three replications were maintained for each concentration. The inoculated plates were incubated at room temperature and observations on the mycelial growth of the fungal antagonist were taken when control plates showed full growth. The relative growth reduction for each fungicide was calculated by the equation below.

$$L= C-T/C \text{ X } 100$$

Where L is percentage of inhibition in growth of *Trichoderma viride*; C is radial growth of the *Trichoderma viride* in control; T is radial growth (mm) of the *Trichoderma viride* in the presence of the fungicides (Rita and Tricita, 2004) [19].

3.12. 2. Data collection on inhibition rate of mycelial growth

Data were collected on the basis of percentage inhibition of radial mycelial growth of identified pathogen responsible for ginger decline against the selected bio-agents and botanicals extract at 2 days, 5days and 10 days.

3.13. Data analysis

The data obtained from all the studied were analyzed by using the Statistix-10 computer based software.

RESULTS

The present study was conducted to evaluate the compatible bio-agents and selected botanicals against the pathogen of rhizome rot disease responsible for ginger decline. The control of rhizome rot disease of ginger in response to different selected treatments, percent disease incidence and severity on the basis of above and below ground symptoms isolation and characterization disease causing agent inhibition of radial mycelial growth of the identified pathogen against the selected treatments, bio-agents and botanicals, yield and yield attributes were recorded. The results have been presented in graphs, figures, and tables under the following heading and sub-heading.

4.1. Effect of selected compatible bio-agents and botanicals on disease incidence (%) on the basis of above ground symptoms at 50, 70 and 90 days after planting (DAP)

In terms of percent disease incidence on the basis of above ground symptoms the selected bio-agents and botanicals were showed promising performances against rhizome rot disease of ginger in comparison to control treatment.

At 50 DAP, the highest disease incidence (66%) was estimated in control treatment and the lowest disease incidence (33%) was recorded in T_1 (*Verticillium lecanii*), T_4 (*Trichoderma viride*) and T_7 (*Allamanda leaf extract*) treatments. There was no disease found in T_2 (*Beauveria bassiana*), T_3 (*Metarhizium anisopliae*), T_5 (Tea wastage) and T_6 (Neem extract) treatments at 50 DAP.

AT 70 DAP, the lowest disease incidence (33.33%) was recorded in T_1 (*Verticillium lecanii*) and T_2 (*beauveria bassiana*) and T_3 (*Metarhizium anisopliae*) treatments. The highest disease incidence (100%) was again recorded in control treatment. Others treatments T_4 (Neem extract) and T_7 (Allamanda leaf extract) showed moderate disease incidence (66.66%) and no disease was found in T_5 and T_6 at 70 DAP.

At 90 DAP the treatments effects were found statistically significant. The lowest disease incidence (33%) was recorded in T_3 (*Metarhizium anisopliae*) and T_5 (Tea wastage) treatments. The highest disease incidence (100%) was found in control treatment and as well as in T_1 (*Verticillium lecanii*) and T_7 (Allamanda leaf extract) treatments. T_2 (*Beauveria bassiana*) and T_4 (Neem leaf extract) treatments showed the moderate disease incidence (66.66) and no disease was found in T_6 up to 90 DAP or up to harvesting. A considerable reduction of disease incidence was achieved by using selected compatible bio-agents and botanicals in the experiment compared to control (Plate 10). Results regarding the disease incidence (%) on the basis of above ground symptoms at 50, 70 and 90 days after planting (DAP) presented in (Figure 1).

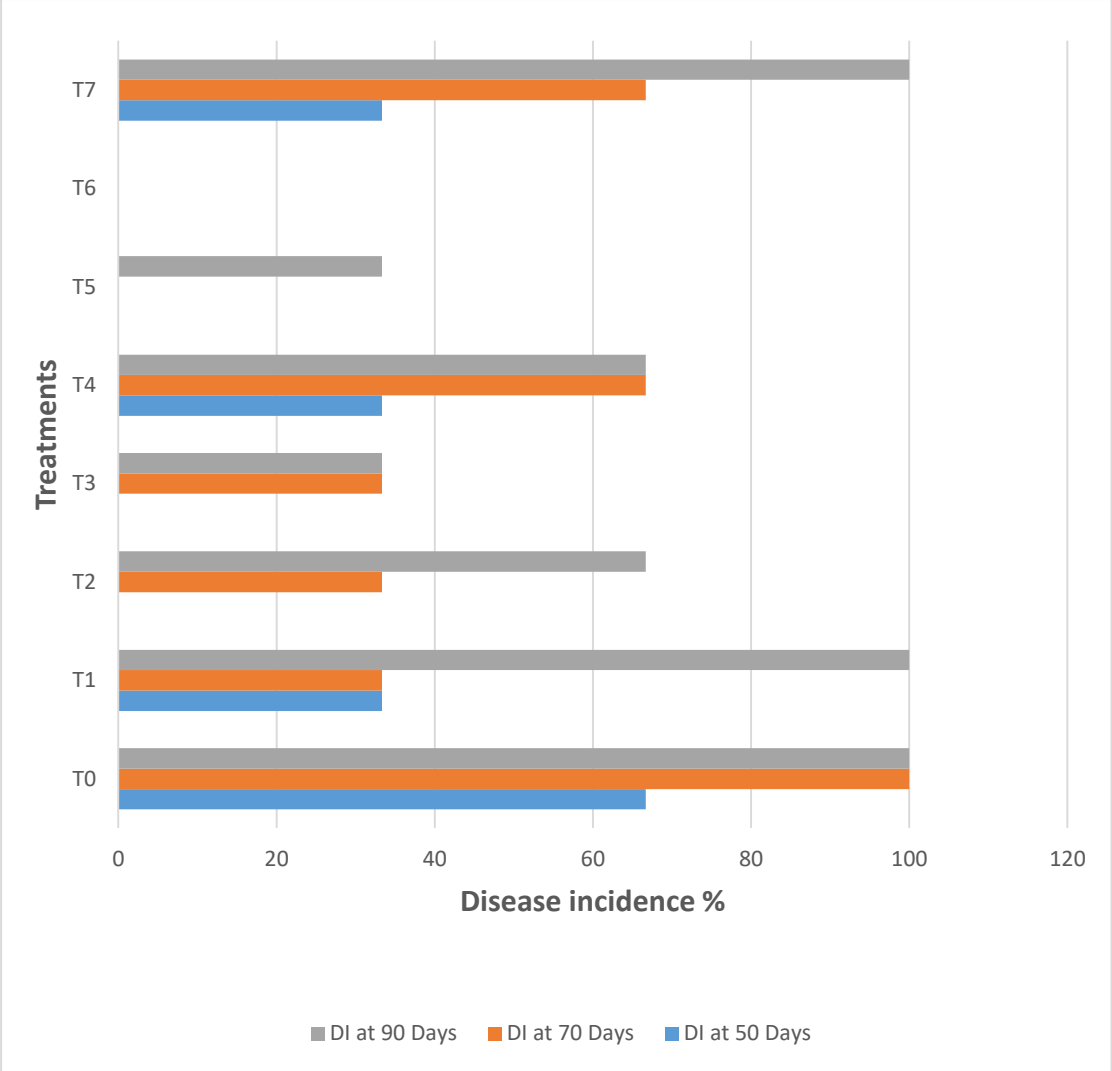


Figure 1: Effect of selected bio-agents and botanicals on disease incidence on the basis of above ground symptom.

Plate 10- Above ground symptom of rhizome rot of ginger plant;in control treatment (A), healthy ginger plant in treatment T_6 (Neem extract)

4.2. Effect of selected compatible bio-agents and botanicals on disease severity (%) on the basis of above ground symptoms at 50, 70 and 90 days after planting (DAP)

In terms of percent disease severity on the basis of above ground symptoms the selected bio-agents and botanicals were showed promising performances against rhizome rot disease of ginger in comparison to control treatment.

At 50 DAP, the highest disease severity (27.77%) was estimated in control treatment and the lowest disease severity (4.16%) was recorded in T_7 (Allamanda leaf extract) and (6.48%) was recorded in T_1 (*Verticillium lecanii*). There was no disease found in T_2 (*Beauveria bassiana*), T_3 (*Metarhizium anisopliae*) , T_4 (*Trichoderma viride*) T_5 (Tea wastage) and T_6 (Neem extract) treatments at 50 DAP.

AT 70 DAP, the lowest disease severity (2.22%) was recorded in T_3 (*Metarhizium anisopliae*) treatments. The highest disease severity (60%) was again recorded in control treatment. Others treatments T_4 (*Trichoderma viride*), T_1 (*Verticillium lecanii*) and T_7 (Allamanda leaf extract) was showed moderate disease severity (8.33%), (10.17%) and (12.72%) and no disease was found in T_2 (*beauveria bassiana*) ,T_5 (Tea wastage) and T_6 (Neem extract) at 70 DAP.

At 90 DAP the treatments effects were found statistically significant. The lowest disease severity (8.88%) was recorded in T_5 (Tea wastage) treatments and the highest disease severity (91.66%) was found in control treatment. T_1 (*Verticillium lecanii*) , T_2 (*Beauveria bassiana*) , T_3 (*Metarhizium anisopliae*), T_4 (*Trichoderma viride*) and T_7 (Allamanda leaf extract) treatments was showed the moderate disease severity. Which were 22.10%,19.76%,11.65%,14.99% and 18.35% respectively. There was no disease found in T_6 up to 90 DAP or up to harvesting (Figure 2). A considerable reduction of disease severity was achieved by using selected compatible bio-agents and botanicals in the experiment compared to control (Plate 11).

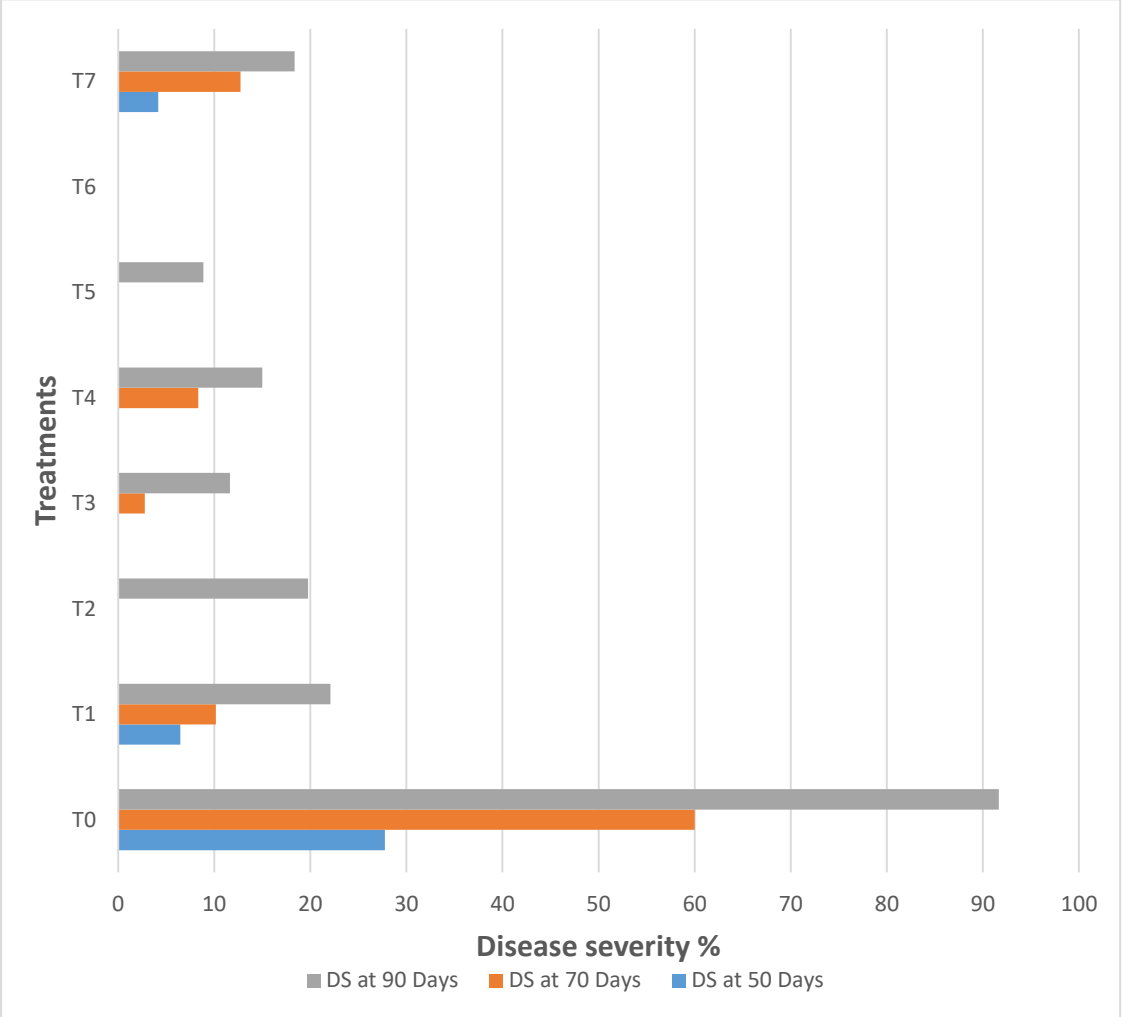


Figure 2: Effect of selected bio-agents and botanicals on disease severity on the basis of above ground symptom.

Plate 11- Above ground symptom of rhizome rot of ginger plant;A. in control treatment, B. healthy ginger plant in treatment T_6 (Neem extract)

4.3. Effect of selected compatible bio-agents and botanicals on disease incidence (%) on the basis of below ground symptoms

In terms of percent disease incidence on the basis of below ground symptoms the selected bio-agents and botanicals were showed promising performances against rhizome rot disease of ginger in comparison to control treatment.

The lowest disease incidence (7.87%) of below ground was recorded in T_5 (Tea wastage). On the other hand the highest disease incidence of below ground (71.10%) was recorded in untreated control. Others treatments T_1 (*Verticillium lecanii*) , T_2 (*Beauveria bassiana*) , T_3 (*Metarhizium anisopliae*), T_4 (*Trichoderma viride*), and T_7 (Allamanda leaf extract) was showed moderate disease incidence and they were 21.43%,21.8%,22.02%,20% and 12.26% respectively. There was no disease found in T_6 (Neem extract) on below ground symptom. Results regarding the effect of selected compatible bio-agents and botanicals on disease incidence (%) on the basis of below ground symptoms and presented in (Figure 3).

4.4. Effect of selected compatible bio-agents and botanicals on disease severity (%) on the basis of below ground symptoms

In terms of percent disease severity on the basis of below ground symptoms the selected bio-agents and botanicals were showed promising performances against rhizome rot disease of ginger in comparison to control treatment.

The highest disease severity of below ground (52.66%) was recorded in untreated control. Others treatments T_1 (*Verticillium lecanii*), T_2 (*beauveria bassiana*) , T_3 (*Metarhizium anisopliae*), T_4 (*Trichoderma viride*), T_5 (Tea wastage) and T_7 (Allamanda leaf extract) was showed moderate disease severity which were 2.47%,2.43%,2.88%,1%,1% and 1.76% respectively. There was no disease found in T_6 (Neem extract) on below ground symptom. Results regarding the effect of selected compatible bio-agents and botanicals on disease severity (%) on the basis of below ground symptoms and presented in (Figure 4).

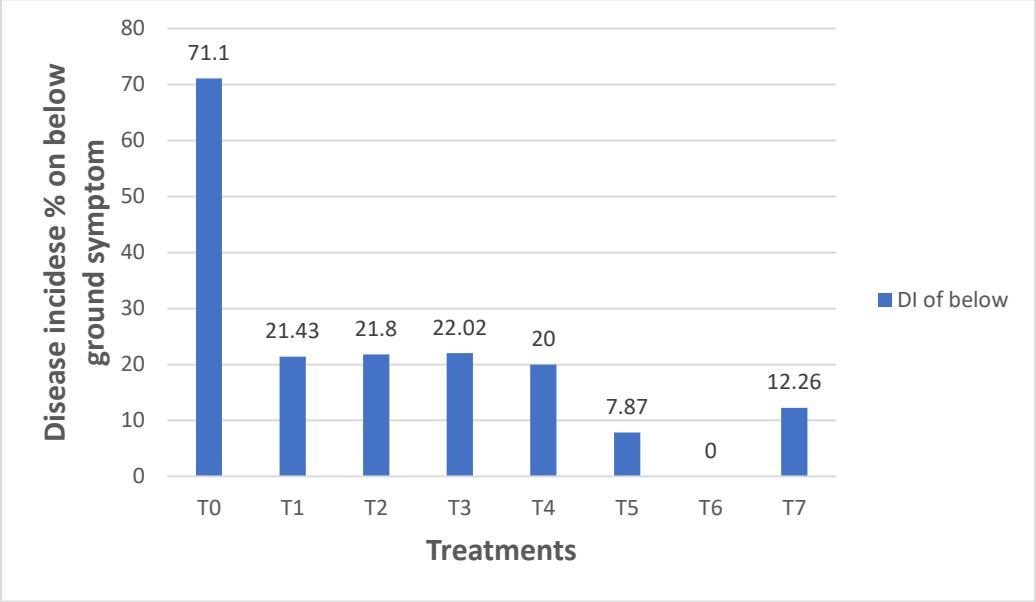


Figure 3: Percentage of disease incidence on the basis of below ground symptom.

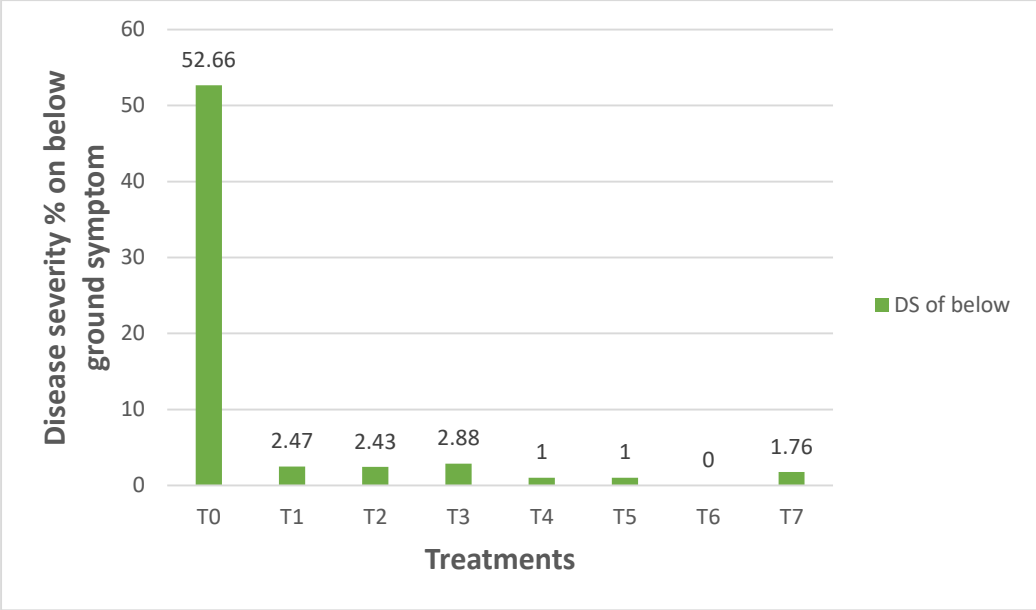


Figure 4: Percentage of disease severity on the basis of below ground symptom.

4.5. Effect of selected treatments on number of rhizomes, fresh and dry weight of rhizomes

The treatments effect on number of rhizomes, fresh and dry weight of rhizomes were found statistically significant. The highest number (13) of rhizomes were counted in T_1 (*Verticillium lecanii*) followed by T_3 (*Metarhizium anisopliae*) treatment which was 9.67. The lowest number of rhizome (4.67) was counted in T_0 (control) treatment. Number of rhizomes were found all most similar in others treatments which were statistically similar.

The highest fresh weight of rhizomes (412.83gm) was recorded in T_3 (*Metarhizium anisopliae*) followed by T_4 (*Trichoderma viride*) and T_1 (*Verticillium lecanii*) that was 379.17gm and 338.0gm which was statistically identical each with other. The lowest fresh weight of rhizomes (116.33gm) was recorded in to T_0 (control) which was statistically different from all of the treatments. The moderate fresh weight was obtained in T_2 (*Beauveria bassiana*), T_6 (Neem extract), T_5 (Tea wastage) and T_7 (Allamanda leaf extract).That were 310.67 gm, 296.67 gm, 253 gm and 251.67 gm respectively, which were statistically identical each with other.

The highest dry weight of rhizomes (304.33gm) was recorded in T_3 (*Metarhizium anisopliae*) followed by T_4 (*Trichoderma viride*) and T_6 (Neem extract) that was 240.63gm and 223.78gm which was statistically identical each with other. The lowest fresh weight of rhizomes (71.60gm) was recorded in to T_0 (control) which was statistically different from all of the treatments. The moderate fresh weight was obtained in T_1 (*Verticillium lecanii*) T_2 (*beauveria bassiana*), T_5 (Tea wastage) and T_7 (Allamanda leaf extract). That were 216.97gm, 212.15 gm, 188.52 gm and 204.74 gm respectively, which were statistically identical each with other. Results regarding the number of rhizomes, fresh and dry weight and presented in table 3.

Table 3: Effect of selected bio-agents and botanicals on number of rhizome fresh and dry weight.

Treatment	Number of rhizome/pot	Fresh Weight (gm)	Dry Weight (gm)
T_0 (Control)	4.67 c	116.33 d	71.60 c
T_1 (*Verticillium lecanii*)	13.00 a	338.00 abc	216.97 b
T_2 (*Beauveria bassiana*)	9.33 b	310.67 bc	212.15 b
T_3 (*Metarhizium anisopliae*)	9.67 ab	412.83 a	304.33 a
T_4 (*Trichoderma viride*)	9.33 b	379.17 ab	240.63 ab
T_5 (Tea wastage)	9.00 b	253.00 c	188.52 b
T_6 (Neem extract)	10.00 b	296.67 bc	223.78 b
T_7 (Allamanda leaf extract)	8.33 b	251.67 c	204.74 b
CV %	24.68	26.89	26.99

4.6. Relationship between number of rhizome and percent disease incidence at 90 DAP (on the basis of above ground symptoms).

From the relationship study between number of rhizomes per pot with percent disease incidence at 90 DAP (on the basis of above ground symptoms), it was revealed that among the botanical treatments, number of rhizome per pot increased with decreased of percent disease incidence (%).Among the bio-agents treatments, although the disease incidence was high but the number of rhizomes was also higher due to use the bio-fortified soil in these treatments. (Figure 5).

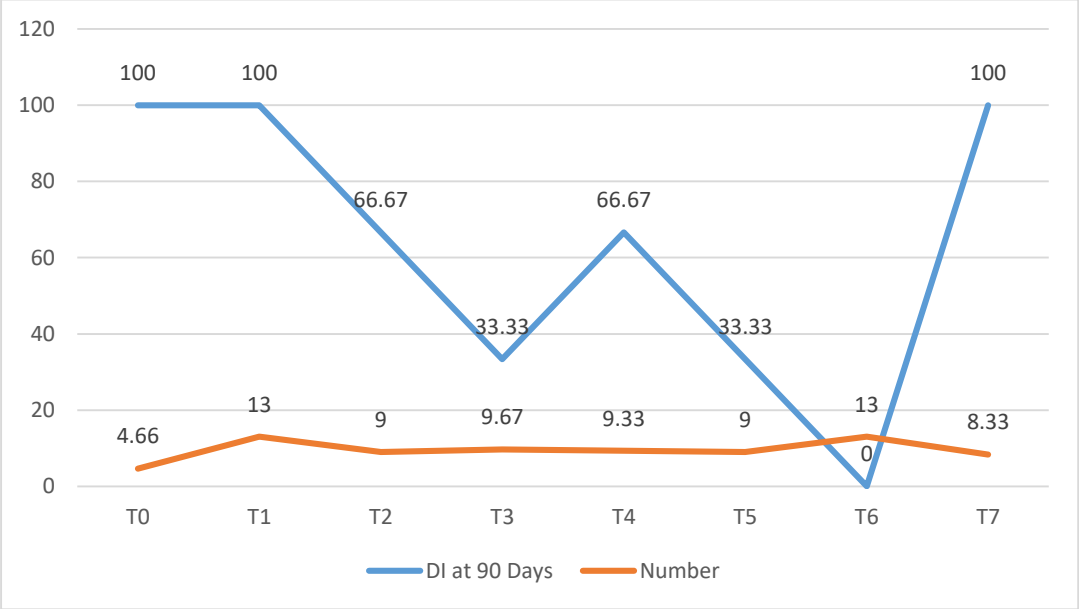


Figure 5: Relationship between number of rhizome per pot and disease incidence (%) at 90 DAP

4.7. Relationship between number of rhizome and percent disease severity at 90 DAP (on the basis of above ground symptoms).

From the relationship study between number of rhizomes per pot with percent disease severity at 90 DAP (on the basis of above ground symptoms),it was revealed that among the botanical treatments, number of rhizome per pot increased with decreased of percent disease severity (%).Among the bio-agents treatments, although the disease severity was high but the number of rhizomes was also higher due to use the bio-fortified soil in these treatments. Relationship between number of rhizome and percent disease severity at 90 DAP (on the basis of above ground symptoms) presented in (Figure 6).

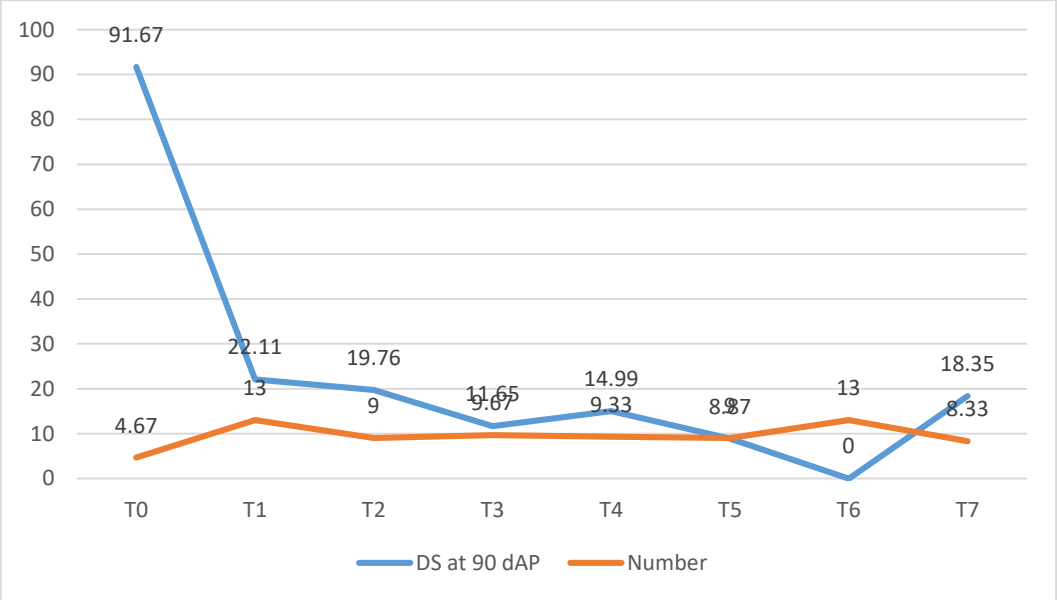


Figure 6: Relationship between number of rhizome per pot and disease severity (%) at 90 DAP

4.8. Relationship between number of rhizome and percent disease incidence (on the basis of below ground symptoms).

From the relationship study between number of rhizome per pot and disease incidence (%), it was depicted that among the botanical treatments number of rhizomes per pot was increased with decreased of disease incidence (%). But among the bio-agents treatments number of rhizome per pot was higher in comparison to botanical and control treatments due to use bio fortified soil, although the disease incidence (%) was also higher in these treatments. Relationship between number of rhizome and percent disease incidence (on the basis of below ground symptoms) presented in (Figure 7).

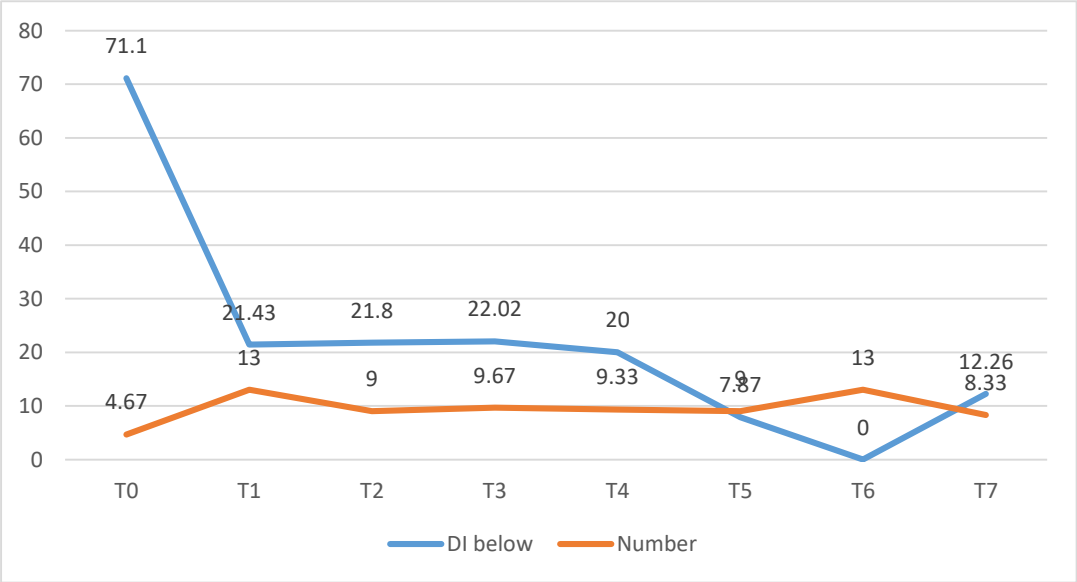


Figure 7-Relationship between number of rhizome per pot and disease incidence (%) on the basis of below ground symptom.

4.9. Relationship between number of rhizome and percent disease severity (on the basis of below ground symptoms).

From the relationship study between number of rhizome per pot and disease severity (%), it was depicted that among the botanical treatments number of rhizomes per pot was increased with decreased of disease severity (%). But among the bio-agents treatments number of rhizome per pot was higher in comparison to botanical and control treatments due to use bio fortified soil, although the disease severity (%) was also higher in these treatments. Relationship between number of rhizome and percent disease severity (on the basis of below ground symptoms) presented in (Figure 8).

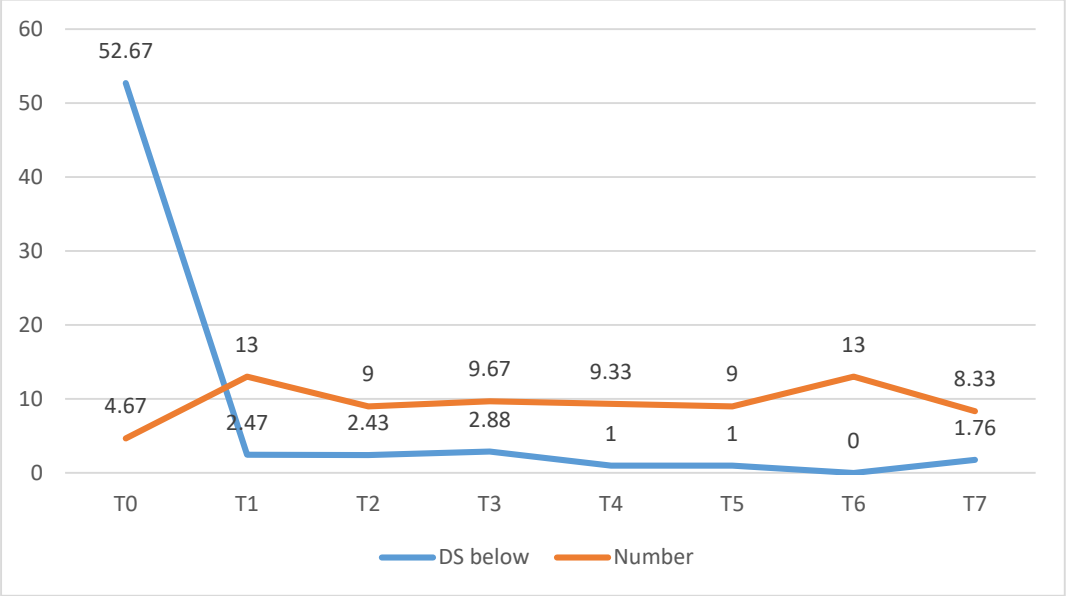


Figure 8-Relationship between number of rhizome per pot and disease severity (%) on the basis of below ground symptom.

4.10. Isolation, identification and characterization of causal organism of rhizome rot disease responsible for ginger decline

Causative agent of rhizome rot disease was isolated from infected rhizome. On the basis of identification and characterization of pathogen of Rhizome rot disease responsible for ginger decline was *Fusarium oxysporum.* Colonies are usually fast growing, pale or bright-colored with or without a cottony aerial mycelium. The color of the thallus varies from whitish to pinkish shades. The microconidia are nearly straight, slender and thin walled. They usually have three or four septa, a foot shaped basal cell and curved and tapered apical cell. The macroconidia are normally only formed on dead or dying host plants.

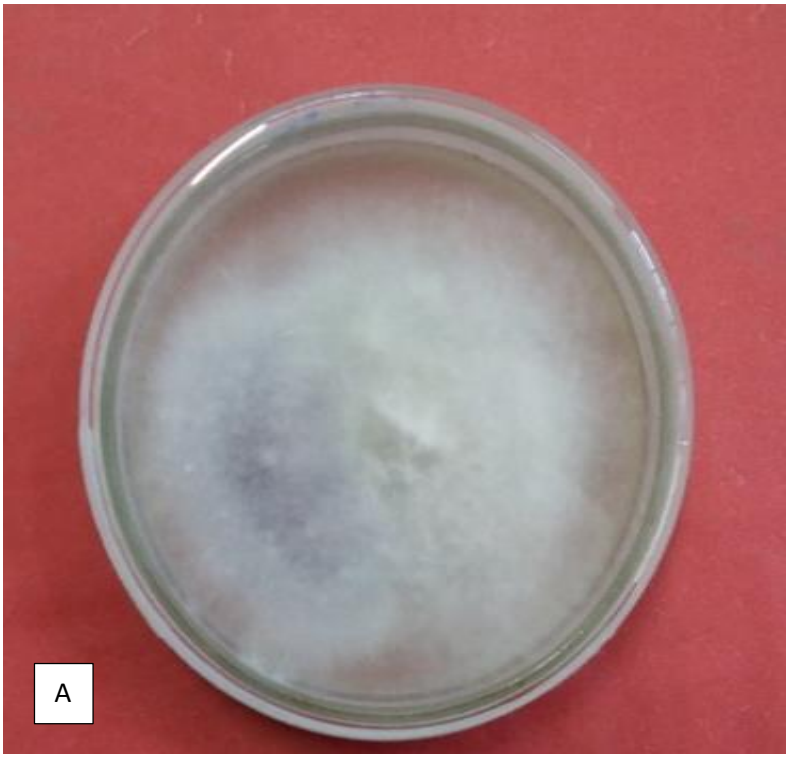


Plate 12-A. Pure culture of *Fusarium oxysporum* in PSA media

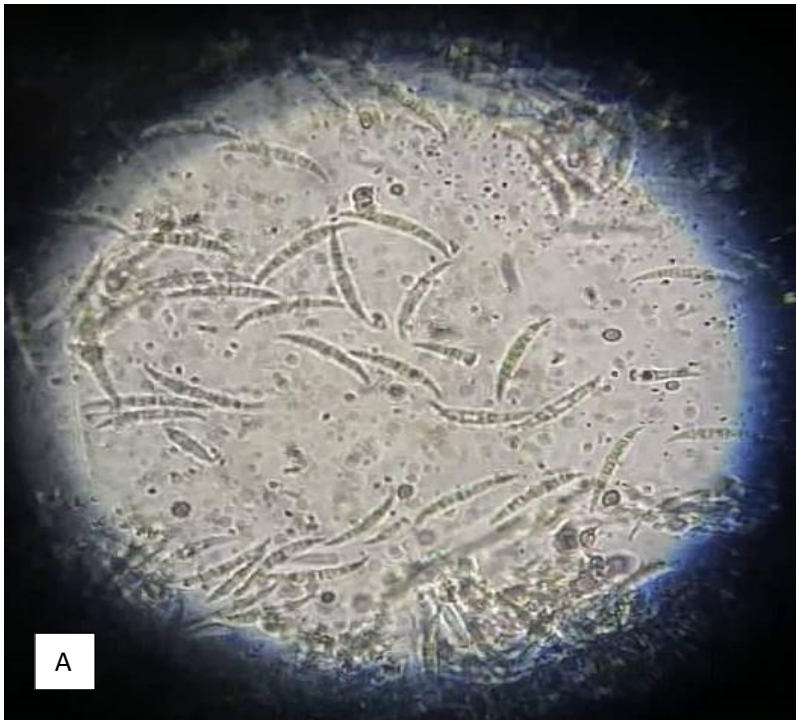


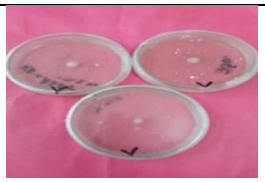
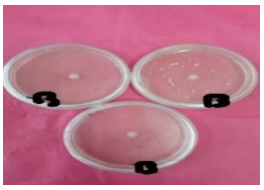
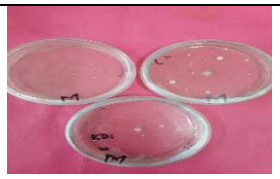
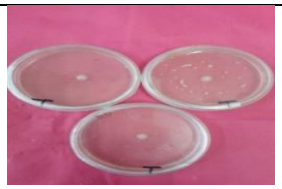
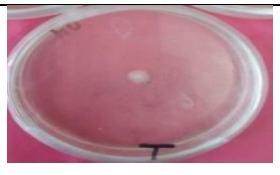
Plate 13- A. Microscopic view of *Fusarium oxysporum* showing two types of conidia; macro conidia and micro conidia.

4.11. Effect of selected bio-agents and botanicals on inhibition to radial mycelial growth in *in-vitro* management

The inhibitory effect of the selected bio-agents and botanicals extract was done following the poison food technique. All the selected treatments showed significant inhibition on radial mycelial growth and spore formation of *Fusarium oxysporum* in comparison to control.

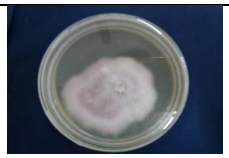
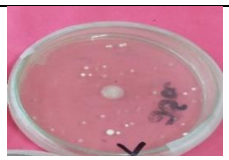
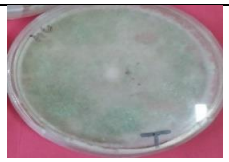
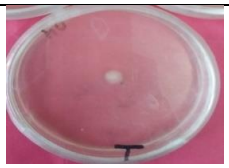
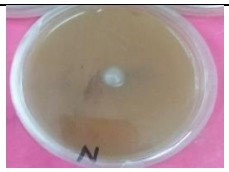
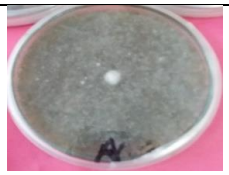
At 2 days after inoculation (DAI), among the bio-agents treated culture plates, *Fusarium* growth was started but the radial mycelial growth was measured less the 1mm.Incase of botanical treated culture plates, radial mycelial growth was found in Tea wastage and Allamanda leaf extract treatment. There was no radial mycelial growth was observed in neem extract treated culture plates.The highest radial mycelial growth (2.00 mm) was measured in control plates. Results are presented in table 4.

Table 4 : Effect of selected bio-agents and botanicals on growth inhibition of *Fusarium oxysporum* in *in-vitro* condition in 2 DAI.

Treatment	Radial growth at 2 DAI (mm)	
T_0 (Control)	2.00 a	
T_1 (*Verticillium lecanii)*	0.43 b	
T_2 (*Beauveria bassiana*)	0.42 b	
T_3 (*Metarhizium anisopliae*)	0.22 bc	
T_4 (*Trichoderma viride*)	0.50 b	
T_5 (Tea wastage)	0.38 b	
T_6 (Neem extract)	0.00 c	
T_7 (Allamanda leaf extract)	0.25 b	

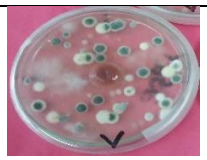
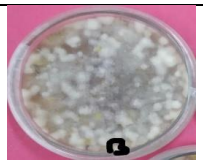
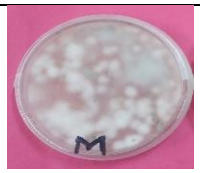
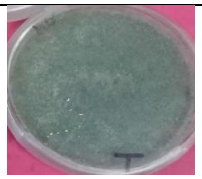
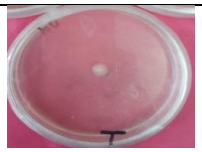
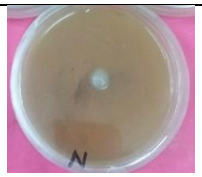
At 5 DAI, radial mycelial growth of *Fusarium* was increased in bio-agents treated culture plates that was T_1 (*Verticillium lecanni*), T_2 (*Beauveria bassiana*),T_3 (*Metarhizium anisopliae*) and T_4 (*Trichoderma viride*)respectively increase of botanicals treated culture plates, radial mycelial growth was also increased that was T_5 (Tea wastage),T_6 (Neem extract)and T_7 (Allamanda leaf extract) increase neem leaf extract treated culture plates radial growth was initiated. Again the highest radial mycelial growth (3.16 mm) was recorded from control plates. Effect of selected bio-agents and botanicals on growth inhibition of *Fusarium oxysporum* in *in-vitro* condition in 5 DAI results are presented in table 5.

Table 5 : Effect of selected bio-agents and botanicals on growth inhibition of *Fusarium oxysporum* in *in-vitro* condition in 5 DAI .

Treatment	Radial growth at 5 DAI (mm)	
T_0 (Control)	3.17 a	
T_1 (*Verticillium lecanii)*	0.63 bc	
T_2 (*Beauveria bassiana*)	0.85 b	
T_3 (*Metarhizium anisopliae*)	0.50 bcd	
T_4 (*Trichoderma viride*)	0.00 e	
T_5 (Tea wastage)	0.62 bc	
T_6 (Neem extract)	0.13 de	
T_7 (Allamanda leaf extract)	0.40 cde	

At 10 DAP, all the were showed bio-agent was suppressed growth of *Fusarium.* Its mean bio-agents were showed full inhibition to radial mycelial growth of *Fusarium oxysporum*. On the other hands botanicals were also gave the better performance regardius to inhibit the radial mycelial growth of *Fusarium oxysporum.* Effect of selected bio-agents and botanicals on growth inhibition of *Fusarium oxysporum* in *in-vitro* condition in 10 DAI results are presented in table 6.

Table 6 : Effect of selected bio-agents and botanicals on growth inhibition of *Fusarium oxysporum* in *in-vitro* condition in 10 DAI .

Treatment	Radial growth at 10 DAI (mm)	
T_0 (Control)	4.16 a	
T_1 (*Verticillium lecanii)*	0.80 b	
T_2 (*Beauveria bassiana*)	0.86 bc	
T_3 (*Metarhizium anisopliae*)	0.52 cd	
T_4 (*Trichoderma viride*)	0.00 d	
T_5 (Tea wastage)	0.00 d	
T_6 (Neem extract)	0.00 d	
T_7 (Allamanda leaf extract)	0.00 d	

DISCUSSION

Ginger (Zingiber officinale) is an important oriental spice crop. It has special significance as spice for tropical countries where it is produced and consumed in large quantities (Rahim. 1992). It is an herbaceous plant and has been cultivated and used in Asia from very ancient time and the useful parts of this crop are the rhizomes (Purseglove et al., 1988). It is among the helthiest and most delicious spices on the planet, it has a very long history of used in various forms of traditional and alternative medicine. It has been used to aid digestion, reduce nausea and help fight the flue and common cold.Ginger is affected by various diseases, such as, Rhizome rot, Bacterial wilt, Soft rot, blight etc. Among all of these, rhizome rot is most damaging one (Chattopadhya,1997). The spice trade generally considers Bangladesh's ginger to be of the best quality and as a result, it commands a premium price on the world market. However, production has steadily declined overtime due mainly to rhizome rot disease in the major production areas.

In this study the disease incidence of rhizome rot on the basis of above and below ground symptoms of ginger in response to different treatments were recorded at 50 DAP, 70 DAP, 90 DAP. All treatments reduced the disease incidence and disease severity of rhizome rot of ginger over untreated control. Based on the disease incidence recorded at 90 DAP the highest disease incidence (100%) was recorded in untreated control. Among the selected bio-agents used for bio-fortification of the pot soil, the highest disease incidence was recorded in T_1 (*Verticillium lecanii*) treatment followed by T_2 (*Beauveria bassiana*) treatment and the lowest disease incidence (33%) was recorded in T_3 (*Metarhizium anisopliae*). Bio-agent *Trichoderma viride* was also gave the satisfactory result over control. Among the botanical used in this study, the highest disease incidence was (100%) was recorded in T_7 (Allamanda leaf extract) treatment and the lowest was in T_5 (Tea wastage) treatment at 90 DAP. There was no disease was found in T_6 (Neem extract) treatment up to 90 DAP or up to harvesting. A considerable reduction of disease severity was found by using the selected

compatible bio-agents and botanicals. Among the selected bio-agents, the highest disease severity was estimated in T_1 (*Verticillium lecanii*) treatments followed by T_2 (*Beauveria bassiana*) treatment while the lowest disease severity was calculated in T_3 (*Metarhizium anisopliae*) treatment. Bio-agent, *Trichoderma viride* was showed better result than untreated control. Among the botanicals Allamanda leaf extract and Tea wastage was showed moderate disease severity. From the study, it was revealed that disease severity was reduced by using the selected bio-agents and botanical. It was also revealed the bio-agents were more compatible than botanical in control the rhizome rot disease of ginger in pot conditions. The result also closely matched with the report of the Ambia (2006) where the lowest disease incidence and disease severity of rhizome rot of ginger was found in case of application of *Trichoderma harzianum* and neem leaf extract at different days after planting and those treatments resulted maximum yield of rhizome. The result also closely matched with the report of the Karuppiyan *et al*., (2007) where soil application of bio-control agents like *Trichoderma harzianun* and Pseudomonas fluorescens during planting time a 2-5% gave effective control of the diseases. These findings corroborate with the findings of Dohroo and Sharma (1984) who stated that rhizome rot of ginger caused by *Fusarium* were control by *Trichoderma viride* and reduced by 80%. Usman et al. (2006) reported that *Trichoderma harzianum* was very effective in controlling the disease.

The performance of the treatments in respect of yield and yield contributing characters against rhizome rot of ginger varied significantly. All the treatments effects were found effective in terms of number of rhizomes, fresh weight and dry weight. The highest number of rhizome found in T_1 (*Verticillium lecanii*) followed by T_3 (*Metarhizium anisopliae*) . Among the bio-fortified treatments with selected bio-agents,the highest number of rhizome was obtained in T1 (*Verticillium lecanii*) treatment followed by T_3 *(Metarhizium anisopliae*) treatment both was statistically similar each other. Number of rhizomes in others bio-fortified treatments were found almost same and both was also statistically similar. Among the botanicals treatments, the highest number of rhizomes were

obtained inT_6 (Neem extract) treatment followed by T_5 (Tea wastage) and T_7 (Allamanda leaf extract) which was statistically similar. The lowest number of rhizomes were obtained in untreated control which was statistically different from both bio-fortified with bio-agents and botanical. In respect of fresh and dry weight, all the selected treatments were varied significantly. Among the bio-fortified treatments with selected bio-agents the highest fresh and dry weight was recorded in T_3 (*Metarhizium anisopliae*) treatments followed by T_4 (*Trichoderma viride*) and T_1 (*Verticillium lecanii*) treatment while the lowest was found in T_2 (*Beauveria bassiana*) treatment. Among the botanical treatments the highest fresh and dry weight was recorded in T_6 (Neem extract) treatment followed by T_5 (Tea wastage) and T_7 (Allamanda leaf extract) treatments. On the other hand the lowest fresh and dry weight was found in control treatment which was statistically different all other treatments. The present findings of the experiment regarding the reduction of disease severity of rhizome rot of ginger and improving the yield attributing characters and yield were supported by the previous reports. Sadanandan and Iyer (1986) stated that, Neem oilcake @ 2MT/ha increased yield by 1.78MT/ha. Meena and Mathur (2005) worked on both bio-control agent and fungicides and showed that rhizomes were treated with fungicides followed by the soil application of bio-agents resulted suppression of the disease and increasing the yield. Anon (2005) observed in integrated management of ginger against *Pythium*, *Fusarium* and *Ralstonia*, the results indicated that Mancozeb, seed solarization and hot water treatments of ginger rhizomes were effective in increasing the emergence and yield of ginger. These findings also supported by Ram *et al.*, (1999), Thakore *et al.,* (1988), Balakrishnan *et al.,* (2000), Ambia (2006) and Bhuyan (2010).

From the *in-vitro* management study, It was found that all the selected treatments showed significant inhibition of mycelial growth and spore formation in comparison to control. The inhibitory effect of the selected treatments against *Fusarium oxysporum* differed significantly among themselves in poison food technique. In poison food technique, the highest mycelial growth of *Fusarium oxysporum* was observed in untreated control. Among the bio-agents used in this

study, the highest radial mycelial growth inhibition of *Fusarium oxysporum* was found in T4 (Neem extract) treatment and other bio-agents were also showed the promosing results in inhibition the mycelial growth and spores formation of *Fusarium oxysporum*. Bharadwaj and Gupta (1987) observed in *in vitro* tests using *Trichoderma viride* and *Trichoderma harzianum* against *Pythium aphanidermatum, Fusarium equiseti* and *Fusarium solani* and found that these antagonists were inhibitory to the pathogens. Among the botanical used in this study also gave better performance in inhibition the radial mycelial growth of *Fusarium oxysporum* in *in-vitro* conditions. In Bangladesh many researchers worked with Bavistin 50 WP, Ridomil Gold MZ-72, Dithane M-45, neem leaf extracts and alamanda leaf extract to inhibit the mycelial growth of *Fusarium oxysporum* in in vitro condition and found promising result (Bhuyan, 2010).

SUMMARY AND CONCLUSION

Experiments were conducted in the net house and laboratory Department of Plant Pathology of Sher-e Bangla Agricultural University, Dhaka, to control rhizome rot disease of ginger through the selected bio-agents and botanical. There were three experiments in this research work; Experiment -1. for bio-fortification with the selected bio-agents, Experiment-2. for evaluation the selected treatments in pot culture, and Experiment-3. for evaluation the selected treatments in *in- vitro* management. In experiment one, some compatible bio-agents viz. *Trichoderma viride, Verticillium lecanii, Metarhizium anisopliae* and *Beauveria bassiana* were used and the selected bio-agents were cultured in artificial media PSA for multiplication, and appearance of mycelium and spores also studied. Quality test performed through analytical method (pour plate method) for morphological and cultural variability test and counting the viable spores/colony forming units on selective medium using Hema-cytometer.

The prepared soil and others selected inputes; cowdung, mustard oil cake, poultry manure, dust and wheat grain were collected to make bio-fortified soil media with bio-agents. Plant materials ginger rhizomes were collected and sown in pots that were prepared with bio-fortified soil and normal soil for botanical application.

Data were collected in respect of percentage incidence and severity. The data obtained for different characters were statistically analyzed to find out the significance of the difference among the treatments. An encouraging performances of the treatments used in the experiment was observed in reducing the disease incidence in terms of hill infection in comparison to control at 50, 70 and 90 DAP. Among the selected bio-agents used for bio-fortification in the pot culture experiment, the highest disease incidence was recorded in T_1 (*Verticillium lecanii*) treatment followed by T_2 (*Beauveria bassiana*) treatment and the lowest disease incidence (33%) was recorded in T_3 (*Metarhizium anisopliae*). Bio-agent *Trichoderma viride* was also gave the satisfactory result over control. Among the botanical used in this study, the highest disease incidence was (100%) was recorded in T_7 (Allamanda leaf extract) treatment and

the lowest was in T_5 (Tea wastage) treatment at 90 DAP. No disease was found in T_6 (Neem extract) treatment up to 90 DAP or up to harvesting. Among the selected bio-agents, the highest disease severity was estimated in T_1 (*Verticillium lecanii*) treatments followed by T_2 (*Beauveria bassiana*) treatment while the lowest disease severity was calculated in T_3 (*Metarhizium anisopliae*) treatment. Bio-agent, *Trichoderma viride* was showed better result than untreated control. Among the botanicals Allamanda leaf extract and Tea wastage was showed moderate disease severity. On the basis of identification and characterization of pathogen of Rhizome rot disease responsible for ginger decline was *Fusarium oxysporum* and it was isolated from infected rhizome. Among the bio-fortified treatments with selected bio-agents, the highest number of rhizome was obtained in T1 (*Verticillium lecanii*) treatment followed by T_3 *(Metarhizium anisopliae*) treatment both was statistically similar to each other. Among the botanicals treatments, the highest number of rhizomes were obtained inT_6 (Neem extract) treatment followed by T_5 (Tea wastage) and T_7 (Allamanda leaf extract) which was statistically similar. The lowest number of rhizomes were obtained in untreated control which was statistically different from both bio-fortified, bio-agents and botanical. The inhibitory effect of the selected treatments against *Fusarium oxysporum* differed significantly among themselves in poison food technique. In poison food technique, the highest mycelial growth of *Fusarium oxysporum* was observed in untreated control. Among the bio-agents used in this study, the highest radial mycelial growth inhibition of *Fusarium oxysporum* was found in T4 (Neem extract) treatment and other bio-agents were also showed the promosing results in inhibition the mycelial growth and spores formation of *Fusarium oxysporum*.

Considering the overall results, application of *Trichoderma viride*, *Metarhizium anisopliae* and the botanical neem leaf extract may be recommended as ecofriendly approach for controlling rhizome rot of ginger. However, further investigation is needed to justify the present findings in different Agro Ecological Zones (AEZ) in the country for consecutive years through conducting the field experiments.

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Appendices

Appendix-I: Effect of selected compatible bio-agents and botanicals on disease incidence (%) on the basis of above ground symptoms at 50, 70 and 90 days after planting (DAP)

Treatment	Disease Incidence		
	50 days	70 days	90 days
T0	66.66 a	100.00 a	100.00 a
T1	33.33 a	33.33 ab	100.00 a
T2	0.00 a	33.33 ab	66.66 ab
T3	0.00 a	33.33 ab	33.33 ab
T4	33.33 a	66.66 ab	66.66 ab
T5	0.00 a	0.00 b	33.33 ab
T6	0.00 a	0.00 b	0.00 b
T7	33.33 a	66.66 ab	100.00 a
CV %	195.96	103.63	65.32
Standard Eror (.05)	33.33	36.43	33.33

Appendix-II: Effect of selected compatible bio-agents and botanicals on disease severity (%) on the basis of above ground symptoms at 50, 70 and 90 days after planting (DAP)

Treatment	Disease Severity		
	50 days	70 days	90 days
T0	27.77 a	60.00 a	91.67 a
T1	6.48 b	10.17 b	22.10 b
T2	0.00 b	0.00 b	19.76 b
T3	0.00 b	2.77 b	11.65 bc
T4	0.00 b	8.33 b	14.99 bc
T5	0.00 b	0.00 b	8.88 bc
T6	0.00 b	0.00 b	0.00 c
T7	4.16 b	12.72 b	18.35 b
CV %	124.96	124.66	36.18
Standard Eror (.05)	6.31	11.71	6.83

Appendix-III: Effect of selected compatible bio-agents and botanicals on disease incidence (%) on the basis of below ground symptoms

Treatment	Disease Incidence(%) of below ground symptom
T0	71.10 a
T1	21.43 b
T2	21.80 b
T3	22.02 b
T4	20.00 b
T5	7.87 b
T6	0.00 b
T7	12.26 b
CV %	63.25
Standard Eror (.05)	11.39

Appendix-IV: Effect of selected compatible bio-agents and botanicals on disease severity (%) on the basis of below ground symptoms

Treatment	Disease Severity (%) of below ground symptom
T0	52.66 a
T1	2.47 b
T2	2.43 b
T3	2.88 b
T4	1.00 b
T5	1.00 b
T6	0.00 b
T7	1.76 b
CV %	24.23
Standard Eror (.05)	1.58