Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; 10(4): 13-23
Received: 07-05-2021
Accepted: 09-06-2021

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Evaluation of compatible bio-agents and selected botanical extracts against the pathogen of rhizome rot disease responsible for ginger decline

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Abstract
A pot and lab experiment was conducted to evaluate the efficacy of selected compatible bio-control agents *viz. Verticillium lecanii* (*T1*), *Beauveria bassiana* (*T2*), *Metarhizium anisopliae* (*T3*), *Trichoderma viride* (*T4*) and botanicals *viz.* Tea wastage (T_5), Neem leaf extract (T_6), Allamanda leaf extract (T_7) against the pathogen of rhizome rot disease responsible for ginger decline. The selected bio-agents were applied as a bio-fortified material. The bio-control 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 compatible bio-control 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 in T_7 (Allamanda leaf extract). No disease symptoms were appear in T_6 (Neem leaf extract) treatment up to 90 DAP or up to harvesting. From the *in-vitro* management study it was found that *Trichoderma viride* (T_4) and *Metarhizium anisopliae* (T_3) gave better performance in inhibiting radial mycelial growth of the pathogen of rhizome rot disease, *Fusarium oxysporum*. Among the botanicals, neem leaf extract gave promising performance in inhibiting 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 effective management of rhizome rot disease of ginger.

Keywords: ginger rhizome rot disease, *Fusarium oxysporum*, *Verticillium lecanii*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Trichoderma viride*, botanicals, bio-fortified material

Introduction
Ginger (*Zingiber officinale*) is one of the earliest species belongs to the family Zingiberaceae. It is an 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) [19]. It has special significance as spice in tropical countries where it is produced and consumed in large quantities (Rahim, 1992) [15]. 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) [14]. In Bangladesh, the annual production of ginger is 80,234 metric tons and the total area of ginger cultivation is about 23,747 acres and the yield per acre is about 3379 kg (BBS, 2020) [8] which is not sufficient for national demand. Thus, the deficit amount has to import from abroad to meet up the national demand. According to FAO (2020) [12], 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. Ginger is a spice crop whose rhizome, ginger root or ginger, is widely used as a spice and a folk medicine. 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 also used in medicine as a carminative and aromatic stimulant to the gastrointestinal tract, externally as an aphrodisiac and internally as a counter irritant. 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 due mainly to rhizome rot disease in the major production areas. 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 yellow, lower leaves dry out and turn brown then eventually all aboveground shoots dry out completely. Plant collapse is 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 seed pieces from the previous crop, although these 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; Meena and Mathur, 2005) [6, 18]. 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) [17]. 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; Ambia, 2006) [1, 11]. Now-a-days *Trichoderma* sp. is frequently used as a bio-agent against soil borne fungal pathogens (Ahmmed and Hossain, 2006) [2]. 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. 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) [9]. 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 exploit 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. The research carried out to achieve the following specific objectives; to isolate, identify and characterize the causal organism of rhizome rot disease of Ginger, to study the compatibility of selected bio-agents and botanicals extract in controlling the rhizome rot disease of Ginger in pot culture and to evaluate the selected bio-agents and botanical extracts against the pathogens of rhizome rot disease of Ginger in *in-vitro*.

Materials and Methods

Design and treatments of the experiment

The experiments were laid out in completely randomized design (CRD). In total eight treatments *viz.* 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 leaf extract, T_7-Allamanda leaf extract were codified with three replications.to achieve the desired objectives.

In-vitro study-1 (Bio-agent multiplication)

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 table 1.

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*

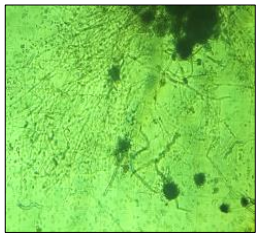
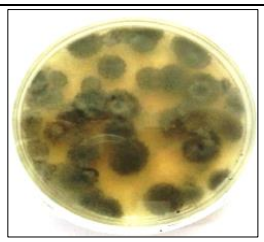
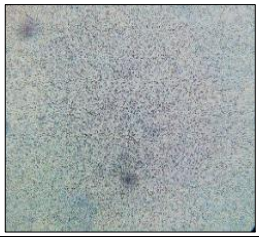
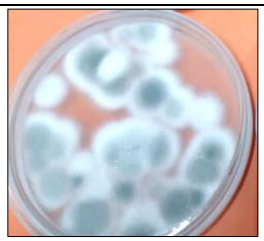
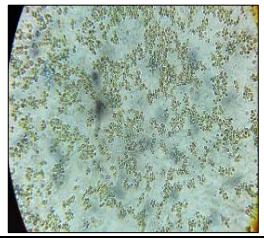
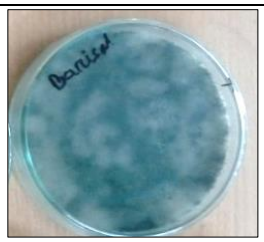
Multiplication of bio-control agents

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

Inoculation of bio-control agents

The pour plate technique was adopted for the inoculation of selected bio-control agents *viz. Verticillium lecani* 1.50% LF (Bio-Catch), *Metarhizium anisopliae* 1.15% WP (Bio Magic) and *Beauveria bassiana* 1.50% LF (Bio-Power) into the solid medium. For liquid formulation, one gram of selected bio-control agents 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 $10^{-6,}$ 10^{-8} and 10^{-8} respectively (Table 2). Four replicates were maintained for each dilution factor. Pipetted out 1ml of supernatant solution from each of the bio-control agents and transferred into petri dish. The melted agar medium was poured into petri dish containing the suspension of selected bio-control agents. 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. In case of bio-control agent, *Trichoderma viride* solution was collected from the available sources and diluted with distilled water. The diluted solution ($1X10^{-10}$ colony per ml) was applied in petridish and followed the cup method for multiplication.

Table 2: Morphological and cultural variability test of selected bio-control agents, viable spores count (CFU's) using hemacytometer and counted 1 X 10^{-6}, 1 X 10^{-8}, 1 X 10^{-8} and 1 X 10^{-10} colony per ml minimum for *Verticillium lecanii*, *Metarhizium anisopilae*, *Beauveriana bassiana* and *Trichoderma viride* respectively

Bio-agents	Pure culture of bio-control agents	Morphological structures
Verticillium lecanii		
Materhizium anisopliae		
Beauveria bassiana		
Trichoderma viride		

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

Prepared bio-fortified compost with selected bio-agents

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

Pot preparation and rhizomes sowing

In this research work, the rhizomes of gingers were used as planting materials which collected from Bangladesh Agricultural Research Institute. The rhizomes of ginger were sown in pots which prepared with bio-fortified soil media and normal soil just the next day of rhizomes treatment with Gibberellic acid (GA3) for enhancing for germination.

Preparation and application of botanicals extract

The selected botanicals *viz*. Neem leaf extract, Allamanda leaf extract and Tea wastage were collected locally and prepared to study its compatibility with selected bio-control agents under *in-vivo* and in *in-vitro* conditions. 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 and filtered through sterile cheese cloth. The supernatant was diluted in equal amount of sterile water for 1:2 solution. Extracted botanicals were added directly to the selected pots/plants as a treatment for checking the compatibility with selected bio-agents.

Intercultural operation

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

Parameters assayed

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

Disease incidence (%) and severity (%) on above ground symptoms

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

Disease incidence (%) and severity in below ground symptoms

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

Parameters on yield and yield contributing characters
Number of tillers, number of rhizomes, fresh & dry weight and yield were recorded.

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 washed with sterilized water thrice and placed in a petridish. The petridish containing rhizomes were incubated at 25±1°C for seven days. When the organism grew freshly on the rhizome then mycelial fragment was transferred on PSA plate to have pure culture. Finally the pure culture of the pathogen was obtained and identified.

***In-vitro* study-2 (Evaluation of bio-control agents and botanicals extract)**
To study the compatibility of selected bio-agents and botanicals extract, the poisoned food technique was applied. To avoid contamination, all bio-control agents and botanicals extract 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 was cut and placed at the centre of each petri dish. Control consisted of PSA medium alone inoculated with the fungus. Three replications were maintained for each concentration. The inoculated plates were incubated at mini-inqubator and observations on the mycelial growth of the fungal antagonist were taken when control plates showed full growth. The relative growth reduction for each treatment was calculated by the equation below.

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

Where L is percentage of inhibition in growth; C is radial growth of the fungal pathogen in control; T is radial growth (mm) of the fungal pathogen in the presence of the treatments. 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 3, 5 and 10 days after inoculation.

Data analysis
The data obtained from all the studied were analyzed using computer based software Statistix-10.0.

Results and Discussion
Effect of selected compatible bio-control agents and botanicals on percent 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-control agents and botanicals 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 while the lowest disease incidence (33%) was recorded in T_1 (*Verticillium lecanii*), T_4 (*Trichoderma viride*) and T_7 (Allamanda leaf extract) treatments. No disease was found in T_2 (*Beauveria bassiana*), T_3 (*Metarhizium anisopliae*), T_5 (Tea wastage) and T_6 (Neem extract) treatments. At 70 DAP, the lowest disease incidence (33.33%) was recorded in T_1 (*Verticillium lecanii*), T_2 (*Beauveria bassiana*) and T_3 (*Metarhizium anisopliae*) treatments. The moderate disease incidence (66.66%) was found in T_4 (Neem extract) and T_7 (Allamanda leaf extract) treatments and T_5 (Tea wastage) and T_6 (Neem extract) treatments. The highest disease incidence (100%) was again recorded in control treatment. 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. 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 (Neem extract) up to harvesting the zinger crops. 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. A considerable reduction of disease incidence (%) was achieved by using selected compatible bio-control agents and botanicals (Fig-1).

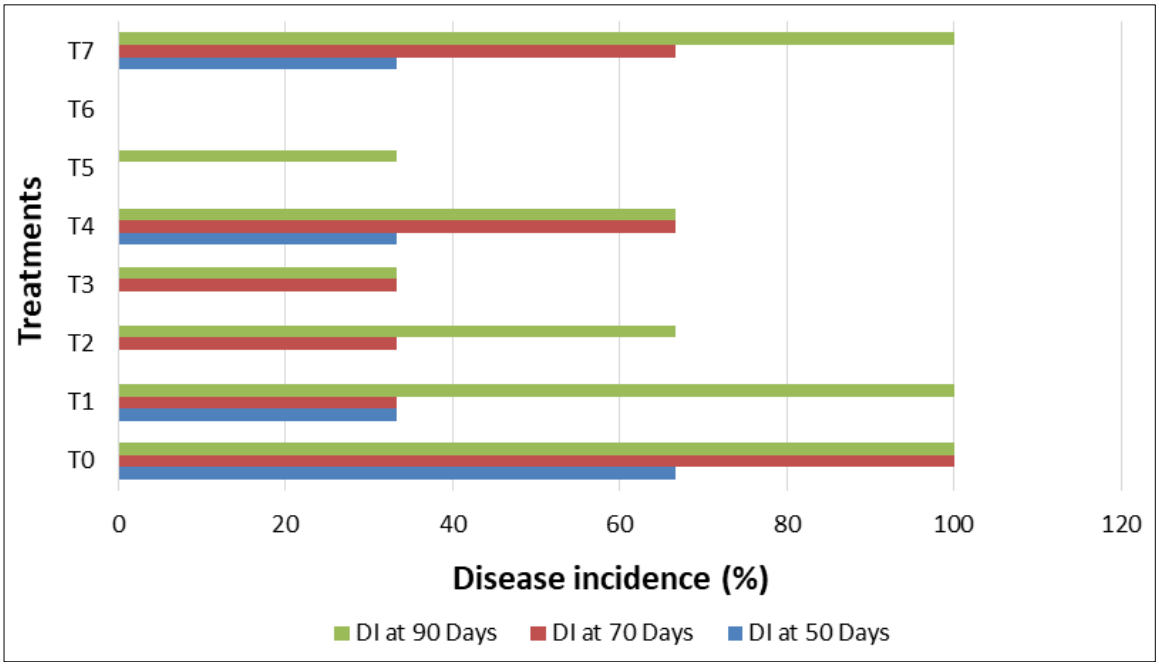


Fig 1: Effect of selected bio-control agents and botanicals on disease incidence on the basis of above ground symptom

Effect of selected compatible bio-control agents and botanicals on percent 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-control agents and botanicals 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 while the lowest disease severity (4.16%) was recorded in T_7 (Allamanda leaf extract) and (6.48%) was recorded in T_1 (*Verticillium lecanii*). 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 recorded in control treatment. Other treatments T_4 (*Trichoderma viride*), T_1 (*Verticillium lecanii*) and T_7 (Allamanda leaf extract) 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 90 DAP the treatments effect 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 showed moderate disease severity which were 22.10%, 19.76%, 11.65%, 14.99% and 18.35%, respectively. No disease was found in T_6 up to harvesting. A considerable reduction of disease severity was achieved by using selected compatible bio-control agents and botanicals in the experiment compared to control (Fig-2).

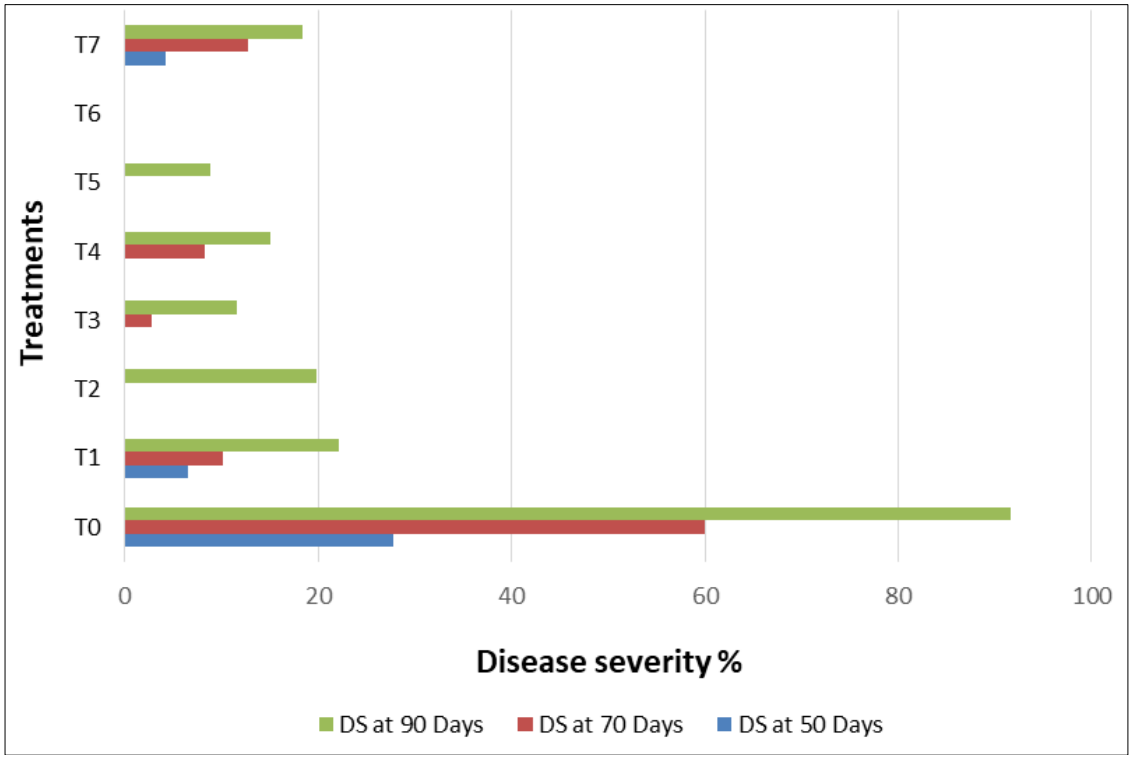


Fig 2: Effect of selected bio-agents and botanicals on disease severity on the basis of above ground symptom

Effect of selected compatible bio-control agents and botanicals on percent disease incidence (On the basis of below ground symptoms)

On the basis of below ground symptoms, the selected bio-control agents and botanicals 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. Other treatments T_1 (*Verticillium lecanii*), T_2 (*Beauveria bassiana*), T_3 (*Metarhizium anisopliae*), T_4 (*Trichoderma viride*), and T_7 (Allamanda leaf extract) showed moderate disease incidence which were 21.43%, 21.8%, 22.02%, 20% and 12.26%, respectively. No disease was found in T_6 (Neem extract) in below ground symptom. Results regarding the effect of selected compatible bio-control agents and botanicals on disease incidence (%) were found statistically significant (Fig-3).

Effect of selected compatible bio-control agents and botanicals on percent 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-control agents and botanicals showed very good 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. Other 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) showed moderate disease severity which were 2.47%, 2.43%, 2.88%, 1%, 1% and 1.76%, respectively. No disease was found in T_6 (Neem extract) on below ground symptom. Results regarding the effect of selected compatible bio-control agents and botanicals on disease severity (%) were found statistically significant (Fig-4).

Isolation, identification and characterization of causative 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 macroconidia were 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 microconidia were oval to global in shaped and thick walled (Fig-5).

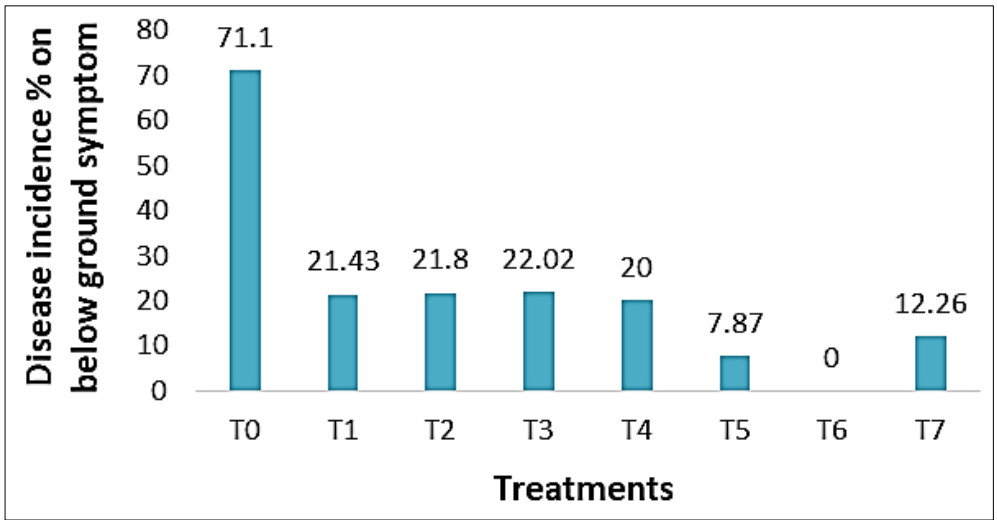


Fig 3: Disease incidence (%) on the basis of below ground symptom

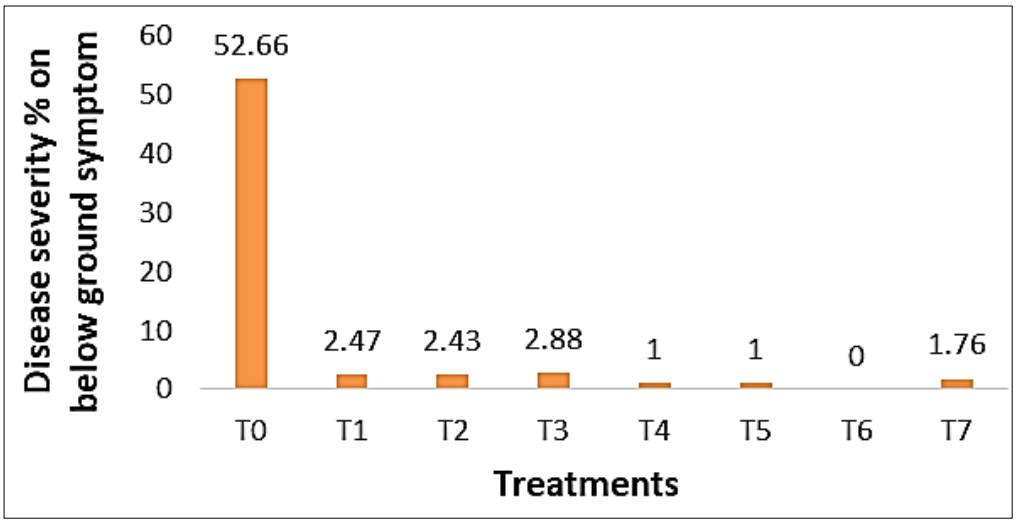


Fig 4: Disease severity (%) on the basis of below ground symptom

Fig 5: Pure culture and microscopic view of *Fusarium oxysporum* showing macro and micro conidia

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 almost similar in other treatments which were statistically similar. The highest fresh weight of rhizomes (412.83 gm) was recorded in T_3 (*Metarhizium anisopliae*) followed by T_4 (*Trichoderma viride*) and T_1 (*Verticillium lecanii*) that was 379.17 gm and 338.0 gm which was statistically identical with each other. The lowest fresh weight of rhizomes (116.33gm) was recorded in 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) which were 310.67 gm, 296.67 gm, 253 gm and 251.67 gm, respectively which were statistically identical with each 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 240.63 gm and 223.78 gm which was statistically

identical with each other. The lowest fresh weight of rhizomes (71.60 gm) was recorded in 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) which were 216.97gm, 212.15 gm, 188.52 gm and 204.74 gm, respectively which were statistically identical with each other. Results regarding the number of rhizomes, fresh and dry weight are presented in table 3.

Table 3: Effect of selected bio-control agents and botanicals on number of rhizomes, fresh and dry weight

Treatment	Number of rhizome/pot	Fresh weight (gm)	Dry weight (gm)
T0 (Control)	4.67 c	116.33 d	71.60 c
T1 (*Verticillium lecanii*)	13.00 a	338.00 abc	216.97 b
T2 (*Beauveria bassiana*)	9.33 b	310.67 bc	212.15 b
T3 (*Metarhizium anisopliae*)	9.67 ab	412.83 a	304.33 a
T4 (*Trichoderma viride*)	9.33 b	379.17 ab	240.63 ab
T5 (Tea wastage)	9.00 b	253.00 c	188.52 b
T6 (Neem extract)	10.00 b	296.67 bc	223.78 b
T7 (Allamanda leaf extract)	8.33 b	251.67 c	204.74 b
CV%	24.68	26.89	26.99

Relationship between number of rhizome and 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 percent disease incidence (%). Among the bio-control agents, although the disease incidence was high but the number of rhizomes was also higher due to the use bio-fortified soil in these treatments (Fig-6).

Relationship between number of rhizome and 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 percent disease severity (%). Among the bio-control agents, although the percent disease severity was high but the number of rhizomes was also higher due to the use bio-fortified soil in these treatments (Fig-7).

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

From the relationship study between number of rhizomes per pot and disease incidence (%), it was depicted that among the botanical treatments number of rhizomes per pot was increased with decreased disease incidence (%). But among the bio-control agents number of rhizomes per pot was higher in comparison to botanical and control treatments due to the use bio fortified soil, although the disease incidence (%) was also higher in these treatments (Fig-8).

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

From the relationship study between number of rhizomes per pot and disease severity (%), it was depicted that among the botanical treatments number of rhizomes per pot was increased with decreased disease severity (%). But among the bio-control agents number of rhizomes per pot was higher in comparison to botanical and control treatments due to the use bio fortified soil, although the disease severity (%) was also higher in these treatments (Fig-9).

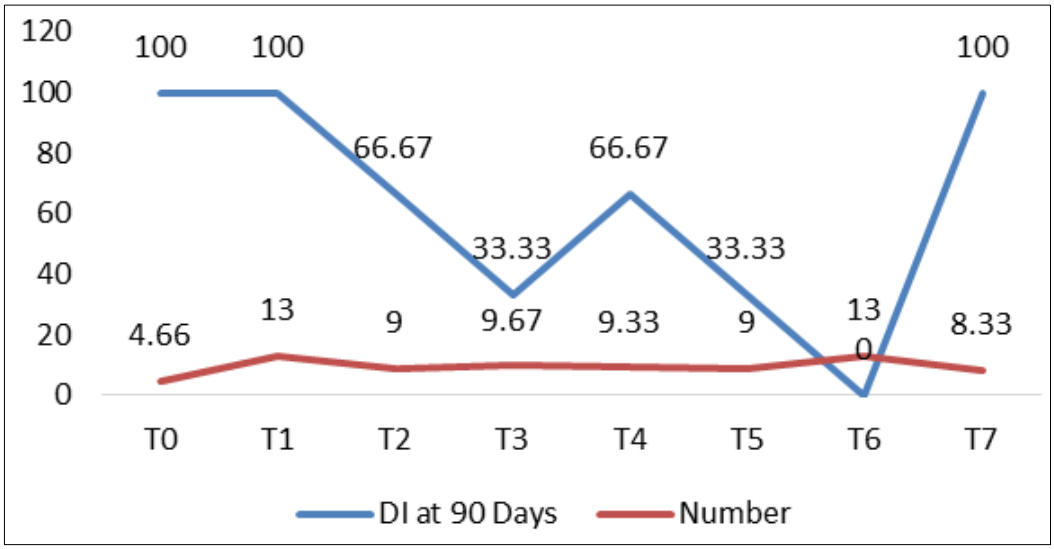


Fig 6: Relationship between number of rhizomes per pot and disease incidence (%) at 90 DAP

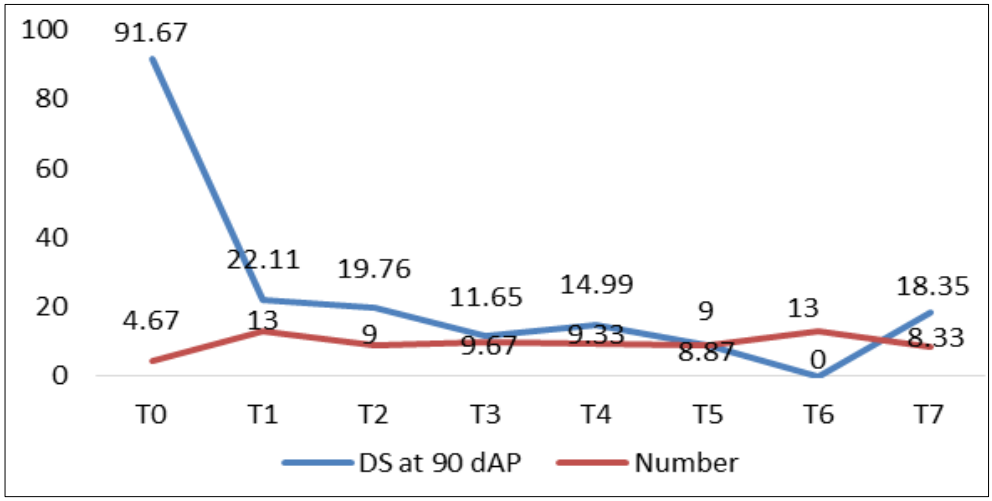


Fig 7: Relationship between number of rhizomes per pot and disease severity (%) at 90 DAP

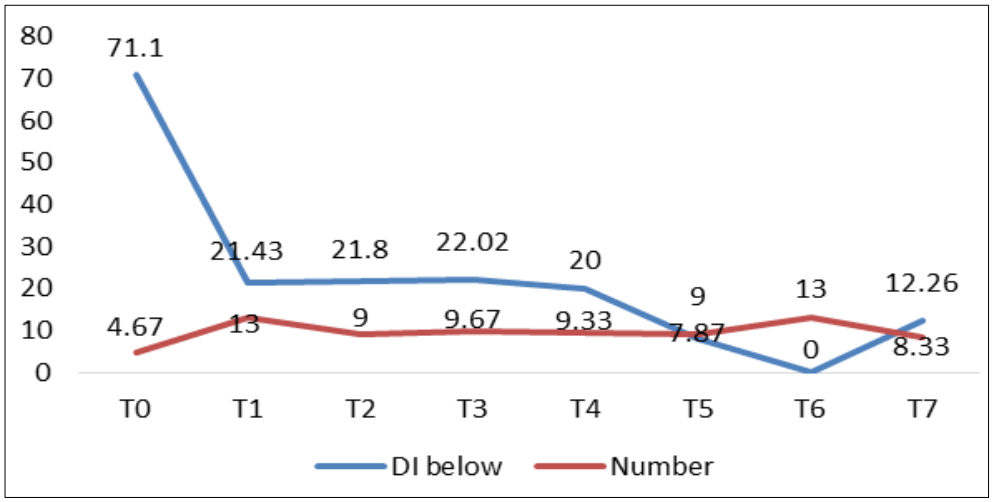


Fig 8: Relationship between number of rhizomes per pot and disease incidence (%) on the basis of below ground symptom

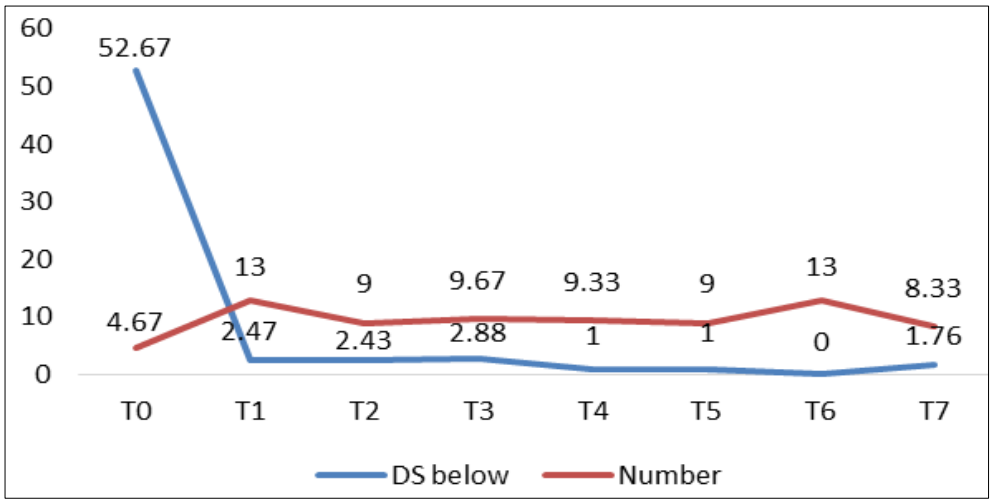


Fig 9: Relationship between number of rhizomes per pot and disease severity (%) on the basis of below ground symptom

Effect of selected bio-control agents and botanicals on inhibition to radial mycelial growth of *Fusarium oxysporum* in *in-vitro* management

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

At 3 days after inoculation (DAI), among the bio-control agents treated culture plates, *Fusarium* growth was started but the radial mycelial growth was measured less than 1mm. In case of botanical treated culture plates, radial mycelial growth was found in Tea wastage and Allamanda leaf extract treatment. 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. At 5 DAI, radial mycelial growth of *Fusarium* was increased in bio-

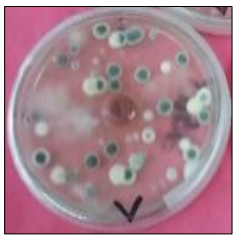
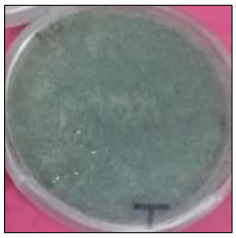
control agents treated culture plates that was T_1 (*Verticillium lecanni*), T_2 (*Beauveria bassiana*), T3 (*Metarhizium anisopliae*) and T_4 (*Trichoderma viride*), respectively. In case of botanicals treated culture plates, radial mycelial growth also increased that was T_5 (Tea wastage), T_6 (Neem extract) and T_7 (Allamanda leaf extract). In case of neem leaf extract treated culture plates, radial growth was initiated. Again the highest radial mycelial growth (3.16 mm) was recorded from control plates. At 10 DAP, all the bio-control agents suppressed the growth of *Fusarium.* It means bio-control agents showed full inhibition of radial mycelial growth of *Fusarium oxysporum*. On the other hands botanicals also gave better performance regarding inhibiting 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 4.

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 and 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* also gave satisfactory result over control. Among the botanicals used in this study, the highest disease incidence (100%) was recorded in T_7 (Allamanda leaves extract) treatment and the lowest was in T_5 (Tea wastage) treatment at 90 DAP. No disease was found in T_6 (Neem leaves extract) treatment up to 90 DAP or up to harvesting. A considerable reduction of disease severity was found by using the selected compatible bio-control 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* showed better result than untreated control. Among the botanicals Allamanda leaves extract and Tea wastage 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 that bio-agents were more compatible than botanical in controlling the rhizome rot disease of ginger in pot conditions. The results closely matched with the report of Ambia (2006) [1] where the lowest disease incidence and disease severity of rhizome rot of ginger was found in case of application of *Trichoderma harzianum* and neem leaves extract at different days after planting and those treatments resulted maximum yield of rhizome. The results also closely matched with the previous reports, where soil application of bio-control agents like *Trichoderma harzianun* and *Pseudomonas fluorescens* during planting time at 2-5% gave effective control of the diseases. These findings corroborate with the findings of Dohroo and Sharma (1984) [5] who stated that rhizome rot of ginger caused by *Fusarium* were controlled by *Trichoderma viride* and reduced by 80%.

The performance of the treatments in respect of yield and yield contributing characters against rhizome rot of ginger varied significantly. All the treatments effect was found effective in terms of number of rhizomes, fresh weight and dry weight. The highest number of rhizomes 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 rhizomes was obtained in T1 (*Verticillium lecanii*) treatment followed by T_3 (*Metarhizium anisopliae*) treatment both was statistically similar with each other. Number of rhizomes in others bio-fortified treatments were found almost same and both were also statistically similar. Among the botanicals treatment, the highest number of rhizomes were obtained inT_6 (Neem leaves extract) treatment followed by T_5 (Tea wastage) and T_7 (Allamanda leaves extract) which was statistically similar. The lowest number of rhizomes was obtained in untreated control which was statistically different from both bio-fortified with bio-agents and botanicals. In respect of fresh and dry weight, all the selected treatments 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 leaves 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 from all other treatments. The present findings of this 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. Meena and Mathur (2005) [6] 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. Ram *et al*. (1999) [7, 16], Balakrishnan *et al.* (2000) [3], Ambia (2006) [1] and Bhuyan (2010) reported that integrated management of ginger against *Pythium*, *Fusarium* and *Ralstonia*, the results found that Mancozeb, seed solarization and hot water treatments of ginger rhizomes were effective in increasing the emergence and yield of ginger.

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 T_4 (Neem extract) treatment and other bio-agents were also showed promising results in inhibiting the mycelial growth and spores formation of *Fusarium oxysporum*. Among the botanicals used in this study, neem leaves extract also gave better performance in inhibiting 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).

Table 4: Effect of selected bio-control agents and botanicals on growth inhibition of *Fusarium oxysporum* in *in-vitro* condition in 10 DAI

Treatment	Radial growth at 10 DAP (mm)	
T0 (Control)	4.16 a	
T1 (*Verticillium lecanii*)	0.80 b	
T2 (*Beauveria bassiana*)	0.86 bc	
T3 (*Metarhizium anisopliae*)	0.52 cd	
T4 (*Trichoderma viride*)	0.00 d	
T5 (Tea wastage)	0.00 d	
T6 (Neem extract)	0.00 d	
T7 (Allamanda leaf extract)	0.00 d	

Conclusion

From the study, it may be concluded that considering the overall results, application of *Trichoderma viride*, *Metarhizium anisopliae* and the botanical neem leaf extracts may be recommended as ecofriendly approach for controlling rhizome rot of ginger.

Acknowledgement

I would like to acknowledge Sher-e-Bangla Agricultural University Research System (SAURES) for research funding and also acknowledge to Department of Plant Pathology for all round assistance and allowing to carry out the research in net house and in the laboratory. All materials used in this research work have been dually acknowledged.

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