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Effect of *Trichoderma asperellum* and *Trichoderma virens* on *Allium cepa* L. Growth, Damping off and Basal Rot Disease Incidence and Severity in Sri Lanka

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Abstract: *Trichoderma* species are frequently used for the biological control of phytopathogenic fungi and they have also been reported as plant growth promoters. In the present study, the effect of two *Trichoderma* spp. *i.e. Trichoderma asperellum* and *Trichoderma virens* isolated from the soils of onion fields on the growth of *Allium cepa* L. plants and suppression of damping off and basal rot diseases was evaluated under field conditions. The two *Trichoderma* spp. were mass cultured in a low cost medium containing molasses and yeast and added to a low cost carrier medium consisting of talc. Two formulations, *i.e. T. asperellum* only and *T. asperellum* in combination with *T. virens* were prepared and the formulations were tested for their effect on onion seedlings at the nursery stage and also on transplanted plants in the field. At the nursery stage, the two formulations were applied using two methods *i.e.* soil application prior to planting of onion seeds or priming of onion seeds with the two formulations separately before planting. Both methods reduced the incidence and severity of damping off disease while increasing the growth of seedlings significantly ($p \leq 0.05$) at the nursery stage. Additional treatment with the two formulations as seedling root dips or soil applications before transplanting the seedlings in the field were effective in controlling basal rot disease of *A. cepa* L. and enhancing the growth of *Allium cepa* L. plants significantly ($p \leq 0.05$) in the field.

Keywords: *Trichoderma asperellum*, *Trichoderma virens*, Damping Off, Basal Rot, Growth Enhancement

1. Introduction

Big onion (*Allium cepa* L.) is a condiment grown for its flavorful bulbs in Sri Lanka as well as a number of other countries in the world. In Sri Lanka about 627 Hactares is cultivated in the Matale and Anuradhapura districts. However, the local cultivation does not meet the annual requirement of big onion approximately 203,993 MT per year [1]. One major factor contributing towards yield reduction is infectious diseases that occur both during the nursery stage and in transplanted field conditions. Some of the more economically significant diseases that reduce yields are caused by fungal pathogens. Diseases caused by fungal pathogens can be broadly divided into seedling diseases, foliage diseases and bulb diseases. The most common fungal genera responsible for big onion diseases are *Fusarium,*

Sclerotium, Pythium, Rhizoctonia, *Colletotrichum* and *Alternaria* [2, 3].

Damping off disease of *Allium cepa* L. that infect young seedlings at the nursery stage is one of the most important diseases caused by soil-borne fungal spp. *Fusarium, Pythium* and *Rhizoctonia* either singly or in combination causing seedling mortality before they are transplanted [3]. This disease may manifest before or after emergence of seedlings *i.e.* pre-emergence or post emergence damping off.

Further, about 10-50% yield loss is caused by basal rot disease, in the transplanted fields and during storage [4]. Basal rot of *Allium cepa* L. is caused by the soil borne fungus *Fusarium oxysporum* f. sp. *cepa* and the symptoms are yellowing or necrosis and withering of the leaves, stunted growth and rotten watery bulbs with decaying root systems.

Chemicals are used extensively for the control of damping off and basal rot pathogens both at nursery stage and the transplanted crop and the more commonly used fungicides in Sri Lanka are Captan, Homai, Mancozeb, Thiram, Brassicol, Benlate, Cresent, Topsin M,Carbendazim [5, 6]. However, the use of chemicals results in deleterious effects on soil organisms and adverse effects on the environment and human health specially as onions are sometimes consumed without cooking [7]. Therefore, safe alternatives should be sought to overcome these problems and the use of microorganisms with the ability to control pathogens has been reported as a safe and viable method. Microorganisms with Biological control ability have been used successfully to control a number of diseases in numerous crops and could be the best alternative especially against soil borne pathogens such as *Fusarium* spp. [8-10]. *Trichoderma* spp. have been used worldwide as effective bio control agents of many fungal pathogens *i.e. F. proliferatum, F. solani, Sclerotium cepivorum* [11]. However, exotic microorganisms which could be potentially damaged the native ecosystems and might feed on non-target hosts. Therefore, the present study aimed at isolating *Trichoderma* spp. occurring naturally in the soils of onion fields in Sri Lanka and testing their effectiveness in controlling the causative agents of damping off and basal rot diseases of onion with a view to utilize them as effective, safe and viable control agents of the two diseases under field conditions. For the preparation of a formulation to be applied under field conditions, the effective *Trichoderma* isolates need to be mass cultured. For the formulations to be economically viable, the mass culture medium must be cheap and easily accessible but at the same time should support a high level of sporulation and growth of the selected *Trichoderma* spp. Rotten Grains, Sugarcane bagasse, vegetable waste, fruit juice waste, Potato dextrose broth, Maltose peptone broth, rotten wheat have been reported as effective mass culture media in a number of studies carried out previously [12, 13] and in the present study, a suitable mass culture medium was developed. A suitable carrier medium has to be selected when applying the mass cultured inoculum/inocula to the field, and a carrier medium should be sufficiently inert to discourage the growth of soil organisms but at the same time retain the viability of the inoculum. A suitable carrier medium that fulfills these requirements was also selected in the present study.

In addition to control of pathogenic fungi, *Trichoderma* spp. have also been reported to enhance growth of plants [14, 15]. Therefore, the effect of the *Trichoderma* formulations on the growth of onion seedlings and plants was also evaluated under field conditions.

This is the first report of preparing locally isolated *Trichoderma virens* and *Trichoderma asperellum* formulations for the effective control of the causative agents of damping off and basal rot diseases prevalent in commercial onion cultivations in Sri Lanka.

2. Materials and Methods

2.1. Biocontrol Agents and Pathogen Isolate

Trichoderma spp. used in this study were previously isolated using the method described by [16] from soil collected from onion fields in Sri Lanka [17] and *Fusarium* sp. was isolated from the *A. cepa* L. seedlings showing characteristic symptoms of damping off disease [17].

The *Trichoderma* isolates were identified as *T. asperellum* and *T. virens*. The *Fusarium* isolate was identified as *Fusarium solani* (NCBI GeneBank accession numbers obtained after deposition of accessions *Trichoderma asperellum, Trichoderma virens* and *Fusarium solani* are MG198706, MG199587 and MF685335 respectively) [18].

2.2. Method of Preparation of Trichoderma spp. Formulations

Formulation I

The mass culture medium comprises a mixture of molasses and yeast. 500 mL of the medium was autoclaved (121 ^{o}C for 20 minutes) and then inoculated with 1 cm diameter eight agar discs of pure culture of *Trichoderma asperellum* only and incubated at room temperature while shaking at 180 rpm for 14 days.

Formulation II

Four discs each of 1 cm diameter were cut from each *Trichoderma* spp. (*i.e. Trichoderma asperellum* and *Trichoderma virens*) and inoculated together into 500 mL molasses and yeast broth and incubated as mentioned above.

Both mass cultured preparations were added to autoclaved talc which served as the carrier medium at a 1:2 (v/w) ratio under aseptic conditions [19, 20].

The field experiment conducted to test the effectiveness of the two formulations against damping off disease caused by *Fusarium solani* was extended up to transplanted stage of onion crop to test their effectiveness against the basal rot disease in the field.

Field experiments to test for the effect of Trichoderma spp. on Allium cepa L. diseases and growth

Tests were carried out during nursery stage of cultivation of the crop in the field.

2.3. Preparation of Seed Beds- Nursery Stage

Field experiment using the seedlings at the nursery stage was conducted in a farmer nursery in Galewela during 17th May-17th June 2015 (*yala* season). Three to four weeks before planting the seeds, the land was ploughed and the soil turned to a 20-25 cm depth several times and left exposed to direct sunlight.

Three standard nursery beds of 3.6 m x 0.9 m x 0.15 m were prepared. Each bed was considered as a block (Figure 1). Each block was divided into 12 plots of 0.3 m X 0.9 m X 0.15 m. Decomposed organic manure was incorporated into a 10 cm depth (10-15 kg/standard bed). Soil was turned once a week about 3 times and rice straw and rice husk was burnt on the beds.

Rows 10 cm apart from each other were marked in each plot. Then a groove of about 12 mm depth was made along each row.

6.5 g of seeds of the onion cultivar *Galewela light red* were planted in each plot by spreading evenly in each groove. Seeds were then covered with a thin layer of soil. The seedbeds were then covered with light mulch and irrigated twice a week. After germination, of seeds, the mulch was removed. Hand weeding and watering was done until the seedlings are 6-8 weeks old when they were ready for transplanting.

Treatments were allocated to each plot in 3 replicate blocks and the distribution of treatments was as indicated in Figure 1. Twelve treatments tested in the trial (Table 1) were distributed as a Randomized Complete Block Design (RCBD), in the three replicate blocks.

During the field trials, soil populations of *Trichoderma* spp. was monitored using serial dilution tests.

The details of treatments and the quantities used are as depicted in Table 1.

Table 1. *Treatments tested in the nursery field trial.*

Treat- ment	Notes
T1	7 g of *Trichoderma asperellum* in talc (Formulation I) was mixed with 200 g of C.C.M. */0.27 m^2 of plot+ artificial soil inoculation with *Fusarium solani* C.C.M. was applied as a 75-mm layer
T2	7 g of *Trichoderma virens* and *Trichoderma asperellum* together in talc (Formulation II) was mixed with 200 g of C.C.M. */0.27 m^2 of plot+ artificial soil inoculation with *Fusarium solani* C.C.M. was applied as a 75-mm layer
T3	*Trichoderma asperellum* in talc (Formulation I) was applied to batches of seed 6.5 g in 50 mL conical flasks. The flasks were gently rotated for 10 min. to distribute the powder homogenously (as seed treatment) + artificial soil inoculation with *Fusarium solani* C.C.M. was applied as a 75-mm layer
T4	*Trichoderma virens* and *Trichoderma asperellum* together in talc (Formulation II) was applied to batches of seed 6.5 g in 50 mL conical flasks. The flasks were gently rotated for 10 min. to distribute the powder homogenously (as seed treatment) + artificial soil inoculation with *Fusarium solani* C.C.M. was applied as a 75-mm layer
T5	Standard fungicide treatments were done according to the recommendations given by the Department of agriculture. The seeds were treated with Thiram at 2 g/kg of seed before sowing, The top soil of nursery was treated with combination of Thiram 80%, Thiophanate methyl 70% WP and nursery was drenched with the same chemical at 2 g/ liter of water at fortnight interval + artificial soil inoculation with *Fusarium solani* C.C.M. was applied as a 75-mm layer
T6	*Fusarium solani* artificial inoculation into soil (Control - artificial soil inoculation with *Fusarium solani* C.C.M. was applied as a 75-mm layer)
T7	7 g of *Trichoderma asperellum* in talc (Formulation I) was mixed with 200 g of C.C.M. */0.27 m^2 of plot+ natural *Fusarium solani* inoculum present in the soil
T8	7 g of *Trichoderma virens* and *Trichoderma asperellum* together in talc (Formulation II) was mixed with 200 g of C.C.M. */0.27 m^2 of plot+ natural *Fusarium solani* inoculum present in the soil
T9	*Trichoderma asperellum* in talc (Formulation I) was applied to batches of seed 6.5 g in 50 ml conical flasks. The flasks were gently rotated for 10 min. to distribute the powder homogenously (as seed treatment) + natural *Fusarium solani* inoculum present in the soil
T10	*Trichoderma virens* and *Trichoderma asperellum* together in talc (Formulation II) was applied to batches of seed 6.5 g in 50 ml conical flasks. The flasks were gently rotated for 10 min. to distribute the powder homogenously (as seed treatment)+ natural *Fusarium solani* inoculum present in the soil
T11	Standard fungicide treatments were done according to the recommendations given by the Department of agriculture. The seeds were treated with Thiram at 2 g/kg of seed before sowing, The top soil of nursery was treated with combination of Thiram 80%, Thiophanate methyl 70% WP and nursery was drenched with the same chemical at 2 g/ liter of water at fortnight interval + natural *Fusarium solani* inoculum present in the soil
T12	Natural *Fusarium solani* inoculum present in the soil-Neither *Trichoderma* spp. in talc (No Formulations) nor standard fungicide application (Control)

*Composted Chicken Manure

Trichoderma asperellum population in Talc-based *Trichoderma asperellum* preparation was $1.2x10^7$ C.F.U./g

Population of *Trichoderma virens* and *Trichoderma asperellum* in combined formulation *i.e.* Talc-based *Trichoderma virens* and *Trichoderma asperellum* preparation were $0.6x10^7$ C.F.U./g and $0.6x10^7$ C.F.U./g respectively.

*Seven day old *Fusarium solani* grown on Molasses yeast extract medium for 12 days and the entire biomass along with medium was incorporated into sterile carrier media *viz.* saw dust- 500 g, Rice bran- 100 g, Soy flour- 5 g, Lime – 10 g, $MgSO_4$- 1g, Glucose-10g at 1:2 ratio maintained in aseptic condition. *Fusarium solani* was mixed with composted chicken manure and applied in the nursery as a saw dust/rice bran preparation to obtain 1% (w/w) inoculum level. CFU of *Fusarium solani* within saw dust/rice bran preparation=$5.0x10^3$ CFU/g

Determination of effect of different treatments (Table 1) on incidence and severity of damping-off disease

The incidence and severity of the damping off disease in the treated seedlings was determined 20 days after sowing. A

scoring system was developed as follows with 0-3 severity scales.

0=no infection;

1=yellowing and wilting of leaves;

2=stunting of seedlings (seedling length ≤ 12.0 cm);

3=collapsed seedlings and/or completely dead seedlings.

Fifty onion seedlings were sampled randomly from each plot. Disease incidence was calculated using the following formula

$$\text{Disease incidence} = \frac{\text{Number of infected seedlings}}{\text{Total number of seedlings assessed (50)}} \times 100 \quad (1)$$

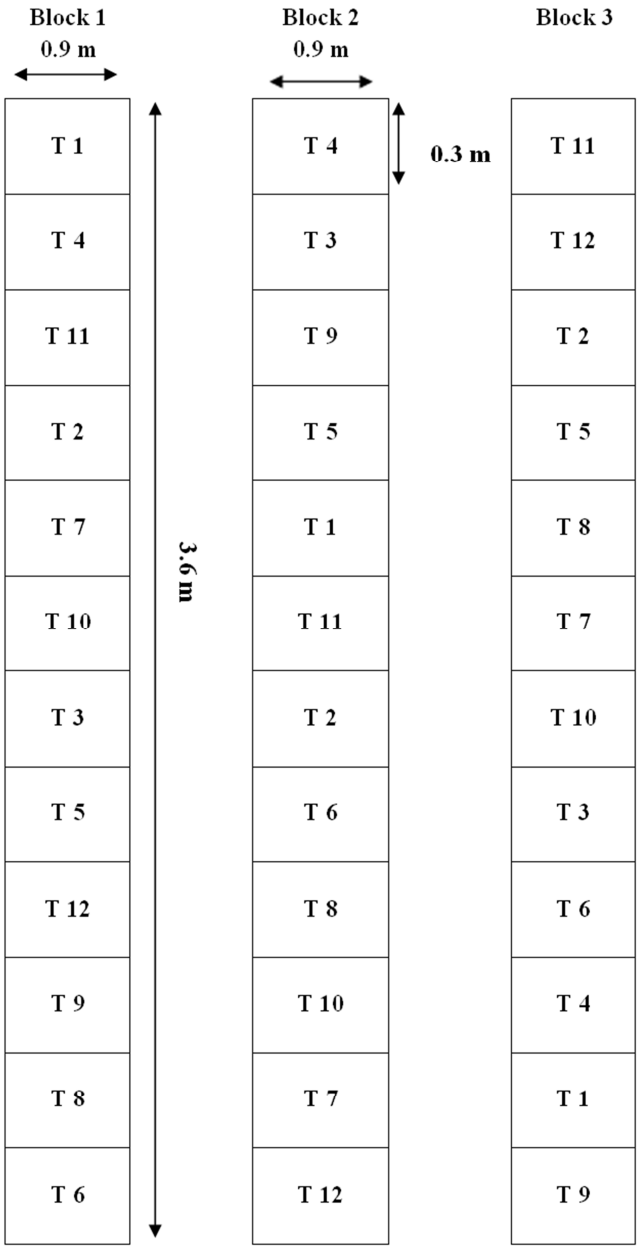

Figure 1. *Experimental design of treatments.*

The disease severity index (DSI) ranged from 0 (no disease) to 100 (all plants killed) and was calculated for each treatment by using the following formula:

$$\text{Disease Severity Index} = \frac{\sum \text{(Class X no.of plants in class)}}{\text{Total no.of plants} \times \text{Maximum disease grade}} \times 100 \quad (2)$$

where class indicates 0-3 and the maximum disease grade indicates 3.

Determination of effect of Trichoderm formulations on the growth of plants

Plants were harvested from each plot and transported to the laboratory to obtain measurements.

The following growth parameters were evaluated:

root length (30 days after seeding, 12 replicates),

number of roots (30 days after seeding, 15 replicates),

seedling length (20 days and 30 days after seeding, 15 replicates),

fresh weight of seedlings (30 days after seeding, 15 replicates).

The length of the starting point of stem to the end point of the flag of the longest leaf of *Allium cepa* L. seedlings was measured as the seedling length. The lengths of the longest root along with two other randomly selected fibrous roots were measured per onion seedling.

2.4. Statistical Analysis

The means were analyzed by analysis of variance (ANOVA) and Tukey's test at 5% significant level with Minitab 16 statistical software.

2.5. The Effect of Formulations on Transplanted Onion Plants

2.5.1. Preparation of Land for Transplanting

Field experiment using the *Allium cepa* L. plants at the field was conducted in a farmer field in Galewela during 17th June 2015-05th September 2015 (*yala* season). The 30 day old seedlings with 3-4 leaves, 18-20 cm height and with slightly marked bulbs were selected prior to transplanting. Well-drained land was selected. The land was ploughed two to three times to bring the soil to a fine tilth. Primary weed control was done 10-14 day prior to land preparation manually and by using selective weed killer oxyfluorfen (Goal). 6 m X 0.9 m standard size, flat, raised beds were prepared and there were six of such beds. The beds were soaked well by giving pre-transplanting irrigation using sprinkler system. Each of these standard beds was divided into two equal plots of 3 m X 0.9 m.

Seedlings grown under treatments T1-T12 mentioned in the previous nursery stage trials were uprooted and separately transplanted in the beds 10 cm distance from each other. 300 seedlings were uprooted from each treated plot and were replanted in the prepared beds in the field.

Eleven treatments were tested in the trial. Initial treatment within the nursery field trial and subsequent treatment within the transplanted field trial are given in the Table 2.

Field experiment to test the effect of Trichoderma spp. on basal rot disease and on the growth of onion plants

The transplanted seedlings were treated as follows:

Table 2. *Treatments tested in the transplanted field trial.*

Treatment within the transplanted field trial (T.F.T.)	Initial treatment within the nursery field trial (N.T.)	Treatment Description
TT1	T12	T.F.T.- Neither treated seedlings with Root Dipping nor Soil infestation with Talc-based *Trichoderma* spp. preparation (No Formulations) + Soil naturally infected with *Fusarium oxysporum* (Control I)
TT2	T12	T.F.T.- Neither treated seedlings with Root Dipping nor Soil infestation with Talc-based *Trichoderma* spp. preparation (No Formulations) + Composted Chicken Manure + Soil naturally infected with *Fusarium oxysporum* (Control II)
TT3	T9	T.F.T.- Soil infestation with Talc-based *Trichoderma asperellum* preparation (Formulation I) mixed with Composted Chicken Manure to obtain 40% (w/w) inoculum level to add in to the soil naturally infected with *Fusarium oxysporum*
TT4	T7	T.F.T.- Soil infestation with Talc-based *Trichoderma asperellum* preparation (Formulation I) mixed with Composted Chicken Manure to obtain 40% (w/w) inoculum level to add in to the soil naturally infected with *Fusarium oxysporum*
TT5	T10	T.F.T.- Roots of *A. cepa* L. 30 days old seedlings were immersed for 15 min. in the suspension of Talc- based *Trichoderma virens* and *Trichoderma asperellum* preparation (Formulation II) at 250 g/l (Either *Trichoderma* spp. population in the suspension - $1.25x10^6$ CFU/ml of suspension) + Composted Chicken Manure, then seedlings were planted in the soil naturally infected with *Fusarium oxysporum*
TT6	T8	T.F.T.- Roots of *A. cepa* L. 30 days old seedlings were immersed for 15 min. in the suspension of Talc- based *Trichoderma virens* and *Trichoderma asperellum* preparation (Formulation II) at 250 g/l (Either *Trichoderma* spp. population in the suspension – $1.25x10^6$ CFU/ml of suspension) + Composted Chicken Manure, then seedlings were planted in the soil naturally infected with *Fusarium oxysporum*
TT7	T10	T.F.T.- Soil infestation with Talc-based *Trichoderma virens* and *Trichoderma asperellum* preparation (Formulation II) mixed with Composted Chicken Manure to obtain 40% (w/w) inoculum level to add in to the soil naturally infected with *Fusarium oxysporum*
TT8	T8	T.F.T.- Soil infestation with Talc-based *Trichoderma virens* and *Trichoderma asperellum* preparation (Formulation II) mixed with Composted Chicken Manure to obtain 40% (w/w) inoculum level to add in to the soil naturally infected with *Fusarium oxysporum*
TT9	T9	T.F.T.- Roots of *A. cepa* L. 30 days old seedlings were immersed for 15 min. in the suspension of Talc- based *Trichoderma asperellum* preparation (Formulation I) at 250 g/l (*Trichoderma asperellum* population in the suspension - $2.5x10^6$ CFU/ml of suspension) + Composted Chicken Manure, then seedlings were planted in the soil naturally infected with *Fusarium oxysporum*
TT10	T7	T.F.T.- Roots of *A. cepa* L. 30 days old seedlings were immersed for 15 min. in the suspension of Talc- based *Trichoderma asperellum* preparation (Formulation I) at 250 g/l (*Trichoderma asperellum* population in the suspension – $2.5x10^6$ CFU/ml of suspension) + Composted Chicken Manure, then seedlings were planted in the soil naturally infected with *Fusarium oxysporum*
TT11	T11	T.F.T.- Roots of *A. cepa* L. 30 days old seedlings were immersed for 15 min. in the suspension fungicides (Homai) and field was drenched with the fungicides (Topsin)

N.T.-Nursery Treatment, T.F.T.-Transplanted Field Treatment.
Trichoderma asperellum population in Talc-based *Trichoderma asperellum* preparation was $1.2x10^7$ C.F.U. /g, Population of *Trichoderma virens* and *Trichoderma asperellum* in combined formulation of *Trichoderma* spp. *i.e.* Talc-based *Trichoderma virens* and *Trichoderma asperellum* preparation were $0.6x10^7$ C.F.U. /g and $0.6x10^7$ C.F.U. /g respectively.

2.5.2. Effect of Treatments on Incidence and Severity of Basal Rot Disease of Allium cepa L.

The incidence and severity of the basal rot was determined nine weeks after transplanting. The infection was identified on basis of symptoms *i.e.* yellow and tan to brown leaves, necrosis of leaf blades, withering of leaves, discoloured and rotten watery bulbs. Disease incidence was calculated as number of infested plants showing anyone of the above mentioned symptoms out of total numbers (300) of *A.cepa* L. plants observed.

$$\text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \times 100 \quad (3)$$

The disease severity index (DSI) ranging from 0 (no disease) to 100 (all plants killed) was calculated for each treatment by using the following formula:

$$\text{Disease Severity Index} = \frac{(\text{Class X no.of plants in class})}{\text{Total no.of plants} \times \text{Maximum disease grade}} \times 100 \quad (4)$$

where class indicates 0-3 and the maximum disease grade indicates 3

The following disease classes were established for the assessment of disease severity

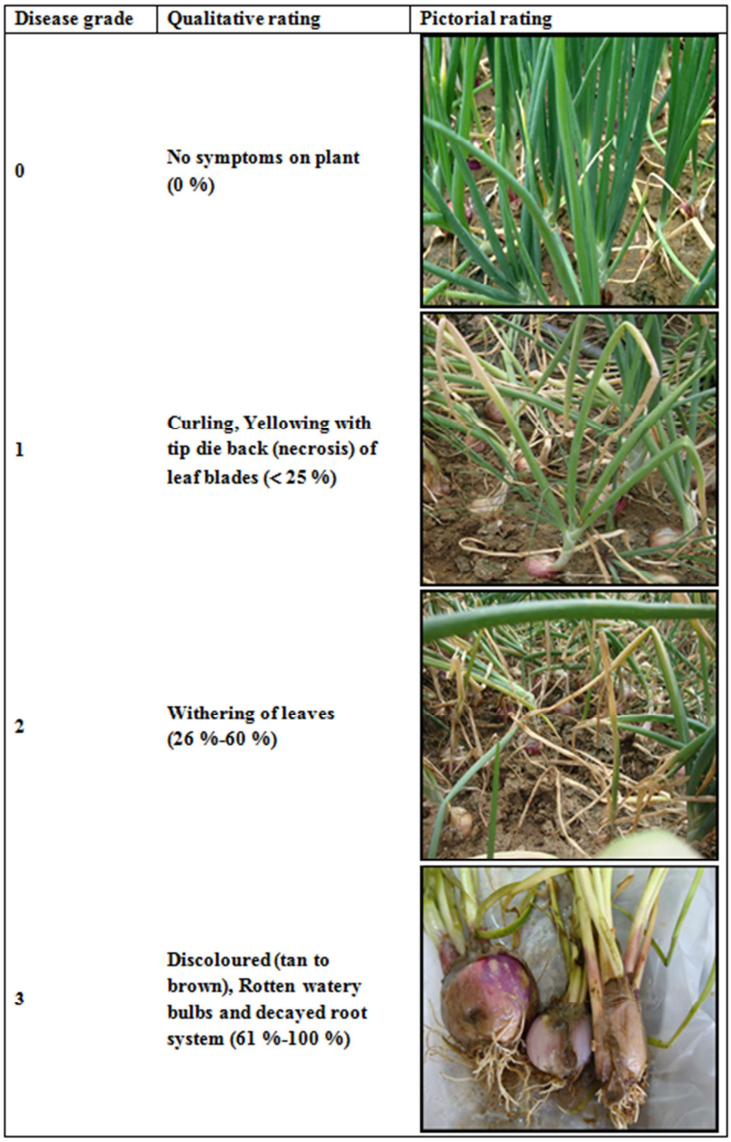

Figure 2. *Disease severity scale for onion basal rot.*

2.5.3. The Effect of Treatments on the Growth of Transplanted Seedlings

The following Growth parameters were evaluated in randomly selected onion plants uprooted carefully after transplanting 10-days old *A. cepa* L. plant fresh weight (5 replicates), dry weight (5 replicates), root length (5 replicates), shoot length (7 replicates), number of roots (5 replicates), 2-months old *A. cepa* L. plant bulb circumference (5 replicates) and fresh weight (5 replicates), healthy bulb weight (15 replicates), diameter (15 replicates) and circumference (15 replicates).

In order to evaluate these parameters, the *A. cepa* L. plants were harvested and transported to the laboratory to obtain measurements. The length of the starting point of stem to the end point of the flag leaf of transplanted *A. cepa* L. plant was measured as the shoot length. The lengths of the longest root along with two other randomly selected fibrous roots were measured per *Allium cepa* L. transplanted plants. Dry weight was measured by oven drying the plants at 60 ^{o}C until a constant weight is reached. Total fresh weight consisting leaves along with fibrous roots were measured as transplanted *A. cepa* L. plant fresh weight.

2.5.4. Statistical Analysis

The means were analyzed by analysis of variance (ANOVA) and Tukey's test at 5% significant level with Minitab 16 statistical software.

3. Results and Discussion

Big onion cultivations in Sri Lanka are mostly concentrated in the Matale and Anuradhapura regions yielding about 75,776 MT annually [21]. However, the amount harvested is insufficient to meet the local demand and one factor that contributes significantly towards reduced yields is diseases that affect onion cultivations deleteriously at different stages of growth.

The survey carried out in a previous study revealed that the more common diseases that affected the plants in all areas of cultivation in Sri Lanka included seedling damping off at the nursery stage and basal rot, leaf and flower stalk anthracnose (twister) under field conditions. The causative agents of these diseases were identified as *Fusarium* spp., *Colletotrichum gloeosporioides*, *Alternaria* sp. and *Sclerotium* sp. Amongst these diseases, damping off and basal rot were the more important production constraints. The pathogenicity of *Fusarium solani* as the causative agent of damping off disease of *A. cepa* L. was confirmed by following Koch's postulates [22, 17].

Fungicides such as Thiram, Homai, Brassicol, Benlate, Captan, Cresent are used currently for the control of onion diseases, Use of agrochemicals has resulted in contamination of the water table and the soil environment resulting in deleterious effects on the environment. Many health problems including the CKDu prevalent in Sri Lanka are suspected to be due to the harmful effect of chemicals used in agricultural practices [23].Therefore, less harmful but effective means of disease management and increased crop productivity should be sought and the use of microorganisms as an alternative to chemicals is a fast developing concept. Microorganims have proven to be effective in the control of pathogens of numerous crops [8, 9].

Trichoderma spp. that are common saprophytic fungi found in almost any environment including many diverse soils, have been used as biocontrol agents due to their ability to reduce the incidence of disease caused by plant pathogenic fungi by means of a wide range of mechanisms. [10, 24-26]. Successful control of soil borne plant pathogenic fungi such as *Rhizoctonia, Sclerotium, Sclerotinia, Fusarium, Pythium, Phytophthora* that cause diseases in onion as well as in other crops by *Trichoderma* spp. have been recorded in Sri Lanka and other countries of the world [27-32].

The ability of *Trichoderma* spp. to control plant diseases are attributed to a number of mechanisms such as competition, mycoparasitism, formation of restrictive structures, antibiosis, and production of secondary metabolites and even induction of resistance in the plant [14, 33, 34].

Introduction of exotic isolates to a soil environment can cause damage to the soil organisms and upset the ecosystem dynamics. Therefore, in the current study, the *Trichoderma* spp. that could be utilized as bio control agents of the pathogens of onion were isolated from local onion fields with a view to select the most effective candidates for the control of causative agents of damping off and basal rot diseases of onion as these two diseases cause considerable economic losses.

Two of the *Trichoderma* isolates *i.e. Trichoderma virens* and *Trichoderma asperellum* isolated from the onion fields in Sri Lanka showed high control ability of phytopathogenic fungi *Fusarium* sp., *Colletotrichum gloeosporioides, Alternaria* sp. under *in vitro* conditions by using mechanisms such as competition, mycoparasitism, formation of restrictive structures [35] and therefore the two spp. were selected for further tests under green house and field conditions. This is the first report of the two *Trichoderma* spp. being present in agricultural fields in Sri Lanka.

In order to prepare formulations of the effective bio control agents, a suitable multiplication medium should be developed. The medium should support a steady state of growth and sporulation of a fungal bio control agent and in the present study, Molasses was developed as a multiplication medium.

Two locally available, natural substrates *i.e.* a sawdust based medium and molasses yeast medium were evaluated as a suitable medium for culture and the molasses yeast medium was selected to be more suitable as it facilitated sufficient sporulation (10^{10} spores/mL) and growth of both *Trichoderma* isolates tested. High numbers of mature chlamydospores were also produced in the molasses yeast medium which may allow prolonged shelf life of the final preparation. As molasses is a byproduct of the sugar industry, it is accessible locally at a fairly low cost which makes molasses yeast a suitable medium in many aspects [20].

Once the selected bio control agent is cultured in the required scale, it has to be prepared as a formulation to be applied in the field. For field applications, the potential bio control agent must be incooporated into a carrier medium with minimum nutrient levels as high nutrients in the carrier medium will enable the growth of other competitive fungal spp in the soil. However, the carrier medium should retain the viability of the bio control agent as well maintaining the required level necessary for control of pathogenic fungi. In the present study, Talc was tested and proved to be suitable as a carrier medium. [20].

Several methods have been recommended for application of bio-fungicides for the successful management of plant diseases by several workers. The most common application strategies are seed biopriming, seedling dip (suitable for the crops where transplanting is practiced), soil application and foliar spray [36]. [9] used coated seeds and soil treatment with different combinations of *Trichoderma* spp. to control *Fusarium* rot of lentil and found out both reduction of disease severity and growth enhancement. According to the investigation carried out by [37], onion basal rot caused by *Fusarium oxysporum* f.sp. *cepa* could be effectively controlled with soil amendment with *Trichoderma* spp.

Based on these reports, two methods *i.e.* seed coating and soil inoculation were tested for the introduction of the prepared *Trichoderma* inoculum under greenhouse conditions and the results showed that seed priming and soil treatment with *Trichoderma* spp. gave the best retention rates and bio control [38].

The field trials were carried out during the *yala* season in a farmer field in the Galewela area. The level of the inoculum in soil was monitored under field conditions and it increased from 10 cfu/g to10^4cfu/g after 1 month and remained at a fairly high level of about (10^4-10^5cfu/g) upto 3 months and reapplications of the inoculum was considered to be unnecessary.

3.1. Effect of Treatments on Damping off Disease in Onion Seedlings at the Nursery Stage

DI and DS of *A. cepa* L. seedlings were evaluated 20 days after sowing (Table 3).

Table 3. *Effect of different treatments on the damping off disease incidence and disease severity of A. cepa L. nursery under field conditions.*

Treatments**	% Disease Incidence (DI%)*	% Disease Severity (DS%)*
T1	10.000 (18.376)^{c}	3.333 (10.464)^{c}
T2	8.667 (17.014)^{c}	2.889 (9.689)^{c}
T3	10.000 (18.433)^{c}	3.333 (10.469)^{c}
T4	10.000 (18.376)^{c}	3.333 (10.464)^{c}
T5	8.000 (16.427)^{c}	2.666 (9.387)^{c}
T6	80.667 (63.961)^{a}	47.667 (43.662)^{a}
T7	0.000 (0.000)^{d}	0.000 (0.000)^{d}
T8	0.000 (0.000)^{d}	0.000 (0.000)^{d}
T9	0.000 (0.000)^{d}	0.000 (0.000)^{d}
T10	0.000 (0.000)^{d}	0.000 (0.000)^{d}
T11	0.000 (0.000)^{d}	0.000 (0.000)^{d}
T12	32.000 (34.399)^{b}	18.222 (25.204)^{b}

* Mean of three replicates

* Mean of three replicates. Figures in parentheses are angular transformed values. DI and DS values within a column followed by different letters are significantly different at $p \leq 0.05$ according to ANOVA. Mean separations by Tukey's test.

The highest average of damping-off disease incidence on *A. cepa* L. seedlings occurred in the T 6 (80.667%) when *Fusarium solani* was inoculated to the plots artificially while T 5 (*Fusarium solani* + standard fungicide application) recorded the lowest damping off disease incidence (8.00%). The indices of severity for untreated plots (T 12) and soil incorporated with *Fusarium solani* alone (T 6) were 18.222% and 47.667% respectively and these values were significantly ($p \leq 0.05$) different from rest of the treatments (T 1-T 11) that had low disease severity indexes. Control of *A. cepa* L. seedling damping off with the effective *Trichoderma* spp. application as either seed coating or soil inoculation was comparable with control obtained with the fungicide treatment.

3.2. Determination of the Level of Trichoderma spp. Inoculum in Soil During the Field Trial

The level of the *Trichoderma* spp. inoculum in soil was monitored under field conditions and remained at 10^4 CFU/g after 1 month.

3.3. Growth Enhancement of A. cepa L. seedlings

Table 4. *Effect of different treatments on Allium cepa L. seedling growth.*

Treatments	Seedling Length (cm) (20 days)	Seedling Length (cm) (30 days)	Root Length (cm) (30 days)	Numbers of roots (30 days)	Seedlings fresh weight (g) (30 days)
T1	14.48±1.477^{cde}	26.20±2.805^{a}	2.64±0.762^{cd}	11.20±1.304^{a}	0.84±0.214^{bc}
T2	14.24±0.877^{de}	25.63±2.866^{a}	3.72±0.737^{abc}	11.00±2.550^{a}	1.20±0.220^{a}
T3	15.88±1.014^{ab}	24.51±3.204^{ab}	3.03±1.250^{bcd}	9.40±1.673^{ab}	0.85±0.133^{bc}
T4	16.25±1.288^{a}	26.47±3.090^{a}	3.07±0.610^{bcd}	9.20±1.643^{ab}	0.10±0.384^{ab}
T5	14.73±1.340^{bcde}	25.44±2.54^{a}	3.10±0.973^{bcd}	8.20±1.095^{ab}	0.83±0.330^{bc}
T6	12.15±1.032^{f}	15.91±1.104^{c}	2.33±0.794^{d}	6.80±0.837^{b}	0.46±0.143^{d}
T7	15.51±0.494^{abcd}	26.79±4.032^{a}	3.44±1.003^{abcd}	10.20±1.304^{a}	0.92±0.395^{abc}
T8	15.73±1.063^{abc}	23.89±2.625^{ab}	2.94±0.707^{bcd}	10.20±1.643^{a}	0.86±0.303^{bc}
T9	16.29±0.473^{a}	25.02±2.267^{a}	3.87±1.045^{ab}	10.80±1.304^{a}	0.99±0.256^{ab}
T10	15.85±0.402^{ab}	26.92±2.235^{a}	4.62±1.109^{a}	11.20±2.168^{a}	0.95±0.230^{abc}
T11	15.73±1.689^{abc}	25.10±1.908^{a}	3.00±0.684^{bcd}	8.40±1.14a^{b}	0.80±0.206^{bc}
T12	13.94±0.630e	21.50±1.878^{b}	2.40±0.486^{d}	8.60±0.894^{ab}	0.67±0.228^{cd}
Number of replicates	n=15	n=15	n=12	n=15	n=15

3.3.1. Effect of Different Treatments on Seedling Length (cm) of Allium cepa L. (20 Days, 30 Days After Sowing)

Treatments *i.e.* T 4 and T 9 had a significantly ($p \leq 0.05$) larger seedling height (16.2533±0.33 cm, n=15 and 16.2933±0.12 cm, n=15 respectively) than other treatments at 20 days of seedling growth. The shortest seedlings were recorded in treatments having no fungicides and bio-agents *i.e.* 12.1467±0.27 cm, n=15 mean value for seedlings infected by *Fusarium solani* (T 6) and to 13.94±0.16 cm, n=15 mean value in the uninfected control seedlings (T 12).

Results revealed that the average of 30 days old *A. cepa* L. seedling height with *Trichoderma* spp. application were in the range of 23.8933±0.68 cm - 26.92±0.58 cm, n=15 compared to 15.9067±0.28 cm, n=15 in the control seedlings infected by *Fusarium solani* and to 21.5±0.48 cm in the uninfected control seedlings. No significant differences were recorded among *Trichoderma* treatments and standard fungicide treatments, while significant differences were recorded between *Trichoderma* treatments and the *Fusarium solani* inoculated and *Fusarium solani* non-inoculated controls. Significantly ($p \leq 0.05$) least seedling height was obtained in pots where *Fusarium* was inoculated alone (control) *i.e.* 15.9067±0.28 cm, n=15 followed by uninfected control seedlings (Table 4).

3.3.2. Effect of Different Treatments on Root Length (cm) of Allium cepa L. (30 Days After Sowing)

Seeds previously treated with both *Trichoderma* spp. (*Trichoderma virens*, *Trichoderma asperellum*) produced the longest fibrous roots. Means comparisons showed that there was significant difference between *Trichoderma* spp. inoculated treatments and the *Fusarium solani* inoculated and *Fusarium solani* non-inoculated controls (Table 4).

3.3.3. Effect of Different Treatments on the Number of A.cepa L. fibrous Raoots

The number of average roots per seedling as result of application of *Trichoderma* spp. were in the range of 9.2±0.73 - 11.2±0.58 roots/seedling, n=5. No significant differences were recorded among *Trichoderma* spp. inoculated treatments, standard fungicide treatments and *Fusarium solani* non-inoculated control (T 12) while significant differences were recorded between Treatments 1,2,3,4,5,7,8,9,10,11,12 and the *Fusarium solani* inoculated control (T 6) (Table 4).

3.3.4. Effect of Different Treatments on Seedling Fresh Weight of Allium cepa L. (30 Days After Sowing)

By looking at fresh weight measurements, a significant difference ($p \leq 0.05$) in fresh weight between all treatments was observed. Lowest mean seedling fresh weight (0.460667±0.04 g, n=15) was recorded by *Fusarium solani* inoculated control (T 6). Higher seedling fresh weight (1.19973±0.06 g, n=15) was recorded with *Trichoderma* spp. (*Trichoderma virens*, *Trichoderma asperellum*) + *Fusarium solani* inoculation (T 2) and were on par with rest of the *Trichoderma* spp. inoculated treatments *i.e.* T 4, T 7, T 9, T 10 (Table 4).

3.3.5. Field Experiments to Test for the Effect of Trichoderma spp. on Basal Rot Disease of Allium cepa L.

The effect of each treatment on DI, DS and growth parameters of *A. cepa* L. plants and bulbs were evaluated at 9 weeks after transplanting.

In all cases DI and DS of *A. cepa* L. plants were evaluated at 9 weeks after transplanting.

Table 5. *Effect of different treatments on the basal rot disease incidence and disease severity of A. cepa L. under field conditions.*

Treatments	Disease Incidence% (DI%)*	Disease Severity% (DS%)*
TT1	83.33	51.00
TT2	79.33	45.22
TT3	12.00	6.89
TT4	13.33	7.78
TT5	12.33	6.78
TT6	11.33	6.00
TT7	11.67	6.33
TT8	13.33	7.56
TT9	11.00	6.00
TT10	11.67	5.89
TT11	11.67	6.00

A. cepa L. basal rot disease incidence and disease severity levels were higher in the treatment TT 1 (neither composted chicken manure nor *Trichoderma* spp.) *i.e.* 83.33% and 51.00% followed by the treatment TT 2 (composted chicken manure without *Trichoderma* spp.) *i.e.* 79.33% and 45.22%. These *A. cepa* L. plants raised from the untreated nursery plots *i.e.* neither *A. cepa* L. seed treatment with *Trichoderma* spp. preparation nor soil inoculation using *Trichoderma* spp. preparation. Lower *A. cepa* L. basal rot disease incidence and severity indexes were recorded from rest of the treatments (TT 3-TT 11). Application of *Trichoderma* spp. *i.e.* either *Trichoderma asperellum* alone or in combination *Trichoderma virens* to the rhizosphere of *A. cepa* L. transplants decreased both disease incidence and severity (85.20% disease incidence reduction and 86.32% disease severity reduction relative to *Trichoderma* spp. non-inoculated controls). Pre-treatment of nurseries and subsequent treatment of seedlings before transplanting with *Trichoderma* spp. could be recommended for controlling both damping-off disease in the nurseries and basal rot disease of *A. cepa* L. in the field.

3.4. Field Experiments to Test for the Effect of Trichoderma spp. on Growth of Allium cepa L.

Table 6. *Effect of different treatments on Allium cepa L. plant growth.*

Treatments	Shoot length (cm) (10 days old plants)	Root length (cm) (10 days old plants)	Number of roots (10 days old plants)	Fresh weight (g) (10 days old plant)	Dry weight (g) (10 days old plants)
TT1	32.36± 2.035^{c}	2.54±0.364^{d}	14.00±0.707^{d}	1.55±0.232^{d}	0.14±0.038^{a}
TT2	32.93± 2.457^{bc}	2.86±0.305^{bcd}	14.60±0.894^{d}	1.57±0.197^{d}	0.14±0.028^{a}
TT3	35.71± 2.628^{abc}	4.10±0.741^{a}	20.00±0.000^{ab}	2.12±0.437^{bcd}	0.24±0.158^{a}
TT4	34.94± 3.272^{abc}	3.80±0.570^{ab}	18.20±0.447^{bc}	1.87±0.201^{cd}	0.21±0.056^{a}
TT5	39.50± 3.753^{a}	4.04±0.730^{a}	21.20±2.387^{ab}	2.70±0.579^{ab}	0.28±0.080^{a}
TT6	39.00± 4.203^{a}	2.80±0.273^{bcd}	19.60±0.894^{ab}	2.22±0.402^{abcd}	0.22±0.049^{a}
TT7	35.07± 2.540^{abc}	3.70±0.447^{abc}	19.40±1.140^{ab}	2.25±0.417^{abcd}	0.22±0.038^{a}
TT8	34.17± 1.266^{abc}	2.70±0.273^{cd}	16.00±1.225^{cd}	1.76±0.404^{cd}	0.18±0.065^{a}
TT9	35.71± 2.343^{abc}	4.20±0.273^{a}	20.00±0.000^{ab}	2.44±0.367^{abc}	0.22±0.042^{a}
TT10	37.49± 1.880^{abc}	4.70±0.273^{a}	22.60±3.362^{a}	2.92±0.321^{a}	0.23±0.057^{a}
TT11	38.57± 6.373^{ab}	3.80±0.570^{ab}	18.80±1.789^{bc}	2.47±0.162^{abc}	0.24±0.040^{a}
	n=7	n=5	n=5	n=5	n=5

Table 6. *Continue.*

Treatments	Fresh weight (g) (2 months old plants)	Bulb circumference (cm) (2 months old plants)	Healthy Bulb weight (g)	Bulb diameter (cm)	Bulb circumference (cm)
TT1	9.55±0.865^{b}	9.06±2.230^{b}	31.18±2.143^{d}	4.03±0.351^{b}	13.07±0.884^{c}
TT2	11.00±1.440^{b}	10.16±1.236^{ab}	31.37±1.995^{d}	4.03±0.639^{b}	13.17±0.617^{c}
TT3	28.62±5.002^{a}	12.18±1.757^{ab}	63.31±8.346^{a}	5.13±0.296^{a}	17.03±1.109^{a}

Treatments	Fresh weight (g) (2 months old plants)	Bulb circumference (cm) (2 months old plants)	Healthy Bulb weight (g)	Bulb diameter (cm)	Bulb circumference (cm)
TT4	27.52±5.222[a]	11.04±1.857[ab]	56.13±6.174[ab]	5.00±0.378[a]	16.13±0.972[a]
TT5	28.53±3.218[a]	12.46±1.777[a]	56.89±7.835[ab]	5.20±0.414[a]	16.77±1.015[a]
TT6	23.19±3.832[a]	12.24±1.016[ab]	51.41±7.413[bc]	4.99±0.493[a]	16.13±1.109[a]
TT7	29.57±1.879[a]	12.36±1.428[ab]	55.00±6.928[bc]	4.83±0.308[a]	16.10±0.761[a]
TT8	24.76±4.157[a]	11.22±1.787[ab]	51.60±3.250[bc]	5.03±0.296[a]	16.20±1.014[a]
TT9	28.10±6.712[a]	12.36±1.031[ab]	55.57±6.321[bc]	5.23±0.371[a]	16.67±1.063[a]
TT10	26.86±4.623[a]	12.16±1.205[ab]	56.32±8.291[ab]	5.17±0.308[a]	16.53±0.719[a]
TT11	27.10±3.759[a]	12.08±1.087[ab]	48.38±4.478[c]	4.80±0.253[a]	14.80±0.841[b]
	n=5	n=5	n=15	n=15	n=15

Shoot growth of treatments TT 5, TT 6 and TT 11 was increased significantly compared to control treatments (TT 1 and TT 2). Mean shoot length of treatments TT 2, TT 3, TT 4, TT 7, TT 8, TT 9, TT 10 and TT 11 were statistically similar, but *Trichoderma* spp. based bio-fungicides multiplied in combination with composted chicken manure *i.e.* TT 3-TT10 were more effective treatments to increase shoot length as composted chicken manure provide a substrate for multiplication of *Trichoderma* spp.

The root growth of 10 day old *A. cepa* L. transplant was significantly enhanced by the different *Trichoderma* spp. based treatments as compared to the untreated controls. The range in root length was 2.7 – 4.7 cm for treatments with *Trichoderma* spp. Number of roots per plant was also increased significantly compared to control treatments TT 1 and TT 2.

Treatment TT 10 significantly increased transplant fresh weight compared with the other treatments. The fungicide treatment (TT 11) mean fresh weight was comparable with the treatment TT 10.

The minimum fresh weight for 2 month old *A. cepa* L. transplants was recorded from TT 1 followed by TT 2. Application of bio-fungicides had significant effect on the *A. cepa* L. 2 month old plants compared with the untreated controls. Efficiency of all treatment strategies to increase fresh weight was statistically similar.

The mean maximum dry weight for 10 day old transplants was recorded in treatment TT 5 (Initial seed treatment with *Trichoderma virens*+ *Trichoderma asperellum* and subsequently seedling roots were treated with *Trichoderma virens*+ *Trichoderma asperellum*) followed by TT 11. All treatments with *Trichoderma* spp. based bio-fungicides had increased the dry weight and fresh weight. Both fungicide treatment (TT 11) and *Trichoderma* spp. inoculated treatments (TT 3, TT 4, TT 5, TT 6, TT 7, TT 8, TT 9, TT 10) significantly increased the weight of healthy bulbs compared with the untreated controls (TT 1 and TT 2). The maximum bulb weight was recorded in treatment TT 3 followed by TT 5.

3.5. Effect of Different Treatments on Allium cepa L. bulb Diameter and Circumference

Both fungicide treatment (TT 11) and application of *Trichoderma* spp. (TT 3, TT 4, TT 5, TT 6, TT 7, TT 8, TT 9, TT 10) significantly increased healthy bulb diameter and circumference ($p \leq 0.05$) compared with the untreated controls. The healthy bulb diameter for *Trichoderma* spp. applied *A. cepa* L. planting was between 4.833 cm and 5.033 cm. The circumference for healthy bulb for *Trichoderma* spp. applied *A. cepa* L. planting was between 16.133 cm and 17.033 cm.

Results of the field trials showed that the inoculation of the *Trichoderma* spp. together and *T. asperellum alone* reduced post emergence damping off disease incidence and the disease level was significantly different ($p \leq 0.05$) from that of the uninoculated controls. A significant disease reduction was evidenced even in the treatments where a high level of pathogen inoculum was added to the soil. However, inoculation of the two *Trichoderma* spp. in combination did not show an increased level of control of the disease when compared with *T. asperellum* inoculation alone. Similar inoculations of more than one *Trichoderma* spp are sometimes reported to be effective in controlling pathogens. By using a combination of *T. harzianum*, *T. asperellum* and *T. virens* it was possible to reduce disease incidence percentage of cucumber fields exposed to *Fusarium* pathogens such as *F. solani* and *F. oxysporum* as agents of root and stem rot cucumber under greenhouse conditions [39]. Similarly, sesame seeds treated with three isolates of *Trichoderma viride* reduced the pre and post emergence damping off caused by *Rhizoctonia solani* and *Fusarium oxysporum* f.sp. *sesame* under pot and field conditions. In the present study the level of control achieved by the *Trichoderma* inocula was not significantly different from that achieved by the fungicide treatment.

Among tested treatment strategies, initial seed treatment with *Trichoderma* spp. would be relatively more effective than soil infestation with *Trichoderma* spp. The amount of *Trichoderma* spp. added using soil application was much greater than that carried by the *A. cepa* L. seed treatment. But with soil application, the *Trichoderma* spp. dispersed in a greater area whereas with seed treatment the *Trichoderma* spp. remained concentrated on or around the seed and later on in the rhizosphere. The *Trichoderma* spp. receive wide range of nutrients *i.e.* amino acids, carbohydrates, organic acids etc. through profuse exudation during *A. cepa* L. seed germination and root exudation. As a result they aggregate in close vicinity of root system and multiply. This may lead to enhancement of *A. cepa* L. plant growth through phosphate solubilization, hormone production [40-43] and better reduction of damping off disease through pathogen suppression by the *Trichoderma* spp. by shielding root zone.

The field trial was continued during *yala* season to evaluate the efficacy of *Trichoderma* spp. in managing basal rot disease of *A. cepa* L. The effect of *Trichoderma* spp. on vegetative parameters of *A. cepa* L. was also assessed.

Results of the current field trial showed that the

inoculation of the *Trichoderma asperellum* alone or *Trichoderma asperellum* and *Trichoderma virens* together had major influences on reduction of *A. cepa* L. basal rot disease incidence, severity and enhancement of growth. The reduction of disease incidence and severity of *Trichoderma* spp. treatments are comparable to the standard fungicide treatments. The satisfactory control of the basal rot of *A. cepa* L. indicates that the used *Trichoderma* spp. *i.e. Trichoderma asperellum, Trichoderma virens* suppressed the basal rot pathogen *i.e. Fusarium oxysporum* f. sp. *cepae* effectively. Additionally, *Trichoderma* spp. inoculation also enhanced *A. cepa* L. plant growth parameters.

Growth promotion activities of *Trichoderma* might be a direct consequence of colonization, enhanced positive interaction with the plant, increased nutrient uptake by plant or due to reduction of pathogen activity [14]. *Trichoderma harzianum* inoculated pigeon pea (*Cajanus cajan* L Millsp) seedlings are reported to show increased dry weight and P uptake per plant over control [44]. Additional evidence for phosphate solubilization has been reported in studies by [25] and [14]. Induction of systemic resistance in plants by *Trichoderma* spp. has also been reported by many workers but these aspects were not investigated in the present study.

4. Conclusions

Trichoderma virens and *Trichoderma asperellum* isolated from soils of *A. cepa* L. growing areas can be effectively used in the management of *A. cepa* L. damping off and basal rot diseases under field condition. Further, application of *Trichoderma* spp. *i.e.* either *Trichoderma asperellum* alone or *Trichoderma asperellum* in combination with *Trichoderma virens* as seed coating or soil inoculation at the time of sowing and subsequent treatment with *Trichoderma* spp. as seedling root dip or soil infestation at the time of transplanting significantly increased growth parameters compared with that in the non-*Trichoderma* spp. inoculated control treatments.

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References

[1] DCS, 2009. Statistical Abstracts. Department of Census and Statistics, Colombo, Sri Lanka.

[2] Conn K E, Jeffrey S L and Rosenberger S A, 2012. Onion Disease Guide Seminis vegetable seeds, inc.

[3] Mishra R K, Jaiswal R K, Kumar D, Saabale P R and Singh A, 2014. Management of major diseases and insect pests of onion and garlic: A comprehensive review. J. Plant Breed. Crop Sci. 6 (11): 160-170.

[4] Rajapakse R G A S and Edirimanna E R S P, 2002. Management of bulb rot of big onion (*Allium cepa*) during storage using fungicides. Annals of the Sri Lanka Department of Agriculture. 4: 319-326.

[5] Edirimanna E R S P and Rajapakse R G A S, 2003. Effect of cultural practices on seed yield of big onion (*Allium cepa* L.), Annals of the Sri Lanka Department of Agriculture 05: 69.

[6] Maankotte K N, 2004. Loku lunu wagawa, pp. 1-46. 2nd edition. Agriculture Department Publication.

[7] Vinale F, Manganiello G, Nigro M, Mazzei P, Piccolo A, Pascale A, Ruocco M, Marra R, Lombardi N, Lanzuise S, Varlese R, Cavallo P, Lorito M and Woo SL, 2014. A Novel Fungal Metabolite with Beneficial Properties for Agricultural Applications Molecules, 19: 9760-9772.

[8] Amal A A, Elsalam K A A, Omar M R and Aly A A, 2005. Antagonistic potential of *Trichoderma* spp. against *Rhizoctonia solani* and use of M13 microsatellite-primed PCR to evaluate the antagonist genetic variation. J plant dis protect. 112 (6): 550–561.

[9] Akrami M, Golzary H and Ahmadzadeh M, 2011. Evaluation of different combinations of *Trichoderma* species for controlling *Fusarium* rot of lentil. Afr. J. Biotechnol. 10 (14): 2653-2658.

[10] Gveroska B and Ziberoski J, 2011. The influence of *Trichoderma harzianum* on reducing root rot disease in tobacco seedlings caused by *Rhizoctonia solani.* International Journal of Pure and Applied Sciences and Technology. 2 (2): 1-11.

[11] McLean K L and Stewart A, 2000. Application strategies for control of onion white rot by fungal antagonists, New Zeal J Crop Hort. 28: 2, 115-122.

[12] Chaudhari P J, Shrivastava P and Khadse A C, 2011. Substrate evaluation for mass cultivation of *Trichoderma viride* Asiat. J. Biotechnol. Resour. 2 (04): 441-446.

[13] Khan, Shafa and Bagwan N and Mohammed, Asef Iqbal and Tamboli R, 2011. Mass Multiplication and Shelf life of Liquid Fermented final Product of Trichoderma viride in Different Formulations. Adv Biores. 2: 178-182.

[14] Harman G E, 2000. Myths and dogmas of biocontrol: Changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Dis. 84: 377-393.

[15] Harman G E, Howell C R, Viterbo A, Chet I and Lorito M, 2004. *Trichoderma* species-opportunistic, avirulent plant symbionts. Nat rev microbiol, 2: 43-56.

[16] Warcup J H, 1950 Nature, Lond, 166: 117.

[17] Gunaratna L N R and Deshappriya N, 2014. Fungi associated with diseases of big onion (*Allium cepa* L.) prevalent in Matale district, Sri Lanka. Journal of the Faculty of Graduate Studies, University of Kelaniya. 3: 92-111.

[18] Gunaratna L N R, Deshappriya N, Jayaratne D L and Rajapaksha R G S A S, 2019. Damping-off disease of big onion (*Allium cepa*L.) in Sri Lanka and evaluation of *Trichoderma asperellum* and *Trichoderma virens* for its control. Trop. Plant Res. 6 (2): 275-292.

[19] Gunaratna L N R, Deshappriya N and Jayaratna D L, 2014. *Trichoderma* sp. as a promising biological control agent against damping off disease in big onion (*Allium cepa* L.), p. 94. Proceedings of 15th Annual research symposium, Faculty of Graduate Studies, University of Kelaniya.

[20] Gunaratna L N R, Deshappriya N and Rajapakse R G S A S, 2017. A low cost mass culture medium for *Trichoderma* spp. for the control of damping off disease of big onion (*Allium cepa* L.), p. 12. Proceedings of the International Research Symposium on Pure and Applied Sciences*,* 20 October 2017

[21] DCS, 2015. Statistical Abstracts. Department of Census and Statistics, Colombo, Sri Lanka.

[22] Gunaratna L N R and Deshappriya N, 2013. Fungal diseases and associated pathogens of big onion (*Allium cepa* L.) prevalent in the Matale district, Sri Lanka, p. 12. Proceedings of 14th Annual Research Symposium, Faculty of Graduate Studies, University of Kelaniya.

[23] Balasubramanya S, Stifel D, Horbulyk T and Kafle K, 2020. Chronic kidney disease and household behaviors in Sri Lanka: Historical choices of drinking water and agrochemical use. Econ Hum Biol. 37: 1-16.

[24] Howell C R and Puckhaber L S, 2005. A study of the characteristics of "P" and "Q" strains of *Trichoderma virens* to account for differences in biological control efficacy against cotton seedling diseases. Biol. Control. 33: 217–222.

[25] Dixit R, Singh R B and Singh H B, 2015. Screening of antagonistic potential and plant growth promotion activities of *Trichoderma* spp. and fluorescent *Pseudomonas* spp. isolates against *Sclerotinia sclerotiorum* causing stem rot of French bean. Legume Res. 38 (3): 375-381.

[26] López-López N, Segarra G, Vergara O, López-Faba A and Trillas M I, 2015. Compost from forest cleaning green waste and *Trichoderma asperellum* strain T34 reduced incidence of *Fusarium circinatum* in *Pinus radiata* seedlings. Biol. Control. 95: 31-39.

[27] Coventry E, Noble R, Mead A and Whipps J M, 2002. Control of *Allium* white rot (*Sclerotium cepivorum*) with composted onion waste. Soil Biol. 34: 1037–1045.

[28] Harman G E, Petzoldt R, Comis A and Chen J, 2003. Interactions between *Trichoderma harzianum* strain T22 and maize inbred line Mo17 and effects of these interations on diseases caused by *Pythium ultimum* and *Colletotrichum graminicola*. Phytopathology. 94 (2): 147–153.

[29] Coventry E, Noble R, Mead A and Whipps J M, 2005. Suppression of *Allium* white rot (*Sclerotium cepivorum*) in different soils using vegetable wastes. Eur. J. Plant Pathol. 111: 101–112.

[30] Coventry E, Noble R, Mead A, Marin F R, Perez J A and Whipps J M, 2006. Allium White Rot Suppression with Composts and Trichoderma viride in Relation to Sclerotia Viability. Phytopathology. 96 (9), 1009-1020.

[31] Rajapakse R G A S, Kahawatta J and Sakalasooriya S M I S K, 2006. Management of Corm Rot of Kiriala (*Xanthosoma sagittifolium* (L) Schott) by Biological Control Agent, *Trichoderma harzianum.* Annals of Sri Lanka Department of Agriculture. 8: 183-191.

[32] Vinale F, Sivasithamparam K, Ghisalberti E L, Marra R, Woo S L and Lorito M, 2007. *Trichoderma* – plant – pathogen interactions. Soil Biol. Biochem. 40 (1): 1-10.

[33] Howell C R, 2003. Mechanisms Employed by *Trichoderma* Species in the Biological Control of Plant Diseases: The History and Evolution of Current Concepts. Plant Dis. 87: 4-10.

[34] Shalini, Narayan K P, Lata and Kotasthane A S, 2006. Genetic relatedness among *Trichoderma* isolates inhibiting a pathogenic fungi *Rhizoctonia solani.* Afr. J. Biotechnol. 5 (8): 580-584.

[35] Gunaratna L N R, Deshappriya N and Rajapakse R G A S, 2015. Determination of the antifungal, biochemical and physiological characteristics of *Trichoderma* spp. isolated from onion fields of Sri Lanka, p. 73. Proceedings of the 35th Annual Sessions, The Institute of Biology, Sri Lanka.

[36] Srivastava P C, Ansari U I, Pachauri S P and Tyagi A K, 2016. Effect of zinc application methods on apparent utilization efficiency of zinc and potassium fertilizers under rice-wheat rotation, J. Plant Nutr. 39 (3): 348-364.

[37] Malathi S, 2015. Biological control of onion basal rot caused by *Fusarium oxysporum* f.sp. *cepae.* Asian Journal of Science. 10 (1): 21-26.

[38] Gunaratna L N R, Deshappriya N, Rajapaksha R G S A S and Jayaratne D L, 2020. The efficacy of *Trichoderma asperellum* and *Trichoderma virens* inocula for the control of *Fusarium solani* the causative agent of damping off disease of *Allium cepa* L. under green house conditions. Adv Biores. (in press).

[39] Akrami M, 2015. Effects of *Trichoderma* spp. in Bio-controlling *Fusarium solani* and *F.oxysporum* of Cucumber (*Cucumis sativus*) Journal of Applied Environmental and Biological Sciences 4 (3): 241-245.

[40] Altomare C, Norvell WA, Bjorkman T. and Harman G E, 1999. Solubilization of Phosphates and Micronutrients by the Plant-Growth-Promoting and Biocontrol Fungus *Trichoderma harzianum* Rifai 1295-22 Appl. Environ. Microbiol. 2926–2933.

[41] Contreras-Cornejo H A, Macı´as-Rodrı´guez L, Corte´s-Penagos C and Lo´pez-Bucio, J, 2009. *Trichoderma virens*, a Plant Beneficial Fungus, Enhances Biomass Production and Promotes Lateral Root Growth through an Auxin-Dependent Mechanism in Arabidopsis. Plant Physiol. 149: 1579–1592.

[42] Martínez-Medina A, Alguacil M D M, Pascual J A and Van Wees S C M, 2014. Phytohormone Profiles Induced by *Trichoderma* Isolates Correspond with Their Biocontrol and Plant Growth- Promoting Activity on Melon Plants. J. Chem. Ecol. 40: 804–815.

[43] Singh V, Upadhyay R S, Sarma B K and Singh H B, 2016. Seed bio-priming with *Trichoderma asperellum* effectively modulate plant growth promotion in pea. Int. J. Agric. Environ. Biotechnol. 9 (3): 361-365.

[44] Dehariya K, Sheikh I A, Dubey M K, Ahirwar S, Shukla A, Singh V and Vyas D, 2013. Interactive effect of *Trichoderma* species with *Glomus intraradices* in growth promotion and wilt disease suppression of *Cajanus cajan.*Int. J. Adv. Res. (1) 8: 867-873.