

**DEVELOPMENT OF *TRICHODERMA* BASED BULB TREATMENT FOR THE MANAGEMENT OF ANTHRACNOSE AND WHITE ROT IN ONION (*ALLIUM CEPA* L.)**

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**ABSTRACT**

Onion (*Allium cepa* L.) is affected to anthracnose and white rot infections in fields. Onion producers rely on fungicides, to treat these diseases despite the high production expense and environmental degradation associated with this approach. Using the commonly used biocontrol agent *Trichoderma* fungus to treat onion bulbs might be a sustainable way to address these diseases. Research was conducted to design a bulb treatment strategy prior to before planting. *MIBO* 01 bulbs were treated as follows: they were mixed with *Trichoderma* powder ( $4 \times 10^6$  CFU per gram) (T1), soaked in *Trichoderma* liquid (T2), and soaked in a fungicide mixture of 50% Thiophanate-methyl + 30% Thiram W/W (T3), along with an untreated control (T4). The pots were set up using a fully randomized design (CRD), with ten replicates for each treatment. Antagonistic tests were conducted with *Trichoderma asperellum*, *Colletotrichum gloeosporioides* and *Stromatinia cepivora* Using SAS software, the data was put through a one-way ANOVA and DMRT at a 5% probability level. Anthracnose Disease Severity index (ADSI) and the white rot disease incidence (WDI) were significantly different among the treatments at  $P < 0.05$ . ADSI values in T1, T2, T3 and T4 were in the order of  $11.1 \pm 0.15\%$ ,  $33.3 \pm 0.35\%$ ,  $39.9 \pm 0.45\%$  and  $63.3 \pm 0.85\%$ , WDI in T1, T2 are 00%, T3 and T4 were in order 30% and 50%, respectively. Repetitive field trials would be helpful in order to optimize the efficacy of the treatment. These findings suggest the pre-planting application of *Trichoderma* on bulbs as a

promising alternative to fungicides in managing white rot infections and the leaf anthracnose of the big onion variety *MIBO 01*. So *Trichoderma asperellum* can use as a alternative method to manage onion anthracnose and white rot disease in onion.

**Keywords:** Anthracnose, Big onion, Bulb treatment, White Rot, *Trichoderma asperellum*

## 1. INTRODUCTION

The onion, which belongs to the Liliaceae family, is a vegetable spice. The big onion is one of the oldest and one of important cash crops grown in Sri Lanka and originated in Central Asia (Selvaraj, 1976). It is called the "Queen of the Kitchen" because of its flavour and taste, as well as the colourful bulbs. Moreover, onion has a lot of uses as a salad ingredient; various recipes as well as recent reports prove that it helps prevent heart disease and other ailments (Chowdry, 2015)

The national total big onion production in Sri Lanka in 2022 will be 15,840.3 metric tons. It is mostly grown in districts like Anuradhapura, Kurunegala, Matale, and Polonnaruwa, and in areas that belong to the Mahaweli H region. Moreover, districts like Mannar, Vavuniya, and Mullativ also cultivate the onion on a small scale (Department of Census and Statistics, 2022).

Anthracnose, purple blotch, white rot, damping off, and bacterial bulb rot disease are the major diseases in Sri Lanka. Anthracnose and white rot can be considered the problematic diseases in big onion bulb cultivation in Sri Lanka. Anthracnose is caused by *Colletotrichum gloeosporioides* and white rot is caused by *Fusarium spp.*, *Pythium spp.*, *Rhizoctonia spp.*, and *Sclerotium spp.* (DOA, 2018).

Symptoms of the anthracnose disease are initially white or yellowish sunken patches on leaves. Leaves may become twisted and sometimes bend from the infected area, stalks can collapse from infected areas, and infected flowers get dried without the formation of seeds. Hence, this can cause a severe yield loss. Infection and development of the disease are high under adverse wet weather conditions. The disease onion anthracnose severely destroys the onion fields, mostly in every cropping season. This causes 80% to 100% yield loss, affects the low supply of onions in the market, and increases the price of onions (Alberto and Aquino, 2010).

White rot has an effect on onions at all stages of the plant's growth. The symptoms of the white rot are the first symptoms are yellowing and dieback of the leaf tips. Then after scales, stem plates, and roots get destroyed, and bulbs become water-soaked and soft. Finally, mycelium gets cottony growth around black sclerotia (Alberto and Aquino, 2010).

Root rot in older plants can result in the death of the plant and bulb rot. Affected plants bulbs are generally slender. Before harvest, some bulbs are rotting, as are others that decay rapidly when stored (Abawi and Lorbeer, 1972).

Common management strategies that can be used to control these diseases are the use of recommended disease-free bulbs, resistant cultivars, proper nursery management adaptations, and fungicidal sprays. Seed or bulb treatment is one of the best curative methods to control diseases at the very initial stage of the crop. A common and most common farmer practice is the use of chemically synthesized fungicide bulb treatments to control onion diseases (Department of Agriculture, 2018). But continuous use of chemical fungicides for a long period of time results in the accumulation of harmful chemicals in the soil, water, and bulbs. Also, it affects human health. Hence, the use of eco-friendly, sustainable biocontrol agents has become an interesting topic nowadays, and a lot of biocontrol agents are used as antagonistic agents to control diseases (Naguleswaran *et al.*, 2014).

Recently, introducing antagonists to control seed borne fungal pathogens has been considered a successful method to control these problems (Shovan *et al.*, 2008). *Trichoderma* spp, also called "plant doctor fungus" is a popular, environmentally friendly bio-control agent against lots of phytopathogenic fungi. It decreases the growth of plant pathogens and helps reduce the use of chemicals. The intimate relationship between *Trichoderma* spp. and the host root cells enhances systemic resistance plant responses to pathogen attack and the development of formulations helps to deliver systems for antagonistic microorganisms and is important in the field of biological control in onion cultivation. (Naguleswaran *et al.*, 2014).

Therefore, this study will be conducted to evaluate the effect of adding different rates of *Trichoderma* spp. as a biocontrol agent on the reduction of onion anthracnose and fungal bulb rot. The objectives of this study are to increase the yield of onions by reducing the anthracnose and fungal bulb rot infections and to identify the bulb treatment rate of a *Trichoderma*-based product and to control the white rot and anthracnose in onions using a *Trichoderma*-based product. So Onion growers can use *Trichoderma* sp. as a alternative method for chemical fungicides.

## **2. MATERIALS AND METHODS**

### **2.1 Isolation of *Colletotrichum gloeosporioides* and *Stromatinia cepivora***

*Stromatinia cepivora* and *Colletotrichum gloeosporioides* were isolated by mycelium or sclerotia from the diseased big onion leaves and the infected big onion bulbs, respectively. Mycelium was used as inoculum for the isolation process. Sclerotia was isolated, 10 mm leaves were chosen for the sample size, and they underwent surface sterilization in 70 percent ethanol for a minute

before being rinsed three times with distilled water. In order to avoid bacterial contamination, sterilized specimens were placed in Petri dishes with PDA media and treated with Amoxicillin as an antibiotic. The specimen was then transferred afterwards when petri plates were kept at 30 °C for the six-day incubation time. After incubation, the produced pure culture was re - suspended, cleaned of any contamination, and kept in a refrigerator at 4 °C for future studies.

## **2.2 Confirmation of *Colletotrichum gloeosporioides* and *Stromatinia cepivora***

The morphology of the culture and the development pattern of the mycelium on PDA under a microscope were used to confirm the symptoms and signs of *Colletotrichum gloeosporioides* and *Stromatinia cepivora*. of onion were confirmed by Sclerotia on infected bulbs and Koch postulated method also followed for confirmation.

## **2.3 *Trichoderma asperellum* Pure Culture Preparation**

*Trichoderma asperellum* pure culture was prepared using the FCDI Mahailuppallama stock culture that is already on hand.

## **2.4 Confirmation of the *Trichoderma asperellum* Bio Control Agent**

The glass rod, Petri plate, curve slide, parchment paper, and autoclaved for 121 °C for 15 minutes. After the liquid PDA had set, a 5 mm *Trichoderma asperellum* block was placed on the curve region, the parchment paper was moistened, and the petri plate was wrapped in parafilms and left at room temperature for 2 days. Under a microscope, its morphological features revealed *Trichoderma asperellum*.

## **2.5 Antagonism Testing for the *Trichoderma asperellum* and the *Stromatinia cepivora***

Using a 5mm cork borer, two plugs each of *Trichoderma asperellum* and Onion *Stromatinia cepivora*. measuring 5mm in diameter were removed from a culture that had been growing for three days. Onions *Stromatinia cepivora*. and *Trichoderma asperellum* were applied separately to PDA media on the edge of Petri plates and positioned on the opposite side of the plate. The parafilms was used to cover the Petri plates, and the same media was used for the control treatment, which involved cultivating only one disk of *Stromatinia cepivora*. on the PDA surface, with no antagonistic fungus present. There were eight replicates used. After that, all cultures were kept at 25 °C for 7 days. Radial development (in centimetres) was measured, and daily mycelium length measurement was taken after two days. The measurement of the untreated control's inhibition percentages was then compared (Küçük and Kivanç, 2004).

## **2.6 Antagonism Testing was Done for the *Trichoderma asperellum* and *Colletotrichum gloeosporioides***

Using a cork borer of 5mm, two plug of *Trichoderma asperellum* and *Colletotrichum gloeosporioides* containing mycelial disks of 5mm in diameter were removed from a 3 days old culture of the two fungi and placed on the opposite side of the plate. The sections based were covered with parafilms, and the control treatment was carried out using the same media but with only one disk of *Colletotrichum gloeosporioides* growing on the PDA surface and no antagonizing fungi present. There were five replicates used. After inoculation, all plates were incubated at 25 °C for 7 days. Radial growth (cm) average measurements were taken after two days, until the control PDA plate was completely covered (Küçük, and Kivanç, 2004).

### **3. FIELD EXPERIMENT**

This study was conducted From March to July 2023, field in the pathology area of the Field Crops Research and Development Institute (FCRDI), Mahailuppallama, Sri Lankaas, a pot experiment using a Complete Randomized block design (CRD) with four treatment and ten replicates to increase the precision of trial. Inthat area average temperature 27 C<sup>0</sup>and annual rainfall is 1000-1500mm. onion bulb selection, vernalization process, pot preparation done with Department of agriculture recommendation. Mass culture of *Stromatinia cepivorawas* done as (Mukherjee *et al.*, 2014) procedure. 20 to 30 seed sorghum was inoculated to the pots for the inoculation of *Stromatinia cepivora*. *Trichoderma asperellum* for bulb treatment was 4\*10<sup>6</sup> CFU per gram. *MIBO* -01 mother bulbs were planted in 40 pots two bulbs per pot as four treatments. Treatment One involved weighing the weight of 80 bulbs, dividing them into 20 bulbs for each treatment, weighing each treatment group to determine the mean weight of the bulbs, and applying the *Trichoderma asperellum* powder that was already on hand and available from FCRDI at the rate of 4g/kg for the 20 bulbs in a uniform amount in each bulb. Two bulbs were then planted in each pot (<https://agritech.tnau.ac.in/horti>, 2015).

4 g/kg dose of *Trichoderma asperellum* liquid was prepared for the second treatment group. 1500 ml of water with *Trichoderma asperellum* powder in FCRDI were added, dipped for 15 minutes, and 2 bulbs were grown for each pot. In the third treatment, two bulbs were planted in each pot while thiophanate-methyl 50 percent (W/W) + thiram 30 percent (W/W) WP (Homai) chemical was applied at the indicated rate of 20 g/5kg with 1500 ml and dipped in 15 minutes. The fourth treatment was control, in which two bulbs were grown in each pot with no further care. In the third treatment, two bulbs were planted in each pot while thiophanate -methyl 50 percent (W/W) + thiram 30 percent (W/W) WP (Homai) chemical was applied at the indicated rate of 20 g/5kg with 1500 ml and dipped in 15 minutes. The fourth treatment was control, in which two bulbs were grown in each pot with no further care.

All other agronomic management practices were done according to the department of Agriculture recommendation.

**Table 1: Treatment of the field trial**

Treatment	Details
T1	<i>Trichoderma</i> powder was mixed in bulbs
T2	Bulbs was soak in <i>Trichoderma</i> liquid
T3	Bulbs was soak in Thiophanate -methyl (50%) W/W + thiram 30 %
T4	Control

### 3.1 Mass Culture of *Stromatinia cepivora*

250 g of Seed Sorghum was soaked in water for 24 hours before being autoclaved for 15 minutes at 121°C in a polythene bag secured with a rubber band. Maintain the items in a laminar flow, transfer the PDA with the mycelium to the sorghum seeds, seal the entrance of the polythene bag with parafilm, and wait three days for the growth of the sclerotia sp. (Mukherjee et al., 2015).

### 3.2 Inoculation of *Stromatinia cepivora* to Pot

First, before the mother bulbs were planted in the containers. The soil of the pots was perforated to allow the insertion of 20 to 30 seed sorghum, which was then coated in mycelium. The holes hardly stood out. Water was being poured.

### 3.3 Data Analysis

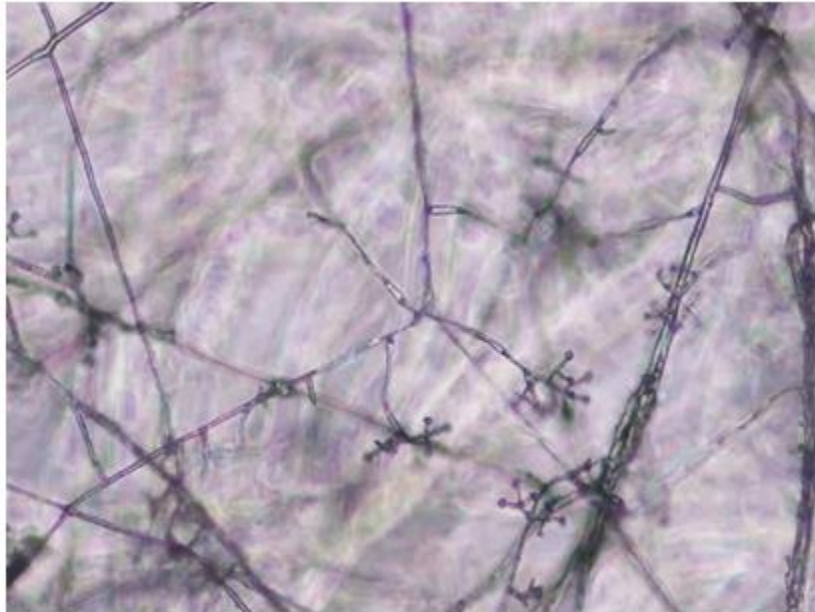
Data collection was done for disease severity index, disease incidence and inhibition percentage. In the pathology area of the FCRDI facilities, a pot experiment using a Complete Randomized block design (CRD) with four treatment and ten replicates. For the antagonistic effect test also CRD design was used with five replicates with respective control and five antagonistic replicates.

Statistically analyze the data using the one-way ANOVA of SAS. The optimum treatment was evaluated using mean comparisons using Duncan's Multiple Range Test (DMRT) at a 5% probability level.

## 4. RESULT AND DISCUSSIONS

### 4.1 Morphological Characters of *Trichoderma asperellum*

After a few days, the white, downy mycelia of *Trichoderma asperellum* that had been cultured on a curve slide with PDA had become pale green and dark green. Secondary branches and branching conidiophores emerged from pustules. Those are paired and asymmetric secondary branches (Harman and Kubicek, 2002).



**Fig. 1: Microscopic view of *Trichoderma asperellum* (Magnification power - \*400)**

#### **4.2 Morphological Characters of *Colletotrichum gloeosporioides***

On potato dextrose agar media, the fungus *Colletotrichum gloeosporioides* developed irregular concentric circles and round, cottony-looking growths that ranged in hue from light gray to dark gray. On the underside of the Petri plate were black concentric circles. Orange specks, called acervuli, which are fruiting bodies, started to appear once the culture had fully developed.



**Fig. 2: *Colletotrichum gloeosporioides* pure culture, Microscopic view of *Colletotrichum gloeosporioides* (Magnification power - \*400)**

#### 4.3 Morphological Studies of *Stromatinia cepivora*

On potato dextrose agar, all of the isolates showed fluffy growth. From milky white to cottony watery white, the hue was altered. There was a smooth, occasionally striped surface texture.



**Fig. 3: Onion *Stromatinia cepivora* pure culture, Microscopic view of *Stromatinia cepivora* (Magnification power - \*400)**

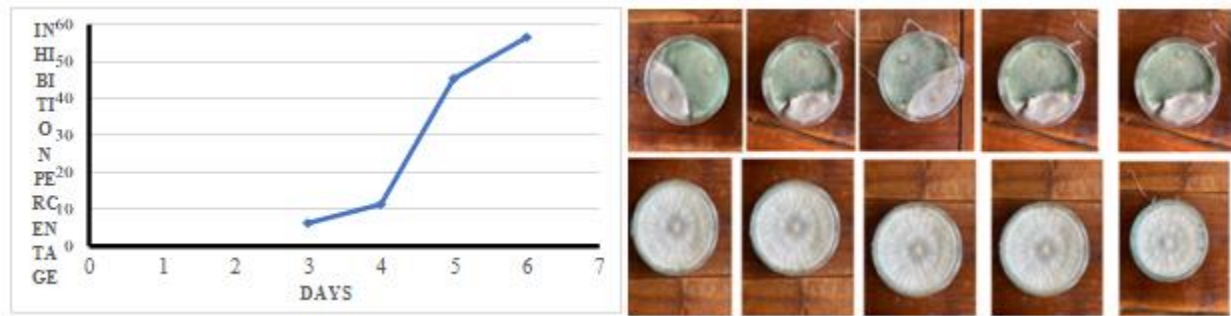
#### 4.4 Results of Dual Culture

Percentage of *Colletotrichum gloeosporioides* and *Trichoderma asperellum* that have been present since the third day. The percentage of residence was lower on the third day and increased daily but not proportionately. The *Trichoderma asperellum* totally covered the dual culture plate in six days, however the *Colletotrichum gloeosporioides* control plate took nine days to fully develop in the petri dish. *Trichoderma asperellum* quickly covers the Petri plate in 6 days at 24 °C incubation temperature because it grows more quickly than *Colletotrichum gloeosporioides*. According to the early studies De Los Santos -Villalobos *et al* reported that *C. Gloeosporioides* inhibit 80% of growth inhibition by *Trichoderma asperellum*. This study also showed that it gets nearly 60% of inhibition percentage which can emphasis that *Trichoderma asperellum* act as a best biological control agent to control onion anthracnose (De Los Santos -Villalobos *et al.*, 2013).

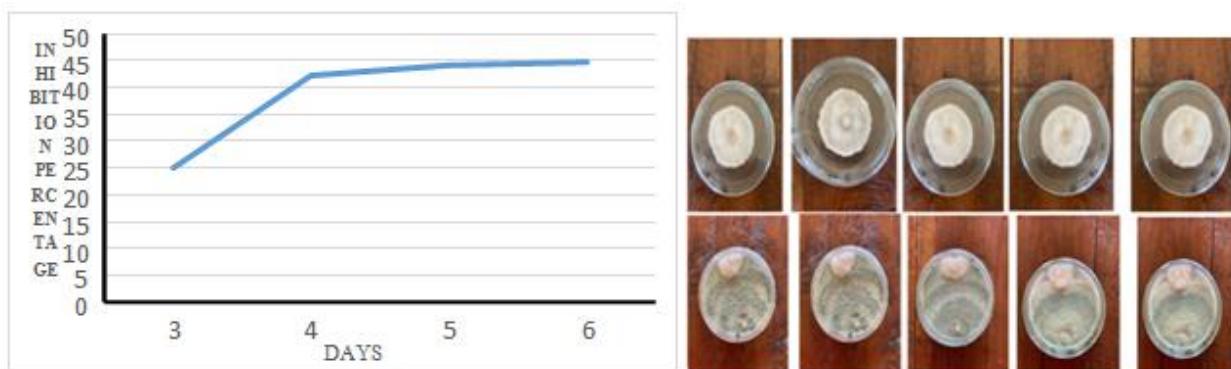
The proportion of *Stromatinia cepivora* and *Trichoderma asperellum* that are present here has a 25% occupancy rate on the third day and increases to a 40% occupancy rate on the fourth day before becoming constant on the fifth day up word. Only five days were needed for *Stromatinia*



*cepivora* to fully develop in a control petri plate at a temperature of 24 C<sup>0</sup>. Based on the previous studies on the antagonistic test of *T. asperellum* showed the best performance against *S. cepivora* as gained in this study (De Los Santos -Villalobos *et al.*, 2013).



**Fig. 4: Inhibition percentage graph *Colletotrichum gloeosporioides* and *Trichoderma asperellum***



**Fig. 5: Inhibition percentage graph of *Stromatinia cepivora* and *Trichoderma asperellum***

#### 4.5 Symptoms Detection of Onion Anthracnose

The chlorotic development, curled, twisted, and chlorotic leaves, as well as the abnormal growth of the pseudo stem, were noted. Mostly white hollow oval lesions could be seen on the bottom leaves. Over time, these lesions expanded until they encompassed the whole leaf. Stunted roots are often the cause of plant demise. These plants produced small, ultimately decaying bulbs. According to (Dutta, 2022), leaf curling, twisting, and chlorosis are the onion twister disease's most visible field symptoms. According to Ebenebe (1980), the harmed plants typically have little bulbs (Before being harvested, some bulbs pass away, while being stored, quickly. Little, elevated acervuline without masses of pinkish orange conidia. According to the early reports of

big onion cultivations being impacted by *Colletotrichum gloeosporioides* (Weerathne, 1996; 1997) also show the same results as this experiment.



**Fig. 6: Onion anthracnose disease symptoms - concentric circle, curling of leaves**

#### **4.6 Fungal Bulb Rot Disease Incidence**

No fungal bulb rot disease incidence was shown in the treatment one *Trichoderma* powder was mixed in bulbs and treatment two bulbs were soaked in *Trichoderma* liquid. Treatment three Bulbs were soaked; in Thiophanate -methyl (50%) W/W + thiram 30% got 30% fungal bulb rot incidences. Control got 50% bulb rot disease incidence which is the highest fungal bulb rot disease incidence. According to Duncan mean separation *Trichoderma* powder was mixed in bulbs and treatment two bulbs were soaked in *Trichoderma* liquid get same group while they give the best management while chemical treatment and homai give the separate groups. Early studies of Alvarado-Marchena and Rivera-Mendez successfully showed that *T. asperellum* consider as successful method to control the *S. cepivorain* onion plant as a biocontrol agent to reduce the mortality of plant due to fungal bulb rot same as found in this experiment (Alvarado-Marchena and Rivera- Mendez, 2016).

**Table 2: Fungal bulb rot disease incidence**

Treatment	Mean
T1: <i>Trichoderma</i> powder was mixed in bulbs	00 <sup>c</sup>
T2: Bulbs were soak in <i>Trichoderma</i> liquid	00 <sup>c</sup>
T3: Bulbs were soak in Thiophanate -methyl (50%) W/W + thiram 30%	30 <sup>b</sup>
T4: Control	50 <sup>a</sup>

#### 4.7 Anthracnose Disease Severity Index

Treatment one exhibited the least disease severity index (11.10%), followed by treatment two with a percentage of 33.32%, the T3 with chemical therapy showing the 2nd highest disease severity index (39.96%), and treatment four (control) with the highest disease severity index. According to mean separation no statistically significant difference between treatments 1 and 2, 2 and 3, or 3. According to the findings, treatment one (*Trichoderma* powder put into bulbs) performed better than treatment two (Bulbs soaked in *Trichoderma* liquid), and treatment two also performed better than treatment four (control). According to the previous records that (Naguleswaranet *al.*, 2014) also showed that *Trichoderma* spp. act as a best promising tool to control the onion anthracnose.

**Table 3: Anthracnose disease severity index**

Treatment	Mean
T1: <i>Trichoderma</i> powder was mixed in bulbs	11.10 <sup>c</sup>
T2: Bulbs were soak in <i>Trichoderma</i> liquid	33.32 <sup>bc</sup>
T3: Bulbs were soak in Thiophanate -methyl (50%) W/W + thiram 30%	39.96 <sup>ab</sup>
T4: Control	63.30 <sup>a</sup>

#### 5. CONCLUSION

Onion bulbs treated with *Trichoderma* powder or bulbs submerged in *Trichoderma* solution showed lower percentages of disease severity indices for anthracnose and fungal bulb rot indices than bulbs treated with chemicals in a field experiment as well as bulbs treated with *Trichoderma* powder or bulbs submerged in *Trichoderma* solution showed the same significant level as the chemical fungicide. *Trichoderma asperellum* had a quicker growth rate, and its inhibition for *Colletotrichum gloeosporioides* and *Stromatinia cepivora* was best. Field trails are recommended for recommendation of the findings of the current investigations. *Trichoderma asperellum* had a quicker growth rate, and its inhibition for *Colletotrichum gloeosporioides* and *Stromatinia*

*cepivora* was best, according to antagonistic tests of above mention. These discoveries make *Trichoderma asperellum* as a potential bio fungicide to control anthracnose and white rot disease in onion. Extensive field studies for recommendation, Comparison of *Trichoderma asperellum* with botanicals and organic manure to enhance the disease suppression can suggest as future line of the work.

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