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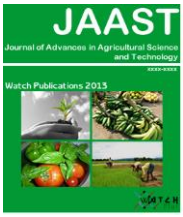

EVALUATION OF *TRICHODERMA* SPECIES FOR THE CONTROL OF GARLIC WHITE ROT DISEASE (*SCLEROTIUM CEPIVORUM*)

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Abstract

The production of Garlic (*Allium sativum L.*) is threatened by white rot disease caused by *Sclerotium cepivorum*; which cause heavy yield losses. The development of biocontrol systems has provided an effective approach for reducing the losses and risk of environmental pollution associated with fungicides and fungicide resistance development. Therefore, the present study was carried out to evaluate the biocontrol potential of seven *Trichoderma species viz., T. harzianum, T. hamatum, T. viride, T. oblongisporum, T. longibrachiatum, T. asperillum, and T. atroviride* by both *in-vitro* and *in-vivo* work for their antagonistic and inhibition potential against garlic white rot pathogen. Dual culture technique was used for the radial growth of the pathogen and antagonists record. All but four isolates were highly antagonistic that they inhibited the growth and colonized the colony of the pathogen and there was statistically significant difference among those *Trichoderma* isolates. The overall inhibition effect of the antagonists on the pathogen‟s colony growth ranged from 43.9 to 59.3 %. *Trichoderma hamatum* had the highest inhibition effect (59.3%) followed by *T. harzianum* (53.3 %), T. *oblongisporum* (52.7 %), *T.viride* (51.8%), *T. asperillum* (50.2%), *T. longibrachiatum* (47.2%) and *T. atroviride* (43.9%) . Therefore, these isolates were further studied in the glasshouse condition by using single and in combination against garlic white rot. The incidence of *S. cepivorum* was significantly reduced in bulbs of garlic and also improved plant growth was observed in plants treated with single inoculation of *T. hamatum* alone followed by *T. harzianum* alone and in combined inoculation of *T.hamatum* and *T. harzianum, T. oblongisporum and T. viride isolates*. Plants inoculated with *S. cepivorum* alone showed pronounced disease symptoms with mean disease incidence of 90.5 %. Combined treatment of *T.hamatum* and *T. harzianum* isolates were significantly reduced (62.22%) the white rot disease of garlic followed by the combined inoculation of *T. oblongisporum and T.harzianum and T. hamatum and T.viride.* The over all reduction in the incidence of white rot was 62.22% in the treatment of *T.hamatum* and *T. harzianum* isolates followed by 53.72% for *T.oblongisporum* and *T. harzianum.* It is concluded that the tested *Trichoderma* isolates (*T.hamatum, T.harzianum, T.oblongisporum* and *T.viride*) have high potential to inhibit the colony growth of *S. cepivorum* and the results clearly pointed out that *T.hamatum, T. harzianum, T* .oblongisporum and *T.viride* isolates can reduce the severity of *S. cepivorum* disease in garlic. Use of these bio-control agents could be promoted as an active component of bio-intensive Integrated Disease Management Program (IDMP) under organic mode.

Keywords: Garlic, *Sclerotium cepivorum*, Biological control, *Trichoderma species.*

INTRODUCTION

Garlic (*Allium sativum* L.), is the second most widely cultivated vegetable and spice crop next to onion (Rubatzky and Yamaguchi, 1997). It is commonly called as „Nech Shinkurt" in Amarharic language and is widely cultivated in Ethiopia. Garlic plays an important dietary, as well as medicinal role for centuries. Even today the medicinal use of garlic is widespread in ethno pharmacology; used in the treatment of headache, bites, worms, tumours and also could reduce risk of cancer and chest pain in Ethiopia (Giday et al., 2003, Keusgen, 2002). It has broad spectrum of antimicrobial property (Rubatzky and Yamaguchi, 1997). Han *et al*. (1995) reported that garlic has antibiotic properties and could be used to treat wound. It can inhibit and kill bacteria, fungi, parasites, lower blood pressure, blood cholesterol and blood sugar, prevent blood clotting, protect the liver and contains antitumor properties (Sovova and Sova, 2004).

Economic significance of garlic in Ethiopia is quite


(A)

Figure 1: Garlic bulbs showing advanced symptoms of white rot

considerable. The spice is the choice of Ethiopian women as the main ingredient for the preparation of pepper paste. It is also used in the preparation of different foods and in therapeutic medicine in Ethiopia and contributes to the national economy as export commodity (Fekadu and Dandena, 2006). Production of cash crops like garlic and other spices is proved to be income generating for farmers, especially for small holder farmers (FAO, 2006). In Ethiopia, the total area under garlic production reaches 9,317 hectares and the production is estimated to be over 1,035,417 quintals annually (MoARD, 2010). In spite of its importance and increased production, garlic productivity, in many parts of the world, is low due to genetic, abiotic and biotic factors (Nonnecke, 1989).

Numerous production problems accounted for the low yield of garlic in Ethiopia; limited improved variety, lack of strong diseases control and insect pest management practices, inappropriate agronomic practices in traditional production system and marketing facilities are the prominent ones. However, the most important constraints for garlic productions are the fungal diseases viz., white rot caused by *Sclerotium cepivorum* Berk. (Coley-Smith, 1987), rust caused by *Puccinia alli*, neck rot caused by *Botrytis alli*, *B.squamosa* and *B. cinerea* (Dennis, 1986). White rot disease is one of the most destructive disease in garlic cultivated fields and causing heavy yield losses (Tamire *et al*., 2007). The management of white rot is very difficult due to its persistence for more than 40 years in the soil.

In Ethiopia, research effort on host resistant against garlic white rot is very limited and fungicides significantly to reduce this disease is not sound very well; therefore, it is more better to focused on environmentally safe, long lasting and effective biocontrol options for the management of this disease. Among microorganisms, fungi are the potential candidate for the biological control of plant pathogens and several antagonistic fungi viz., *Trichoderma*, *Gliocladium* and *Talaromyces* have shown enormous potential as biocontrol agents against diffirent soil borne and foliar diseases. *Trichoderma* as a potent fungal biocontrol agent against a range of plant pathogen has attracted considerable scientific attention (Rini and Sulochana, 2007). *Trichoderma* spp. are considered as the most important because of their potential to control various soil borne and seed borne diseases caused by a wide range of fungal pathogens. *Trichoderma* species are considered as multi-potential organisms and their biological properties are being exploited. The biocontrol mechanisms exercised by *Trichoderma spp.* could be attributed to competition for nutrients, release of toxic metabolites and extra cellular hydrolytic enzymes (Elad, 2000). They also produce volatile and non volatile antibiotic compounds, which inhibit fungal growth at very low concentration (Chaube *et al*., 2002; Harman *et al*., 2004). Thus, this research work was undertaken to evaluation the *in vitro* and *in vivo* effects of *Trichoderma* spp. for the control of garlic white rot disease.

MATERIALS AND METHODS

Isolation and culturing of *Sclerotium cepivorum*

In vitro and *in vivo* studies were conducted at Ambo Plant Protection Research Center (APPRC), West Showa Zone, Ethiopia. The pathogen was isolated from diseased bulbs of (Figure 1) collected from different sites at Ambo, West Showa. Cultures were obtained garlic bulb was first surface sterilized with 70% alcohol for 90 seconds followed by three times wash in sterile distilled water and then plated on PDA medium. The plates were incubated at 26^0C for five days. After incubation, the development of colonies on the medium was observed (Figure 2). Mycelial suspension of *S. cepivorum* was obtained by adding 10 ml of sterile distilled water (SDW) in two-week-old agar cultures and mycelium was scraped from the agar surface with a spatula; mixed into 490 ml of sterile distilled water (SDW). This culture was used to inoculate five polythene bags (100 ml per bag), each containing a mixture of washed and sterilized sand (20g, <2mm particle size), ground maize meal (80g) and water (175 ml).

The bags were heat-sealed and incubated at 26^0C for 6 weeks after which sclerotia were formed (Figure 3).

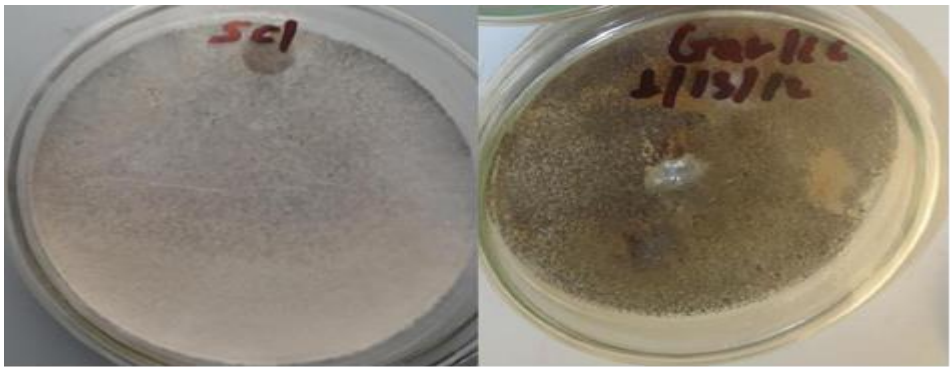

Figure 2: *Sclerotium cepivorum* colonies on plate

Figure 3: Sclerotia of *S. cepivorum* produced in polythene bags (a) and harvested Sclerotia (b)

Sclerotia were harvested by floatation in water and retrieval in a sieve (200µm mesh diameter). The harvested sclerotia were air dried at room temperature, after which they were stord at 4^0C for further use.

Mass production of *Trichoderma* spp.

Mass multiplication of *Trichoderma* species were followed the method of Tewari and Mukhopadhyay (2001) and Rini and Sulochana (2007). Multiplication of *Trichoderma* spp. were done by inoculating half-liter sterile glass flax containing wheat bran (50g), sand (50g), and water (20ml) and added with spore suspensions. Spore suspensions were obtained by adding 20 ml sterile distilled water to a three-week-old cultures and scraping gently with a spatula. The spore suspension of *Trichoderma* spp. were inoculated into prepared growth medium and incubated for 3 days at 20^0C (Figure 4).

***In vitro* study *Trichoderma* spp against garlic white rot pathogen:**

Seven *Trichoderm* species viz., *T. harzianum* J6, *T. hamatum* W3, *T. viride* PPRC1, *T. oblongisporum* J3, *T. longibrachiatum* J9, *T. asperillum* SNNP2 and *T. atroviride* SNNP12 that were obtained from APPRC and *in-vitro* evaluated for their antagonistic and inhibition potential against the garlic white rot pathogen (*S.cepivorum)* using dual culture technique with direct confrontation test as done by Kucuk and Kivanc, 2004. Five day old cultures of *Trichoderma* species and *S. cepivorum* were used in which mycelial discs (6mm in diameter) of each of the *Trichoderma* isolates were placed separately on one edge of a Petri-dish containing 20ml of fresh PDA and mycelial discs of *S. cepivorum* of the same size were put on the opposite side of the Petri-dish. Control plates of the pathogen and the antagonist were also prepared. A Completely Randomized Design (CRD) was used. There were three replications per treatment and the plates were incubated at 25 ^{o} C for 5 days.

The isolates were then scored for degree of antagonism using the rating system of Bell *et al*. (1982) on a scale of 1-5: where 1 = *Trichoderma* completely overgrow the pathogen and cover the entire medium surface; 2 = *Trichoderma* overgrow at least 2/3 of the medium surface; 3 = *Trichoderma* and Sclerotium each colonize 50% of the medium surface and neither of them appear to dominate the other; 4 = *Sclerotium* colonizes

Figure 4: *Trichoderma* cultures mass produced in conical flasks.

Figure.5: Inhibition effect of Trichoderma. spp in dual culture and control/*S. cepivorum* alone

at least two-thirds of the medium surface and appear to withstand encroachment by *Trichoderma* and 5 = *Sclerotium* completely overgrow the entire medium surface. According to this rating system, a *Trichoderma* isolate is considered as antagonistic if the mean score was less or equal to 2 and not antagonist if the number was greater than 2. In addition, the inhibition percentage of the pathogen by each of the test isolates and growth of *S. cepivorum* was recorded by measuring the diameter of the colonies with digital caliper. Percentage inhibition of colony growth was calculated using the following formula employed by Whipps. (1987) :

$$I\ \% = \left(1 - \frac{\text{average diameter of the } \textit{Treated}}{\text{Average diameter of the } \textit{Control}}\right) \times 100$$

Where I (%) represents the average inhibition percentage; *Treated*, indicates the average colony diameter of *S. cepivorum* in the presence of the antagonist and *Control* is the average colony diameter of *S. cepivorum* without the antagonist (Figure.5).

***In vivo* study *Trichoderma* spp. against garlic white rot pathogen:**

The experiment was conducted under glass house condition adjusted with AC in APPRC, EIAR. The seven species of *Trichoderma* isolates were evaluated singly and in combination for the biological control of white rot disease of garlic under pot culture condition as stated by Srivastava *et al*., 1991. The bulbs of garlic (*Allium sativum* L. var. PB-206) were used. Sterilized sand, decomposed animal dung and sterilized sandy clay loam soil (1:1:2 ratio) were used. Each pot (21cm diameter and 20 cm height) was filled with 3kg of mixed soil. Sclerotia of *S. cepivorum* were thoroughly mixed with surface soil (100 Sclerotia per pot) and wheat bran: sand cultures of *Trichoderma* spp. (1g/100g soil) separately as well as in combination was added to each pot and thoroughly mixed with surface soil. Three garlic seedlings were planted in each pot. The experiment was laid out in completely randomized block design with three replications and 8 treatments in Experiment I and 28 treatments in Experiment II consisting of various combinations of different isolates of *Trichoderma* spp. with *S. cepivorum* were maintained in pot cultures (Figures 6). Pots treated without *S. cepivorum* were used as control checks.

The per cent incidence was calculated using the formula: Incidence of (%) white rot infection = (Total No. of diseased plants / Total No. of health plants) x 100.

The white rot severity was assessed and rated on 0-5 scale where 0 = healthy; 1 = bulb covered with mycelium

Figure 6: *In vivo* experiment of *Trichoderma spp*. against garlic white rot pathogen.

Table 1. Antagonistic effect of *Trichoderma* isolates against *S. cepivorum* within 10 days inoculation/plating

Trichoderma species	Mean score of antagonism
T. oblongisporum	1.75[a]
T. longibrachiatum	3.75[b]
T. asperillum	3.00[b]
T. hamatum	1.25[a]
T. harzianum	1.50[a]
T. atroviride	3.00[b]
T. viride	1.50[a]

"a" degree of antagonism and "b" not antagonistic on a 1-5 scale according to Bell *et al.,*1982.

but not rotted; 2 = 1-25% of the bulb rotted; 3= 25-50% of the bulb rotted; 4 = 50-75% of the bulb rotted and 5 = 75-100% of the bulb rotted as mentioned by Tian and Bertolini, 1995.

Disease severity scores were converted into percentage as follows for analysis.

$$\text{Disease severity (\%)} = \frac{\text{Total points score}}{\text{Total number of bulbs scored x Highest score on the scale}} \times 100$$

After 90 days inoculation, matured plants were uprooted, fresh and dry biomass of bulbs data were recorded after drying the samples at about 30^{o} C for 10 days to its constant weight.

Data analysis

Data were subjected to analysis of variance and the treatment means were further separated by Duncan‟s Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

In vitro* study of *Trichoderma* isolates against *Sclerotium cepivorum

The antagonism test revealed that amongst seven species of *Trichoderma*, four (*T.hamatum*, *T.hazianum*, *T.oblongisporum* and *T.viride*) were scored less than 2 and were antagonistic to the white rot pathogen. However, *T. asperillum*, *T. atroviride*, and *T.longibrachiatum* which exhibited a score of 3.00-3.75, were not antagonistic (Table 1). These isolates grew only up to 1 cm in 5 days indicating that it was not antagonistic, whereas all the other isolates colonized and covered the colonies of *S. cepivorum* on the respective plates. The control plates in the inhibition test was used to calculate the percentage inhibitions of the respective *Trichoderma* species only. The results of the data recorded within 5-10 days after plated was showed that there is a significant difference among the *Trichoderma* species in suppressing the colony growth of the pathogen and inhibition percentage (Table 2). *Trichoderma hamatum* and *T. harzianum* isolates covered the plates completely on the 5th day of incubation while the two other species (*T.oblongisporum and T. viride*) grew and cover the plates on the 6th day of incubation but the three other species grew and cover the plate on the 8th day of incubation. Mean while in the control treatment, *S. cepivorum* grown covered the plates on the 10th day of inoculation and producing

Table 2: Inhibition effects of Trichoderma species on colony growth of S. cepivorum (5-12 days after inoculation).

Trichoderma species	Mean colony growth *of S. cepivorum (cm)* in presence of *Trichoderma* species	Mean % inhibition of growth
T. asperillum	2.95	33.7
T. harzianum	2.25	53.3
T. hamatum	2.00	59.3
T. viride	2.95	51.8
T. atroviride	3.45	43.9
T. *oblongisporum*	2.75	52.7
T. *longibrachiatum*	3.75	47.2
S.cepivorum alone (Control)	4.45	-----

sclerotia after the 12th day. Therefore, growth of *S. cepivorum* was grew comparatively limited when inoculated after 48 hours inoculation of *Trichoderma* species. Each of the antagonistic *Trichoderma* isolates limited the colony growth of the pathogen differentially by overgrowing the pathogen colony.

Trichoderma isolates are known to rapidly colonize medium surface and substrates (Kucuk and Kivance, 2004). The mean colony growth of *S.cepivorum* in plates containing *Trichoderma T. hamatum* and *T.longibrachiatum* isolates ranged in diameter from 1.25 cm to 3.75 cm. In contrast, its colony growth reached 4.45 cm after 10th day in control plates.

Trichoderma hamatum and T. harzianum showed much more influence on the pathogen. *T. hamatum* inhibited the colony growth of *S. cepivorum* by 59.3% followed by *T.harzianum* (53.3%), *T. oblongisporum* (52.7 %) and *T.viride* (51.8 %) which was also significantly different from each other (Table 2). Up to 36% reduction of colony growth of *F. oxysporum* was obtained by using *Trichoderma harzianum* isolates (Sahi and Khalid, 2007). In a similar study done by using culture filtrates of *T. Viride*. Tesfaye (1999) found that *F. solani* could grow only for 1.5 mm in 96 hrs (4 days). Thus, *Trichoderma* species are among the most promising bio control agents against many fungal pathogens (Akrami *et al.*, 2009). Antagonistic interactions of *Trichoderma* species with other fungi and mechanisms involved in the bio control process are based on antibiosis, parasitism, induced resistance and competition (Hoitink *et al.,* 1997). Bio control agents also produce enzymes such as chitinase, protease and cellulose that have been proved to be involved in the antagonistic activity (Howell, 2003). However, antagonistic fungi are specific in their antagonistic activity against specific fungi (Saleem *et al.,* 2000). *In-vitro* tests are suitable for selecting antagonistic organisms with a particular mode of action, but are very poor predictors of the activity of the organisms in the field (Campbell, 1989). According to Mpika *et al.,* 2009, efficiency of *Trichoderma* species in antagonizing plant pathogens is closely linked with local conditions. *T.atroviride*, *T.asperillum* and *T.longibrachiatum* used in the present study not showed high antagonistic activities as indicated by inhibition of mycelial growth of pathogen in dual culture experiments, which was further confirmed by inhibiting the mycelial growth and sclerotial germination on culture filtrates of these bio-agents. Arya and Kaushik (2001) reported maximum inhibition of *Fusarium oxysporum* and *Rhizotonia solani* (74.7-75.2%) with *Gliocladium virens* followed by *T. harzianum* (65.9-72.4%). Kapoor, 2008 reported that maximum inhibition growth by *T. harzianum* against *R. solani*, *Pythium debaryanum*, *Sclerotinia minor* and *Fusarium oxysporum f.sp .pisi.* In the present study *T.hamatum and T.harzianum* isolates also showed maximum inhibition of *S. cepivorum* (Table 2). This could be obviously attributed to several possibilities of existence of microbial interactions such as higher competitive ability, stimulation, antibiosis by these *Trichoderma* isolate over test pathogen.

The antagonism of *Trichoderma* spp. against many fungi is mainly due to production of acetaldehyde, a carbonyl compound (Robinson and Park, 1966).This may also be the reason for its antagonistic effect on *Trichoderma* and *S.cepivorum*.

In vivo test of *Trichoderma* spp. against *S. cepivorum* pathogen

Both single and combination inoculation effect of *Trichoderma isolates* increased the plant growth parameters over the control while less plant growth was observed in the plants inoculated with *S. cepivorum* alone. The single treatments, *T. hamatum* alone with pathogen showed significant growth response and reduced the disease incidence when compared to not inoculated or *S. cepivorum* alone used as control (Table 3). Among the different combination treatments, *T.hamatum* and *T.harzianum* with pathogen showed significant growth response and reduced the disease incidence when compared to uninoculated and *S. cepivorum* alone control plants (Table 4). *T. hamatum* combination with *T. harzianum* isolates were significantly

Table 3: Effect of individual *Trichoderma* spp. on garlic white rot disease development and yield enhancement.

Treatments	White rot incidence (%)	Severity of white rot (%)	Average fresh biomass weight of bulbs (g/plant)	Average dry weight of bulbs (g/plant)
T.harzianum	16.66	(36.6)	40.14b	28.13b
T. hamatum	15.45	(34.6)	41.33a	30.08a
T. viride	17.7	(39.6)	27.01d	18.22d
T. oblongisporum	16.85	(38.6)	33.90c	20.02c
T.asperillum	26.7	(46.8)	23.02e	11.14f
T. longibrachiatum	35.5	(50.4)	20.10f	15.50e
T. atroviride	33.6	(47.9)	12.85h	7.50g
Untreated/Control	95.5	(92.6)	23.00e	10.05f

Means with the same letter are not significantly different

Table 4: Effect of combined *Trichoderma* spp. on garlic white rot disease development and yield enhancement.

Treatments	Average fresh biomass weight of bulbs (g/plant)	Average dry weight of bulbs (g/plant)	White rot incidence (%)	White rot severity (%)
Untreated *(S.cepivorum* alone)	10.04j	8.05n	95.5	(92.6)f
A with B *T.harzianum +T hamatum*	45.6a	34.26a	16.66	(35.7)a
A with C *T.harzianum +T.viride*	39.56b	24.6 b	16.5	(37.5)a
A with D *T.harzianum + T. oblongisporum*	40.6b	24.08b	16.7	(38.4)a
A with E *T. harzianum + T. longibrachiatum*	32.5c	15.6g	23.33	(45.6)c
A with F *T.harzianum + T. asperillum*	20. 0f	14.6h	36.66	(50.2)d
A with G *T. harzianum + T. atroviride*	12.5i	9.6m	33.33	(46.6)d
B with C *T hamatum + T.viride*	34.5 c	23.6c	20.66	(45.8)c
B with D *T hamatum + T. oblongisporum*	38.6b	17.8f	20.3	(43.6)c
B with E *T hamatum + T. longibrachiatum*	29.7 d	19.9d	36.7	(47.8)d
B with F *T hamatum + T. asperillum*	29.7d	17.8f	33.33	(49,6)d
B withG *T hamatum + T. atroviride*	24.5e	14.6h	36.66	(50.2)d
C with D *T.viride + T. oblongisporum*	15.48h	10.75l	36.7	(50.4)d
C with E *T.viride + T. longibrachiatum*	12.58i	9.32m	38.66	(59.5)e
C with F *T.viride + T. asperillum*	22.8e	13.6i	33.33	(56.5)d
C with G *T.viride + T. atroviride*	20.68f	13.96i	36.66	(57.8)d
D with E *T. oblongisporum + T. longibrachiatum*	17.5g	12.4j	37.6	(53.4)e
D with F *T. oblongisporum + T. asperillum*	21.5f	20.8d	38.5	(56.4)e
D with G *T. oblongisporum + T. atroviride*	23.6e	18.6e	33.5	(48.6)d
E with F *T. longibrachiatum + T. asperillum*	14.21h	11.4k	34.5	(56.8)d
E with G *T. longibrachiatum + T. atroviride*	18.6g	12.5j	35.6	(48.6)d
F with G *T. asperillum + T. atroviride*	18.7g	12.6j	36.66	(56.8)e

Means with the same letter are not significantly different

increased the growth and biomass and reduced the disease incidence and severity of white rot disease in garlic plants caused by *S. Cepivorum*. The over all reduction in the incidence of white rot was 59.3 % for the single treatment of *T. hamatum* alone with pathogen while the combination treatments of *T. hamatum and T. harzianum* isolates, the incidence of white rot *was reduced by 62.22 %.* The lowest shoot, root and bulb dry weights were recorded in plants inoculated with *S. cepivorum* alone. *Trichoderma* species can also be used as biological deterrent of soil borne diseases of vegetable crops (Gopinathan and Selvaraj, 2008). In the

present study, the wheat bran: sand formulation of *Trichoderma* isolates particularly *T. harzianum* isolate combined with inoculum of *T.hamatum were* significantly reduced the white rot of garlic caused by *S. cepivorum* followed by the combination treatment of *T.oblongisporum* with *T. hamatum, T. oblongisporum with T.harzianum and T. harzianum with T.viride* (Table 4).

These results clearly pointed out that *T.hamatum*, *T. harzianum, T.oblongisporum and T.viride* isolates were effective (both in single and in combination treatments) in reducing the severity of the disease caused by *S. cepivorum* in garlic and increased the growth parameters when compared with control.

CONCLUSIONS AND RECOMMENDATIONS

The efficacy test results of *Trichoderma* spp is indicated that the *T. harzianum, T. hamatum, T. viride* and *T.oblongisporum* isolat*es* showed inhibition effects on *S. cepivorum* under *in vitro* condition*.*Under pot culture experiment, the single and combined *Trichoderma s*pp. has reduced the incidence and severity of white rot and also increased the plant growth and biomass of garlic. Further ecological studies to be made and also these antagonistic isolates to be exploited as bio-fungicides and could be recommended to the farmers or producers for the management of garlic white rot disease. Considering the Ethiopian farmers concern, new low cost technology could be developed for the mass cultivation of *Trichoderma* spp. which ultimately resulted in achieving very low cost production per unit of bio-fungicide product.

Trichoderma spp., as a potential bio-control agent has gained greater attention and extensive research could be carried out for developing these organisms as bio-fungicides as alternative or supplement to chemical pesticides. Use of these biocontrol agents could be promoted as an active component of bio-intensive Integrated Disease Management (IDM) programme under organic mode.

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