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Evaluation of Arbuscular Mycorrhizal Fungi and *Trichoderma* Species for the Control of Onion White Rot (*Sclerotium cepivorum* Berk)

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Abstract

The present study was carried out to evaluate the indigenous Arbuscular Mycorrhizal Fungi (AMF) and *Trichoderma* species isolated from rhizosphere soils of onion cultivated fields at Ambo and Toke Kutaye districts of West Showa, Ethiopia, and their effect on plant growth, and their biocontrol against onion white rot caused by *Sclerotium cepivorum* Berk. Five species with twenty isolates of *Trichoderma* were isolated and screened *in vitro* for the inhibition of *S. cepivorum*. Out of these, four isolates of *Trichoderma* spp *viz*. *T. harzianum* (ATh1), *T. viride* (ATv1), *T. hamatum* (NThm3), and *T. koningii* (QTk2), were found potent antagonists with mean percent inhibition of the pathogen, 65.4, 64.8, 54.3 and 53.5, respectively. Altogether, 10 AMF species representing four genera *viz. Acaulospora, Gigaspora, Glomus*, and *Scutellospora*, were isolated and identified. Six dominant species of AMF were selected and mass multiplied by using *Sorghum vulgare* Pers. as the compatible host plant. Among six AMF species, the potential efficient strain, *Glomus aggregatum* (Awaro isolate), was selected for using as bio control agent. The bio-control potential of these bio-agents against white rot pathogen was carried out under pot culture condition, using *G. aggregatum* alone or in combination with four isolates of *Trichoderma* spp. Incidence of *S. cepivorum* was significantly reduced in bulbs of onion (66.19%), and also improved plant growth was observed in plants inoculated with combined inoculation of G. aggregatun and *T. harzianum* (ATh1 isolate), followed by the combined inoculation of *G. aggregatum* and *T. viride* (ATv1) with pathogen (59.75%). Plants inoculated with *S. cepivorum* alone showed pronounced disease symptoms with mean disease incidence of 90.5%. The overall reduction in the incidence of white rot was 56.22% in the treatment of *T. harzianum* ATh1 isolate with pathogen, followed by 53.72% for *G. aggregatum* with pathogen. These results clearly pointed out that *G. aggregatum* and *T. harzianum* ATh1 isolate can block the severity of disease caused by *S. cepivorum* in onion. 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: Arbuscular mycorrhizal fungi; *Trichoderma* spp.; Bio-control; Onion; White rot; *Sclerotium cepivorum*

Introduction

Allium crops are the most indispensable vegetable crops used as condiments in most Ethiopian cuisine. Among them, onion (*Allium cepa* L.), rightly called as "queen of kitchen", is one of the oldest known and an important bulbous vegetable crop grown in Ethiopia. It is used in preparation of different foods, and in therapeutic medicine in the country. Besides, it is rich in flavonoids like quercetin and sulfur compounds, such as allylpropyl disulphide, that have been perceived benefits to human health [1]. Onion has also medicinal value, as a possible cancer preventive [2,3]. The best growing altitude for onions under Ethiopian condition is between 700 and 1800 MASL (Meters Above Sea Level) [4]. A survey made in 1987 estimated the total area under onions and shallot in Ethiopia to be about 10,000 ha, with the total production of about 75-500 tones. But, now-a-days the area under the production of onion is increased by far and productivity also increased up to 80-120 kg/ha under optimum condition [4]. However, the productivity of onion is affected by many biotic and abiotic stresses, resulting in yield reduction, low quality and less storability of the crop, and other constraints in the production of onion in Ethiopia, including the use of low quality seeds, imbalanced fertilizers, and uneven irrigations.

Among the diseases of onion, white rot (*Sclerotium cepivorum* Berk.) is identified as the most important disease of onion in Ambo and Toke Kutaye districts of West Showa, Ethiopia, causes breaking of floral stalks, and thus, the bulb yield and seed production is significantly reduced. In Ethiopia, the management methods of the diseases of onion are followed as mainly crop rotation, avoidance and sanitation to proper storage methods [5]. Conventional methods of control of diseases of onion include the use of chemicals, organic means, and through integrated management efforts. As a result, attempts to manage the disease have focused on reducing the populations of sclerotia in the soil through biological control. Research on biological control of plant pathogens has received much attention in recent years, as a means of increasing crop production by avoiding a number of problems related to chemical control, and hence, developing practices compatible with sustainable agriculture [6].

Arbuscular mycorrhizal fungi (AMF) are widespread in nature, and a fundamental component of agro ecosystems. AM symbioses may also improve plant health through a more specific increase in protection (improve resistance, and/or tolerance against biotic and abiotic stresses) [7,8]. *Trichoderma* spp. as a potential bio control agent was recognized in the early 1930's [9]. Among different strategies for plant growth promotion and disease suppression, biological approaches are very useful, and an ominous choice. The use of AMF and an antagonistic fungus, *Trichoderma* spp. are the major players,

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which could play active role in plant growth promotion and disease suppressions. Attempts have been made to know the importance of white rot disease on some onion growing areas, but not much detailed studies on disease survey works have been carried out, particularly in Ambo and Toke Kutaye districts of West Showa, Ethiopia. Hence, the present study was carried out to evaluate the native strains of AMF and *Trichoderma* spp. isolated from rhizosphere soils of onion cultivated fields at Ambo and Toke Kutaye districts of West Showa, Ethiopia, and their effect on plant growth promotion, and biological control activities against white rot disease.

Materials and Methods

Description of the study areas

The screening experiment was conducted in Ambo and Toke Kutaye districts of West Showa zone, Oromia Regional State, Ethiopia, during the academic year, 2008–2009, to evaluate the indigenous AMF and *Trichoderma* species from rhizosphere soils of onion cultivated fields at Awaro and Qora from Ambo district, and Nagafile and Imala Dawe (I/D) Ajo from Toke Kutaye district of West Showa, Ethiopia. Ambo district has total geographical area of 83598.69 sq.kms, which is located at 8°57' North latitude and 38°07' East longitude, at an average elevation of 1380-3300 meter above the sea level, and the Toke Kutaye district has total geographical area of 78887 sq.kms and is located at 8°57' North latitude and 38°07' East longitude, at an average elevation of 1800-2300 meter above the sea level. In both districts, the annual rainfall ranged from 800-1000 mm and the temperature of the districts ranged between 15°C and 29°C, with average temperature of 22°C.

Sample collection

The soil and root samples were collected from four different onion cultivated field study sites. At each study site, an area of 500 sq. meters was chosen for sampling. Five healthy and diseased plants were selected, and their roots, bulbs and rhizosphere soil samples were collected at 0-30 cm soil depth. Approximately, 2 kg of rhizosphere soil was collected in triplicates from each study site, and the samples were brought to Ambo Plant Protection Research Center (APPRC) laboratory in sealed plastic bags, and stored at 5-10°C. The roots of the healthy test plants were separately washed thoroughly, free of attached soil particles, and cut in to 1 cm bits and fixed in Formalin: Acetic acid: Alcohol (FAA) in the field itself [10]. The diseased bulbs were collected from the field in sealed plastic bags and stored at 5–10°C, until processed for use. Soil samples were thoroughly mixed and a portion of soil samples were analyzed for soil texture, pH, EC, N, P, K, Ca, Mn and Fe [11]. The remaining soil samples were used to isolate AM fungal spore populations and *Trichoderma* species.

Identification and mass production of AMF

Spore population density and species richness of AMF from each soil sample was estimated by the method of wet sieving and decanting [12]. Assessment and the percentage of AMF colonization in the roots of onion were determined by the method of trypan blue in lactophenol [10]. For identification of AMF, intact spores were picked up from the filter paper and mounted on micro slides in lactophenol, and were observed under binocular research microscope. The morphology of spores and sporocarps of AMF were observed, and then their characters were used for identification by using Manual for identification of AM fungi [13]. Mass inoculum production of AMF was carried out in the roots of *Sorghum vulgare* Pers. under glass house condition, by the method of Selvaraj et al. [14]. The selected dominant six species of AMF *viz., Acaulospora scrobiculata, Glomus aggregatum, G. fasciculatum, G. deserticola, Gigaspora margarita* and *Scutellospora heterogama* collected from four different study sites were only mass multiplied. Among them, *Glomus aggregatum* was selected for the efficient strain for further experimental use.

Identification and mass multiplication of *Trichoderma* spp.

Trichoderma species were isolated from rhizosphere soils of four different onion cultivated fields of West Showa, Ethiopia, using standard serial dilution plating technique on *Trichoderma* Selective Agar (TSA) medium [15], and identified down to species level based on standard mycological methods, with the help of the Manual of Soil Fungi [16], and the manual of Hypomycetes and identification of *Trichoderma* species [17]. Mass multiplication of *Trichoderma* species was followed by the method of Gopinathan and Selvaraj [18].

In-vitro evaluation

The pathogen, *S. cepivorum*, was isolated from bulbs of infected onion plants grown in well developed sick plot at APPRC, Ambo, and showing typical symptoms of the disease [19]. The isolated pathogen and the *Trichoderma* species were maintained as pure cultures at 4°C in refrigerator, until used. Twenty isolates with five species of *Trichoderma* were evaluated *in-vitro* for their antagonistic and inhibition potential against the onion white rot, using dual culture technique with direct confrontation test. Each of the twenty *Trichoderma* isolates and *S. cepivorum* were inoculated on to PDA (Potato Dextrose Agar) medium separately, and incubated at 25°C for 5 days. Control plates of the pathogen and the antagonist were also prepared. A Completely Randomized Design (CRD) was used. Isolates were then scored for degree of antagonism after five days, using the rating system of Bell et al. [20], on a scale of 1-5, where class1=*Trichoderma* completely overgrow the pathogen and cover the entire medium surface; class 2=*Trichoderma* overgrow at least 2/3 of the medium surface; class 3=*Trichoderma* and Sclerotium each colonize 50% of the medium surface, and neither of them appear to dominate the other; class 4=*Sclerotium* colonizes at least two-thirds of the medium surface, and appear to withstand encroachment by *Trichoderma*, and class 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 class 2 and not antagonist if the number was greater than class 2. Another set of experiment was prepared for inhibition and colony growth tests. To determine the inhibition percentage of the pathogen by each of the tested antagonists, growth of *S. cepivorum* was recorded by measuring the diameter of the colonies. Percentage inhibition (I%) of its colony growth was then calculated using the following formula used by Whipps [21].

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

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.

Efficacy test *in vivo*

The experiment was conducted under glass house condition (12–15°C minimum and 26–30°C maximum temperature), at APPRC, Ambo, Ethiopia. The potential native AMF, *G. aggregatum* (Awaro isolate) and four species of *Trichoderma* isolates were evaluated for the biological control of white rot disease in onion, under pot culture condition [22]. Sterilized sand, decomposed animal dung, and sterilized

sandy clay loam soil (1:1:2 ratio) (5 kg per pot) was used. Sclerotia of *S. cepivorum* was thoroughly mixed with surface soil (100 sclerotia per pot) and wheat bran: sand cultures of *Trichoderma* spp. (1 g/100 g soil) and AMF inoculum (50g/pot) were added to each pot, and thoroughly mixed with surface soil. Two onion seedlings (Variety Adama red) were planted in each pot. The experiment was laid out in Completely Randomized Design (CRD), with four replications and 12 treatments consisting of various combinations of *G. aggregatum*, and different isolates of *Trichoderma* spp. with *S. cepivorum* were maintained in pot culture. Uninoculated and inoculated with *S. cepivorum* alone were used as control check.

The following treatments were:

- Control (without *Sclerotium cepivorum*, AMF, and *Trichoderma* spp.)
- Control (*Sclerotium cepivorum* (Sc) alone),
- *Glomus aggregatum* (Ga) alone
- Sc+Ga
- *Sc+T. harzianum* ATh1 isolate
- *Sc+T. koningii* QTk2 isolate
- *Sc+T. hamatum* NThm3 isolate
- *Sc+T. viride* ATv1 isolate
- Sc+Ga+ATh1 isolate
- Sc+Ga+QTk2 isolate
- Sc+Ga+NThm3 isolate
- Sc+Ga+ATv1 isolate

Data analysis

After 90 days of inoculation, the plants were uprooted and the data were recorded on plant height of healthy and diseased plants, shoot, root, and bulb dry biomass of plants, after drying the samples at 60°C to constant weight in a hot air oven. AMF root colonization and spore numbers in root zone soils were assessed by grid line intersection [10], and wet sieving and decantation methods [12], respectively. The per cent white rot infection was calculated using the following formula:

Per cent white rot infection=Total No. of diseased plants/Total No. of seedlings planted×100

The data were subjected to analysis of variance, and the treatment means were further separated by Duncan's Multiple Range Test (DMRT), for significant differences at the level of P>0.05%.

Results and Discussion

Edaphic characteristics

Edaphic characteristics of the soils of the study areas indicated that the changes in soil moisture ranged from 9.2-10.5%, were in accordance with the climatic changes during different seasons, and the soil pH was acidic (pH 6.4–6.8) in all the four study sites, with low to moderate electrical conductivity (EC 1.4–1.8). The soil types were black sandy clay loam in Awaro, Qora and Nagafile sites, whereas dark grey clay loam in I/D Ajo. Generally, the soils were nutrient deficient, particularly high organic carbon (1.95–2.95%), less P-level (2.2–2.8 mg/kg), moderate to high level of K (296–346 mg/kg), and also with other micronutrient content such as zinc (1.4–2.2 g/g), calcium (1.2–1.8 g/g), manganese (2.8–3.4 g/g), and iron (58.5–98.5 g/g). The available N content of the soils, irrespective of the study sites, were invariably high (625-860 mg/kg).

AMF colonization

The test plant was positive for AMF colonization in the roots of onion, although the species of AMF colonizing the organism was varied. Percent colonization of AMF in the roots of onion differed in each locality. But there was a definite trend in AMF colonization between the different characteristics of the soil. There was a certain degree of specificity among the different AMF species in those four study sites (Table 1). The presence of high degree of AM colonization with various AMF structures, such as infection pegs, hyphal coils (pelotons), hyphal dimorphism, intracellular arbuscules, inter and intracellular vesicles, were observed in the root cortical cells of onion. Rajeshkumar and Selvaraj [23] kept in mind while describing a species as mycorrhizal, if roots contained one of the following combinations of AM fungal structures in the primary cortex, hyphae+arbuscules, hyphae+pelotons, or hyphae+vesicles. Totally, ten AM fungal species were isolated from rhizosphere soils of the onion (Table 2). Of the ten AMF, only two species, *G. aggregatum* and *G. fasciculatum*, were found to be colonized in the roots of onion. The difference in colonization could be due to variation in the species and the soil type, upholding the view expressed by Schenck and Kinlock [24].

Spores and sporocarps of AMF

Altogether, ten AMF species representing four genera *viz., Acaulospora, Gigaspora, Glomus,* and *Scutellospora* were recorded (Table 2), of which *Glomus* was the dominant genus. Based on the frequency of occurrence, the AMF species were identified and grouped as dominant (above 50%), and common (blow 50%) forms. Accordingly, *Gigaspora margarita. Glomus aggregatum, G. deserticola, G. fasciculatum, Acaulospora scrobiculata* and *Scutellospora heterogama*, were dominant forms, whereas *A. delegata, G. geosporum, G. macrocarpum* and *S. calospora* were constitute common forms

Location	Percent AMF root colonization	Total No. of AMF spores/100 g of soil	Positive for AMF in the roots	Positive for AMF in the root zone soils
S1-Awaro	96.5 a	1085 a	*Glomus aggregatum*	*Glomus aggregatum, G. deserticola, Acaulospora delegata, A. scrobiculata, Gigaspora margarita.*
S2-Qora	84.5 b	986 b	*Glomus aggregatum*	*Glomus aggregatum, G. fasciculatum, Acaulospora scrobiculata, G. margarita*
S3-Nagafile	56.5 c	425 c	*Glomus fasciculatum*	*G.aggregatum, G. fasciculatum, G. geosporum, G. macrocarpum, G. margarita, Scutellospora heterogama.*
S4-I/D Ajo	32.6 d	154 d	*Glomus fasciculatum*	*Glomus aggregatum, G. fasciculatum, G. margarita, S. calospora, S. heterogama.*

Means in the same column followed by the same superscript letter do not differ significantly, according to DMRT (P<0.01).

Table 1: Colonization and spore density of AMF from rhizosphere soils of onion.

List of AMF	S1*	S2	S3	S4	Species frequency %
Acaulospora delegata	+	--	--	--	25
A. scrobiculata	+	+	--	--	50
Gigaspora margarita	+	+	+	+	100
Glomus aggregatum	+	+	+	+	100
G. deserticola	+	+	_	_	50
G. fasciculatum	--	+	+	+	75
G. geosporum	--	--	+	--	25
G. macrocarpum	_	--	+	--	25
Scutellospora calospora	--	--	--	+	25
S. heterogama	--	--	+	+	50

*Study sites- S1-Awaro; S2-Qora; S3-Nagafile ; S4-I/D Ajo.

Table 2: Occurrence of AMF from rhizosphere soils of onion.

Inoculation treatment	Plant height (Shoot and root length) (cm)	Plant (Shoot and root) biomass (g/ plant)	% AMF root colonization	No. of AMF spores/100 g soil
Un inoculated control	85.0 d	24.90 d	0.00 e	0.00 e
Acaulospora scrobiculata	94.1 c	26.68 c	62.6 d	486.00 c
Glomus aggregatum	106.4 a	35.53 a	94.8 a	878.00 a
Glomus fasciculatum	98.9 b	30.88 b	72.2 b	568.00 b
Glomus deserticola	96.8 b	29.46 b	68.4 b	493.00 b
Gigaspora margarita	100.8 b	32.69 b	78.4 b	596.00 b
Scutellospora heterogama	89.7 c	26.99 c	58.5 c	345.00 d
SEM	2.8	1.4	2.8	14.6
CD (P<0.05)	1.2	0.8	1.2	7.4

Means in the same column followed by the same superscript letter do not differ significantly, according to DMRT (P<0.01).

Table 3: Effect of AMF on plant growth of *S. vulgare*.

(Table 2). The variation in spore population in onion also might be due to soil types. Higher number of AMF species was observed in black sandy clay loam soil study sites. The mean spore number per 100 g of soil varied from 365–1085 in root zone soils of onion. The lowest number of spores was recorded in root zone soil of onion (365/100 g) at I/D Ajo study site, whereas the highest number (1085/100 g) was observed in Awaro study site (Table 2). The AMF spore abundance was reported to be determined by the host plant species and environmental variables, than by AMF species [25]. Edaphic characteristics such as soil type [26], soil depth, soil pH [27], and soil fertility [28], were reported to influence AMF sporulation. The present study clearly highlights the soil edaphic factors that favor root colonization and sporulation of AMF, associated with onion crop. Rajeshkumar and Selvaraj [23] studied that AMF distribution is dependent on the host plant and certain ecological factors, such as organic carbon, soil pH, soil nutrients, and soil fertility. Although AMF is not host specific, they exhibit certain host preferences [29]. Also, the root colonization patterns may regulated by the host. McGonigle and Fitter [30] reported from the study of two native grasses and forbs in England, that AMF in the field showed a degree of ecological specificity.

Mass production of AMF

Plants inoculated with *G. aggregatum* had significantly higher percent root colonization, extra-matrical spore count, shoot and root length, and shoot and root biomass (Table 3). *Gigaspora margarita* and *G. fasciculatum* were found to be the second best, next to *G. aggregatum*. All the parameters were found to be significantly least in plants inoculated with *Scutellospora heterogama* and *A. scrobiculata*.

Isolation of *S. cepivorum* from diseased bulbs

Fluffy white cottony growth of the fungal mycelium was noticed on the surface of the medium. As the growth of the mycelium progresses, the mycelium becomes more compacted with numerous small spherical black bodies (Sclerotia), forming on this mycelial mat, which are approximately the size of a poppy seed or pinhead.

Mass multiplication of *Trichoderma* spp.

Twenty isolates of *Trichoderma* spp. were isolated from rhizosphere soil samples of Ambo and Toke Kutaye districts study sites. Isolates were numbered based on substrate from which they were isolated (Table 4). The five species of *Trichoderma* viz., *T. harzianum*, *T. viride, T. virens*, *T. koningii* and *T. hamatum* were identified. The species identities of all the isolates are still under determination. The isolates were mass multiplied on wheat bran and sand substrate. As reported by Singh et al. [31], wheat bran as the good substrate used for multiplication of the *Trichoderma* antagonists.

In vitro evaluation

The mean colony growth of *S. cepivorum* in plates containing *Trichoderma* isolates ranged from 2.0 cm (*T. harzianum*) to 3.45 cm (*T. virens*). The antagonism test revealed that four of the twenty isolates

Test organisms	Mean colony growth of *S. cepivorum (cm)* and *Trichoderma species*	Mean % inhibition on *S. cepivorum*
T. harzianum		
ATh1	2.00 d	65.4 a
QTh2	2.10 d	64.8 a
NTh3	2.15 d	61.4 a
ITh4	2.25 d	60.8 a
Sclerotium cepivorum(Control)	4.45 a	----
T. viride		
ATv1	2.10 d	64.8 a
QTv2	2.35 c	54.3 b
NTv3	2.43 c	48.6 c
ITv4	2.54 b	43.6 c
Sclerotium cepivorum (Control)	4.45 a	----
T. hamatum		
AThm1	2.34 c	54.3 b
QThm2	2.43 c	48.6 c
NThm3	2.48 c	45.4 c
IThm4	2.56 b	43.5 c
Sclerotium cepivorum(Control)	4.45 a	----
T. koningii		
ATk1	2.38 c	53.5 b
QTk2	2.39 c	53.2 b
NTk3	2.56 b	43.5 c
ITk4	2.45 c	48.5 c
Sclerotium cepivorum (Control)	4.45 a	---
T. virens		
ATvn1	3.15 a	35.6 d
QTvn2	3.24 a	33.8 d
NTvn3	3.45 a	32.5 d
ITvn4	3.23 a	33.5 d
Sclerotium cepivorum (Control)	4.45 a	-----
C.V.	7.66	12.4
LSD (0.01)	0.48	8.2
SE	0.14	2.2

Means in the same column followed by the same superscript letter do not differ significantly, according to DMRT (P<0.01).

Table 4: Interaction between *S. cepivorum* and *Trichoderma species* under *in vitro*.

which were scored less than 2 were highly antagonistic to the white rot pathogen. However, *T. virens*, which exhibited a score of 3.15-3.45, was not antagonistic at all. This isolate 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. This isolate was, therefore, not tested further. In contrast, its colony growth reached 4.45 cm after 7 days in control plates. The results of the data recorded 5-7 days after inoculation showed that there was a significant difference among the *Trichoderma* species, in suppressing the colony growth of the pathogen and inhibition percentage (Table 4). *T. harzianum* ATh1 isolate covered the plate completely on the 5th day incubation, while the other species grew and cover the plate on the 6th day of incubation. Meanwhile, in the control treatment, *S. cepivorum* grown, covered the plates on the 7th day of inoculation and producing sclerotia after the 10th day. Therefore, *S. cepivorum* did not grow at all when inoculated after 48 hours inoculation of *Trichoderma* species. *Trichoderma* isolates are known to rapidly colonize medium surface and substrates [32]. *Trichoderma harzianum* inhibited the colony growth of *S. cepivorum* by 65.4%, followed by *T. viride* (64.8%), *T. hamatum* (54.3%), and *T. koningii* (53.5%, which was also significantly different from each other. Akrami et al. [33] also found that *T. asperillum* and *T. harzianum* reduced 43.2% and 51.5% reduction in disease incidence, respectively. Up to 36% reduction of colony growth of *F. oxysporum* was obtained by using *Trichoderma harzianum* isolate [34]. In a similar study done by using culture filtrates of *T. viride*, Tesfaye [35] found that *F. solani* could grow only for 1.5 mm in 96 hrs (4 days). Alemu and Kapoor [36] also found that *T. viride* and *T. harzianum* inhibited the fungus, *Botrytis gladiolorium* that causes corm rot on *Gladiolus*. 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 [37], and also produced enzymes that have been proved to be involved in the antagonistic activity [38]. However, antagonistic fungi are specific in their antagonistic activity against specific fungi [39]. *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 [40]. According to Mpika et al. [41], efficiency of *Trichoderma* species in antagonizing plant pathogens is closely linked with local conditions. Arya and Kaushik [42] 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 [43] reported that the maximum inhibition growth by *T. harzianum* against *R. solani*, *Pythium debayanum, Sclerotinia minor* and *Fusarium oxysporum f.sp .pisi*. In the present study also, *T. harzianum* ATh1 isolate showed maximum inhibition of *S. cepivorum* (65.4%).

In vivo evaluation

Inoculation with *Glomus aggregatum* alone increased the plant growth parameters to the maximum, while less plant growth was observed in the plants inoculated with *S. cepivorum* alone. In all treatments, *G. aggregatum* either alone or in combination with isolates of *Trichoderma* spp. and pathogen, showed significant growth response and reduced the disease incidence, when compared to un inoculated and *S. cepivorum* alone control plants (Table 5). *G. aggregatum* combination with *T. harzianum* ATh1 isolate was significantly increased the growth, biomass, and reduced the disease incidence and severity of white rot disease in onion plants caused by *S. cepivorum*. The overall reduction in the incidence of white rot was 66.19% for *G. aggregatum*, with *T. harzianum* ATh1 isolate. The lowest shoot, root and bulb dry weights were recorded in plants inoculated with *S. cepivorum* alone. Singh et al. [31] reported that native species of *Trichoderma* was controlled red rot of sugarcane by the enzymatic action of the metabolites released by bio-agent. Some workers also showed that AMF can also be used as biological deterrent of soil borne diseases of vegetable crops [7,18]. In the present study, the wheat bran: sand formulation of *T. harzianum* ATh1 isolate combined with inoculum of *G. aggregatum,* significantly reduced the white rot of onion caused by *S. cepivorum*.

Conclusion

From the results of this study, it is concluded that the tested indigenous *Trichoderma* isolates have high potential to inhibit the colony growth of *S. cepivorum*. The efficacy test results of AMF and *Trichoderma* spp. clearly indicates that AM fungal inoculation alone, or in combination with *Trichoderma* spp. reduced the incidence and severity of white rot, and increased the plant growth of onion. Also, these two bio-control agents commercially exploited as bio-fungicides, and could be recommended to the farmers in Western Showa, Ethiopia for the management of white rot in onion. The outcome of bio-fungicides (*Trichoderma* and AMF) distribution under the lab to land

Treatments	Average height of healthy plants (cm)	Average height of diseased plants (cm)	Shoot and root dry weight of healthy plants (g/plant)	Average bulb weight of healthy plants	Per cent root rot incidence and severity*
Control (without inoculants)	62.0 b	----	6.98 b	12.4 b	----
Control check (*Sclerotium cepivorum* (Sc) alone)	24.5 a	12.5 a	1.24 a	6.8 a	90.5 (50.84) a
Glomus aggregatum (Ga) alone	68.8 e	----	9.88 e	20.8 e	----
Sc+Ga	64.4 d	46.5 c	8.76 d	17.6 d	46.28 (21.62)c
Sc+Trichoderma harzianum (ATh1 isolate)	68.2 e	48.8 c	9.85 e	18.2 d	43.78(13.97)c
Sc+*T. koningii* (QTk2 isolate)	60.5 c	34.7 b	7.82 c	16.5 c	45.03(23.11)c
Sc+*T. hamatum* (NThm3 isolate)	59.6 c	32.6 b	7.45 c	16.4 c	56.25(35.74)b
Sc+*T. viride* (ATv1 isolate)	66.5 e	46.8 c	8.45 d	17.8 d	39..65(14.68)d
Sc+Ga+ATh1 isolate	69.5 e	52.4 d	9.95 e	21.2 f	33.81(11.31)e
Sc+Ga+QTk2 isolate	66.8 e	49.6 d	8.24 d	20.3 e	45.03(19.5)c
Sc+Ga+NThm3 isolate	64.5 d	40.6 c	8.75 d	19.8 e	56.28(23.11)b
Sc+Ga+ATv1 isolate	67.5 e	50.8 d	8.65 d	20.6 e	40.25(20.56)d
LSD 0.01	8.6	6.8	1.4	3.4	8.6
CD (%)	2.4	3.4	0.4	0.8	2.4

*Percent severity–the data in parenthesis.
Means in the same column followed by the same superscript letter do not differ significantly, according to DMRT (P<0.01).

Table 5: Effect of Glomus aggregatum and Trichoderma spp. on growth and the incidence of onion white rot.

program has clearly brought out the role of AMF and *Trichoderma* spp. in the management of plant disease in field conditions, at Western Showa, Ethiopia, which merit commercialization.

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