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# Biocontrol Potential of *Trichoderma Sp.* And Rhizosphere Bacteria from Infected Garlic (*Allium Sativum L.*) In North Gondar, Ethiopia

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## Abstract

Garlic (*Allium sativum L.*) is a vital vegetable crop used for both nutritional and medicinal purposes worldwide. However, it is highly susceptible to various diseases, including the destructive white rot caused by Sclerotium cepivorum Berk. This study aimed to isolate and evaluate the efficacy of *Trichoderma* and Rhizosphere bacteria against Sclerotium cepivorum Berk in garlic. Samples were collected from three districts known to be affected by the pathogen. In vitro tests demonstrated that nine *Trichoderma* isolates effectively inhibited the growth of Sclerotium cepivorum Berk mycelia. Among these isolates, five exhibited significant inhibition (>50%) with a p-value of 0.000 in dual culture assays. The most potent isolate, GUT-6, showed a remarkable 90% inhibition, followed by GUT-3 and GUT-5 with 88.57% and 85.71% inhibition, respectively. In contrast, isolate GUT-7 exhibited the lowest inhibition efficiency (40%) compared to the control. Evaluation of bacterial isolates revealed lower inhibition efficiency against the radial growth of Sclerotium cepivorum Berk compared to *Trichoderma* isolates. Out of the six bacterial isolates tested, only two (GUR-1 and 12) showed inhibition (>50%), while the other four exhibited poor efficiency (<50%). The highest and lowest inhibitions were recorded by isolates GUBI-12 (58.13%) and GUBI-3 (13.95%), respectively. Overall, this study highlights the potential of *Trichoderma* species as an effective biocontrol agent against Sclerotium cepivorum Berk and recommends further evaluation in greenhouse conditions.

**Keywords:** Antagonist; Garlic; Mycelia; Rhizosphere bacteria; Sclerotium cepivorum; *Trichoderma*; Biocontrol

## Introduction

Plant fungal diseases are the most important issues in agriculture and food production in the world. It is estimated that the crop losses due to plant diseases in developing countries is about 50% and one third of these damages are due to fungal diseases (Mitra et al., 2018). Management of plant fungal diseases is a significant cost component in crop production (Bastakoti et al., 2017). However, increasing concerns about the effects of fungicides in the environment and residues in food have resulted in deregistration of a number of fungicides. The need to replace these has increased interest in biological control of plant diseases in recent years (Philip et al., 2017). Recent efforts have focused on developing economically safe, long lasting and effective bio-control methods for the management of plant diseases. The use of biocontrol agents has been shown to be eco-friendly and effective against many plant pathogens (Chemeda Dilbo et al., 2015). Among the fungi that constitute effective Biocontrol Agents (BCAs) species of the genus *Trichoderma* are well represented (Naher et al., 2014; Philip et al., 2017) [1,2].

Garlic is one of the main Allium vegetable crops known worldwide with respect to its production and economic value which requires good production and management practices (Diriba Shiferaw, 2016). Despite its importance and increased production, garlic yield and quality is affected by various biotic and abiotic stresses (Jaleel et al., 2007; Cheruth et al., 2008). Globally, there are 66 diseases that are attacking Allium crops. Among them, white rot disease caused by Sclerotium cepivorum Berk is one of the most destructive disease-causing heavy losses in onion and garlic (Coley-Smith, 1987). Researchers around the world have prospected potential microorganisms for the biological control of white rot to increase the number of tools for disease control and reduce the use of fungicides (Clarkson et al., 2002; Stewart and McLean, 2007) [3,4].

Biological control is the suppression of disease by the application of a Biocontrol Agent (BCA) usually a fungus, bacterium, or virus, or a mixture of these to the plant or the soil. The main advantage of using a BCA is that they are highly specific for a pathogen and hence are considered harmless to non-target species. Over the past decade there have been many reports of the identification of effective BCAs for fungal and bacterial diseases in crops and a number of BCAs are in commercial production (Philip et al., 2017). Recently the use of BCAs has attracted a lot of attention due to the ability of some species to suppress different plant disease and the possibility of combining with other control methods (Mitra et al.,2018) and also there is a great demand for safer, alternative and effective control agents (Hussain et al., 2017) [5,6].

Allium crops are the most indispensable vegetable crops used as condiments in most Ethiopian cuisine. Among them, onion *(Allium cepa L.)* and garlic (*Allium sativum L.*) belongs to the family Alliaceae, considered as the most important vegetables and spice crops produced on large scale in Ethiopia, cultivated during dry and rainy seasons (Rubatzky and Yamaguchi, 1997; Mohammed et al., 2014) [7]. In Ethiopia, Garlic production was increased from 6,042 ha in 2001/02 to 21,258 ha of land in 2012/13 with a total production increment from 79,421 to 222,548 tons of bulbs, but its productivity was decreased from 13.20 and 10.47 ton/ha, respectively (CACC, 2002; CSA, 2012/13). However, the productivity of onion and garlic is affected by many

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biotic and abiotic stresses that accounted for the low yield of onion and garlic in Ethiopia (Mohammed et al., 2014). In Ethiopia, the main limiting factor for onion and garlic production is the white rot disease resulted in breaking of floral stalks, and thus, the reduction of the bulb yield and seed production (Mohammed et al., 2014) [8].

In Ethiopia, research efforts have been done on the distribution of white rot disease in the country. Mohammed et al. (2014), reviewed the distribution, incidence, and prevalence of garlic white rot in major growing districts of south east and east Tigray (Zeray and Yesuf, 2013), in Ambo and Toke Kutaye districts of Western Showa (Ararsa and Thangavel, 2013), and in Northern Showa (Tamire et al., 2007) in Ethiopia. However, the presence and distribution of white rot in eastern part of Amhara region is not assessed so far [9].

The use of *Trichoderma* for biological control of white rot disease is not only safe for the farmers and consumers but also environmentally friendly. Therefore, the present study focused on filling the gap on the distribution of white rot on the given study area and adding another biocontrol frame work through isolating, characterizing and in vitro testing of the best *Trichoderma* species for controlling white rot disease of garlic [10].

## Material and Methods

### Description of the study area

This research was carried out in University of Gondar, Microbiology laboratory. The research samples were collected from Lay Armacheho, Denbia and Gondar Zuria districts of Central Gondar Zone [11].

Lay Armacheho district is one of the districts of Central Gondar Administrative Zone; it covers an area of 129,272 ha. The altitude of the district ranges between 980 and 2820 meters above sea level and the average annual rainfall ranges between 1223mm-1700mm. The annual maximum and minimum temperature of the district is 38°C and 10 °C, respectively. The agro-ecology of the district is highland 7%, midland 65% lowland 32% and the soil type is claylome 25%, vertisoli 8%, and red brown 45%. Lay Armacheho is bordered on the north by Tachi Armacheho, on the south by Gondar town, to the west by Chilga, to the east by Wogera districts. Major crops in the district are teff, wheat, finger millet and maize (LDAO, 2015) [12].

Denbia district is one of the districts of Central Gondar Administrative Zone; it covers an area of 148,968 ha. The altitude of the district ranges between 1750 and 2100 meters above sea level. Denbia is bordered on the south by Lake Tana, on the southwest by Takusa, to the west by Chilga, to the north by Lay Armacheho, and to the east by Gondar Zuria district. Its administrative town Kolla Diba is 35 km from Gondar town. In the Woreda, there are four small urban centers including kola Diba, Aynba, Chuahet and Robet towns as well as rural Kebeles and peasant associations. The topography of the district is 87 % plain, 8 % mountain 2.8% plateau and 2.2% covered by water and the soil type is clay 65 clay lome 35%. The area has a summer rain fall with mean annual rain fall and mean annual temperature of 1600mm and 20°C respectively. The district has 64% is arable or cultivable (49118 ha) and another 25% under irrigation, 6% pasture, 4% forest or shrub land, and the remaining 1% is considered degraded or other. This district is adjacent about 287 square kilometers to Lake Tana which is subjected to regular and extensive flooding. Major crops in the district (in order of importance) are teff, sorghum, finger millet and maize (DDAO, 2015) [13,14].

Gondar Zuria district is one of the districts of Central Gondar Administrative Zone, it covers an area of 114,983ha.The altitude of the district is 1107-3022 meters above sea level and the average annual rainfall range between 950mm-1035mm. The annual temperature of the Woreda is 330c maximum and 270c minimum. Its administrative town Maksegnete is 42 km from Gondar town. In the district, there are four small urban centers including Maksegnete, Teda, Enfranze and Degoma towns as well as rural Kebeles and peasant associations. Regarding the economic activity, agriculture is the dominant source of income for the farmers in the area. The major crop produced includes, Teff, Maize, Sorghum and Barley (Mulugeta & Achenef, 2015) (Figure 1) [15].

### Sample collection

Soil samples for isolation of antagonists and disease infected plants for isolation of the pathogen were collected from garlic grown soil and infected garlic plant. These samples were collected from the 3 districts of Central Gondar, (Denbia, Lay Armacheho, and Gondar Zuria) that were under garlic cultivation. Both soil and garlic plant samples were collected carefully and placed in separated clean polyethylene bags (Somasagaren and Hoben, 1984) [16].

### Isolation of the pathogenic agent (Sclerotium cepivorum)

The parts of diseased garlic plant stem and bulbs that showed symptoms were cut and surface sterilized with 4% sodium hypochlorite solution for 5-10 minutes followed by subsequent washings with sterile distilled water cited in Krishna et al. (2016) and placed on plate media prepared from potato dextrose agar (PDA), containing g/L; Potato infusion (200), Dextrose (20), Agar (15), pH (5.6±0.2) (Nikan et al., 2018). The plates were incubated at 28 ± 2 $^0$C for 3-5 days. The fungal pathogens grown on the media were purified through repeated hyphal tip culturing technique (Elsheshtawi et al., 2014) and identified using the standard identification keys such as Colonies on PDA are white, usually with a fairly even sheet of aerial mycelium, dichotomously branched hyphal tips, black spherical sclerotia and mycelium appears as septate white hyphae (Barnett and Hunter, 1998). The purified pathogenic agents were kept at 4 0C refrigerator for further diagnostic tests [17,18].

## Confirmation Test

### Preparations of pathogen inoculum

Fungal mass of infected garlic isolate Sclerotium cepivorum for soil infestation in laboratory experiments was obtained by growing the

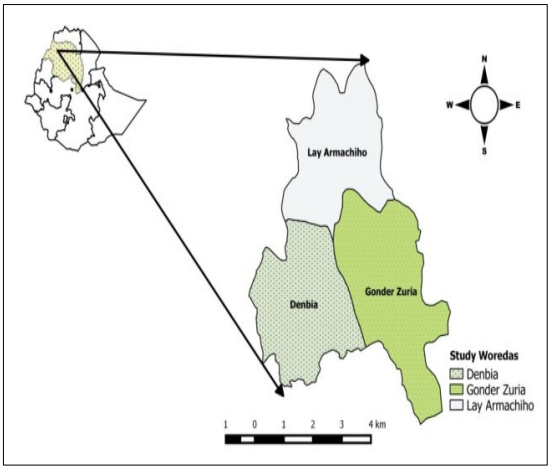



**Figure 1:** Map of the study area (using GIS version 9.2 software).

isolate on a sand-barley medium (Abd El-Moity, 1976). The medium was prepared by mixing 50 g wheat grains, 50 g sand, and 40 ml water; then the mixture in glass bottles (500 ml capacity) with cotton plugs was sterilized at 121 °C for 30 min. The autoclaved medium was inoculated with a 5 mm mycelial disk of Sclerotium cepivorum and incubated at 18±2 °C for 5 weeks (Elshahawy et al., 2019) [19].

## Pathogenicity test

The experiment was carried out in pots under greenhouse conditions. Each pot was filled with 3 kg of soil. The soil was pre-infested with Sclerotium cepivorum at the rate of 2% (w/w) 2 weeks before sowing. Then, the cloves of garlic were first surface sterilized using 70% ethanol for five minute and rinsed three times with sterilized distilled water. Seeds were sown in sterilized soil infested with the pathogen into 10-cm-diameter pots [20].

The Control pots were not inoculated with pathogen (Zewide et al. 2007; Elshahawy et al, 2019). The pathogenicity test (Koch postulate) was also conducted and performed to confirm the role of the isolated fungus in disease occurrence and symptom appearance. The symptoms observed, the leaves turn yellow and wilt, snow white mycelia on the bulb surface, small rounded shape sclerotium and destroyed root system, in the newly grown garlic crop were recorded and the pathogen was re-isolated for confirmation (Mahdizadehnaraghi et al., 2015) [21].

## Isolation of bio control agent, trichoderma species and other rhizosphere bacteria

### Isolation and purification of trichoderma

For isolation of Trichoderma species, serial dilution technique was followed and 1 ml of each solution was taken from 10-4- 10-7 and inoculated onto PDA (g/L; Potato infusion (200), Dextrose (20), Agar (15), pH (5.6±0.2)) adjusted plates (Rifai, 1969; Watanabe, 1994) [22]. The plates were incubated at 28 ± 2 0C for 3-5 days. The culture plates observed daily and based on the colony morphology distinct colonies were isolated into new plate and purified. The growth of Trichoderma spp. was identified using (Barnett and Hunter, 1998) and re-confirmed according to a taxonomic key for the genus Trichoderma such as, the mode of mycelia growth, colour, changes of medium colour, yellow pigment secreted into the agar, and loosely or compactly tufted mycelia and formation of distinct concentric rings for each isolate were examined (Rifai, 1969; Watanabe, 1994). All the isolates obtained were maintained on their PDA slants for short term storage at 40C refrigerator for antagonistic potential test. To prevent unwanted contaminants the PDA was amended with 100 µg/mL chloramphenicol (Ramazan et al., 2015) [23].

## Isolation of rhizosphere bacteria and purification

Rhizosphere bacteria were isolated from the rhizosphere soil of healthy garlic plant collected from the three districts of North Gondar using serial dilution technique. Approximately 1 g rhizosphere soil is homogenized with 9 mL sterilized distilled water and shaken for 30 min. and suspensions were serially diluted until 10-5 with sterile water Han et al. (2015), after which 0.1 ml of diluted soil suspension was plated on respective medium plates that contain nutrient agar medium (NA) ( g/L; Peptone (5), Sodium chloride (5), Yeast extract (1.5), Beef extract (1.5), Agar poGZer (20)) supplemented with 100 µgml-1 of cycloheximide to suppress fungal growth as described in Somasagaren and Hobben (1994) and incubated at 28 ± 2 0C for 3-5 days [24]. Based on the color and colony morphology single and seemingly different colonies were selected and purified through repeated re-streaking until pure isolates obtained and incubated at 28 ± 2 0C for 3- 5 days.

## Testing the antagonistic activity of trichoderma and rhizosphere bacteria isolates

The antagonistic activity of Trichoderma which was isolated and identified morphologically and the rhizosphere bacteria were tested using dual culture technique as described by (Landa et al., 1997) [25].

## Dual culture test for antagonistic fungal isolates

The antagonist activities of the isolates of Trichoderma were examined against the pathogen using dual culture technique. The mycelia discs (5mm, using crock borer) of 7 day old pure culture of the pathogen were placed on the PDA plates 1 cm far from the edge and the same size mycelia disc of Trichoderma isolate were placed on the opposite edge of the Petri-plate, whereas control plates were inoculated with only pathogen disc and incubated at 25± 200C for 7 days (Krishna et al., 2016) [26]. The colony diameter of radial growth of targeted fungal pathogens were measured after 1st, 2nd, 3rd and 4th days of incubation at two locations from center of the test plate and average diameter were calculated. Finally, the percent inhibitions of average radial growth were calculated by using the following formula in relation to the growth of the controls (Srijina et al., 2017).

L= (C-T/C) 100

Where, L = inhibition percentage;

C = radial growth measurement of the control

T = radial growth of pathogen in the presence of Trichoderma.

## Dual culture antifungal inhibition test for rhizosphere bacteria isolates

From the pure rhizosphere bacteria broth culture (48hr) a loop full of the bacterial isolates were equidistantly spot inoculated on the margins of potato dextrose agar (PDA) plates amended with sucrose (0.5%) and incubated at 28 ±2°C for 48 h cited in (Mussa Adal et al., 2018) [27]. After 48 hrs of incubation of the bacterial isolate, 5mm fungal pathogen was placed at the center of the plate, whereas the control plate was with only pathogen mycelia disc and incubated 28 ±2°C for 5- 7 days. The presence and absence of inhibition zone were recorded. The growth diameter of the pathogen (distance between the point of placement of fungal disk and actively growing edges of the fungus) was measured (Amini et al., 2012). The percentage inhibition of fungal growth was measured as the distance of clear zone between the bacterial colony and the fungus using the formula described by (Han et al., 2015) [28].

Inhibition of growth (%) = (1-TD×) 100

CD

Where, TD =distance between the center and fungal hyphae edges in the treatment

CD =distance between the center and fungal hyphae edges in the control plates.

Designation of the antagonists

The purified isolates were designated as GUT (Gondar University Trichoderma isolates) for fungal antagonist and GUR (Gondar University Rhizosphere bacteria Isolates) for bacterial antagonist followed with consecutive numbers representing each isolate [29].

## Characterization of bacterial antagonist

### Cultural characterization of the rhizosphere bacterial isolates

The isolates were examined for their colony and cell morphology. Different morphological features including size, shape, margins, colour and consistency of colony, cell shape, and cell arrangement were examined as per the standard procedures described by Aneja (2003) and Cappuccino and Sherman (2010) [30].

## Gram reaction test

The rhizosphere bacterial isolates were tested for determining their Gram reaction type using the KOH method (Buck, 1982). A drop of 3% KOH was placed on a clean microscope slide and loop full rhizosphere bacterial isolates were picked, dipped and mixed properly for l minute. The mixtures were then lifted with inoculating loop about 1 cm from the slide and presence and absence of obvious stringiness (viscosity) were recorded as Gram negative and Gram-positive bacteria, respectively cited in (Mussa Adal et al., 2018) [31].

## Biochemical characterization

Rhizosphere bacterial isolates were subjected to biochemical tests for characterization including the oxidase test, catalase test, carbohydrate fermentation and starch hydrolysis test, (Cheesbrough, 2006).

## Catalase activity

Catalase test was performed by taking a drop of 3% hydrogen peroxide was added to 48hr old bacterial colony on a clean glass slide and mixed using a sterile tooth-pick. The effervescence indicated catalase activity (Kumar et al., 2012) [32].

## Oxidase activity

Oxidase test was determined using Kovac„s oxidase reagent (1% dimethyl-p- phenylenediamine dihydrochloride). Aliquots of the reagent were dropped on a filter paper and fresh rhizosphere bacterial cells from NA agar slants were streaked up on the filter paper. The appearance of lavender color which turns dark purple to black within 1-3 minutes was considered as positive test for oxidase enzyme production (Kaur, 2014) [33].

## Starch hydrolysis activity

The ability of isolates to utilize starch as carbon and energy source has been tested as described by (Aneja, 2003). The isolates were inoculated on the starch agar media (SAM) and incubated at 28 ± 2°C for 48 hrs. After incubation, drops of iodine solution were poured on surface of the plates and Presence or absences of clear zone around the colonies were recorded [34].

## Hydrolytic enzyme production of antagonistic rhizosphere bacteria

## Amylase production

Rhizosphere bacterial cultures were screened for amylase enzyme activity by starch hydrolysis test on starch agar plate (Sharman, 2010). The pure isolated colonies were streaked on starch agar plates using starch as the only carbon source. After incubation at 37°C for 24-48 hrs, the individual plates were flooded with Gram's iodine to produce a deep blue colored starch-iodine complex. In the zone of degradation the appearance of clear zone was recorded as the basis for detection and screening of amylase producing isolates (Vaidya and Rathore, 2015) [35].

## Protease production

Rhizosphere bacterial isolates grown 48 hrs were tested for production of protease by growing them on Skim milk agar (Skim milk poGZer 10g ml-1, agar 15g ml-1) and were incubated at 28±20C for 3-5 days (Simbert and Krieg, 1994). An ability to clear the skim milk suspension in the agar was taken as evidence for the secretion of protease. Non- bacteria inoculated plates were used as the control (Chantawannakul et al., 2002) [36].

## Cellulase production

The rhizosphere isolates (48 hr old) were spot inoculated on Carboxymethyl cellulase CMC agar medium with yeast extract plates containing (g L-1) NaNO3 (2), K2PO4 (1), MgSO4 (0.5), KCl (0.5), CMC sodium salt (2), peptone (0.2), and agar (17) to evaluate their cellulase activity according to Kasana et al. (2008). The plates were incubated at 28±20C for 3-5 days. The clear zone surrounding the colonies was recorded as an indicative of cellulase production (Samanta et al. 1989) [37].

## Chitinase production

Chitinase production potential of Rhizosphere bacteria isolates was determined by growing the bacterial isolates on chitin agar medium containing (g L-1) chitin (4), MgSO4.7H2O (0.5), K2HPO4 (0.7), KH2PO4 (0.3), FeSO4.7H2O (0.01), MnCl2 (0.001),

NaCl (0.3), yeast extract (0.2) and agar (20) using the method described by Renwick et al. (1991). The bacterial cells (106 ml-1) were spot inoculated on the chitin medium and were incubated at 28±20C for 3-5 days. The clear zone formed around colonies was recorded as a positive test for chitinase activity (Nisa et al., 2010) [38].

## Hydrogen cyanide (HCN) production

Rhizosphere isolates were streaked on nutrient agar slant medium (48hr). Filter paper strips were dipped in picric acid and 2 % sodium carbonate were inserted in the tubes. The test tubes were incubated at 28 ± 2°C for 3-5 days after sealing them with parafilm (Ahemad and Khan, 2012). HCN productions were checked on the basis of changes in colour from yellow to light brown, moderate brown or strong brown of the yellow filter paper strips (Mussa Adal et al., 2018) [39].

## Ammonia production

Each isolate were tested for the production of ammonia in peptone water. Freshly grown bacterial broth cultures (48hr.) were inoculated in 10 ml nutrient broth and incubated in a rotatory shaker at 28 ± 2°C for 72 hrs. Afterwards, 0.5ml of Nessler's reagent was added to each culture. Development of deep yellow to brown color was recorded as a positive test for ammonia production (Cappucino and Sherman, 1992).

## Carbohydrate and amino acid utilization for rhizosphere bacteria

Carbon utilization of the isolates was determined on a basal medium containing 10% (w/v) of one of the 8 carbohydrates: starch, dextrin, lactose, sucrose, galactose, maltose, glucose, by reducing the yeast extract to 0.05 g l-1 following the method developed by Somasegaren and Hoben (1994).The basal medium containing (g l-1): KH2PO4 (1), K2HPO4 (1), FeCl3.6 H2O (0.01), MgSO4. 7H2O (0.2), CaCl2 (0.1), agar (15) and supplemented with 1 g l-1 of mannitol. Similarly, the ability of isolates to utilize different nitrogen sources was tested on the

same basal medium containing one of the following 4 amino acids at a concentration of 0.5 g l -1 according to Amarger et al. (1997). The amino acids were L-alanine, L-arginine, L-asparagine and L-glycine. The plates were incubated at 28 ± 2°C for 3-5 days presence as well as absence of growth was recorded as positive and negative respectively [40].

### Characterization of fungal antagonist

### Hydrolytic enzyme production of trichoderma isolates

#### Amylase activity

To examine the production of amylase enzyme, the myclial disc of trichodema isolate were inoculated onto the medium (Glucose: 1g Yeast extract: 0.1g Peptone: 0.5g Agar: 16g Soluble starch: 2% Distilled water: 1000ml). Following incubation for 48 hrs. at 28 ± 2°C, the plates were flooded with 1% iodine in 2% potassium iodide solution. The clear zone formed surrounding the colony was considered positive for amylase activity (Maria, 2005) [41].

### Protease activity

Trichoderma isolates grown 48 hrs were tested for production of protease by growing them on Skim milk agar (Skim milk poGZer 10g ml-1, agar 15g ml-1) and were incubated at 28±20C for 3-5 days (Simbert and Krieg, 1994). An ability to clear the skim milk suspension in the agar was taken as evidence for the secretion of protease. Non-inoculated plates were used as the control (Chantawannakul et al., 2002).

### Cellulase activity

For cellulase assay, the Trichoderma isolates were grown on yeast extract peptone agar (g/L; Yeast extract (0.1), Peptone (0.5), Agar (16)) medium supplemented with 0.5% carboxymethyl cellulose (CMC). The mycelial disk was inoculated onto the medium. Following incubation at 280C for 48hrs the plates were flooded with 0.2% aqueous Congo red and destained with 1M NaCl for 15 minutes. The clear zone surrounding the colony was considered positive for the cellulase activity (Kathiresan and Manivannan, 2006) [42].

### Chitinase production

Chitinase production potential of Trichoderma isolates was determined by growing the isolates on chitin agar medium containing (g L-1) chitin (4), MgSO4.7H2O (0.5), K2HPO4 (0.7), KH2PO4 (0.3), FeSO4.7H2O (0.01), MnCl2 (0.001), NaCl (0.3), yeast extract (0.2) and agar (20) using the method described by Renwick et al. (1991). The trichoderma mycelia discs were spot inoculated on the chitin medium and were incubated at 28±20C for 3-5 days. The clear zone formed around colonies was recorded as a positive test for chitinase activity (Nisa et al., 2010) [43].

### Physiological tolerance test for antagonists, trichoderma and rhizosphere bacteria

Antagonistically effective isolates were selected for their in vitro ecological characteristics; tolerance to pH, temperature, salt and resistant to antibiotics (Somasegaren and Hoben, 1994). All tests were carried out in triplicates.

### pH, salinity and temperature tolerance test

Each isolate were grown to determine their capacity to grow at different pH ( 4, 5, 8, 9, 9, 10); salt concentrations of 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, of NaCl (Bernal and Graham, 2001) and their resistance to different incubation temperature of 5°C, 10°C, 15°C, 35°C, 40°C and 45°C as indicated in (Mussa Adal et al., 2018) and were incubated 28±2°C for 3-5 days.

### Antibiotic resistance test

The inherent antibiotic resistance (IAR) of the Trichoderma and rhizosphere bacterial isolates were determined by inoculating on solid medium PDA for Trichoderma isolate and NA for rhizosphere bacteria containing antibiotics (µg/ml): Chloroamphinicol (40), Erythromycin (30), Neomycin (20) and Tetracycline (30) (Adal et al., 2018).

### Screening for heavy metal tolerance of trichoderma

Salts of heavy metals, chromium, mercury, nickel, zinc and lead were used to test the heavy metal tolerance of the culture. The heavy metals were separately incorporated in Sabouraud‟s dextrose agar at a concentration of 100 ppm. The culture was inoculated and following incubation the plates were observed for fungal growth. The mycelial growth was recorded (Kathiresan and Manivannan, 2006) [44].

### Heavy metal tolerance test for rhizosphere bacteria

The isolates were tested for their resistance to heavy metals namely Ni, Hg, Pb, Zn and Cr by agar dilution method. Nutrient agar plates amended with various soluble heavy metal salts at concentrations 100 µg/ ml were inoculated and incubated for 3 days at room temperature. Heavy metal tolerance was indicated by the appearance of bacterial growth and results recorded (Kumar et al., 2012).

### Data analysis

Results on each experimental treatment were collected every day in its respective time from the experiment. The experiments were carried out in triplicate and the average data were used for each calculation. The result of in vitro antagonistic efficiency test was analyzed and interpreted using one way ANOVA. The experimental treatments were compared and contrasted against their controls following Duncan‟s multiple range test (DMRT) using SPSS ver. 20 at a significance level of $P < 0.05$. The mean maximum and minimum value of individual results has been analyzed.

## Result

### Isolation of the pathogen

After isolating and growing the fungus from the infected garlic on PDA media, the morphological (Figure 2) and microscopic examination (Figure 3) appearance of the pathogen indicated that the fungal pathogen resembled white and were similar to the morphology of white rot pathogen.

### Confirmation test of the pathogen

As described in Lupiens et al. (2013) the characteristic symptoms and signs such as dying and dead leaves, cortical rot of roots, the presence of white mycelium on bulbs (Figure 4) and the presence of many small sclerotia (Figure 5) in the outer scale of basal plant confirmed white rot pathogen, Sclerotum cepivorum. Bioformulation were formed from the isolated pathogen using wheat bran and used to infect the soil in the pot on which garlic seed planted later. The pathogens clearly affect the garlic crop and the symptoms were observed (Figure 6). The pathogen again re-isolated from the infected plant for confirmation and it was similar with the original isolates [45].

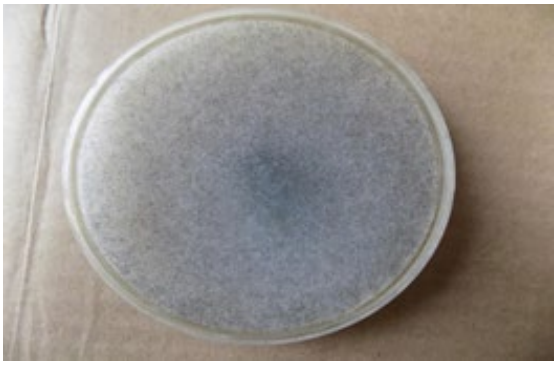


**Figure 2:** The isolated pathogen on PDA.

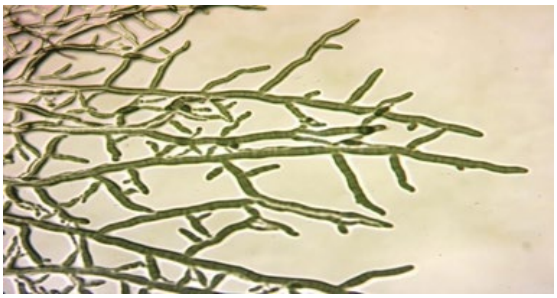


**Figure 3:** Microscopic image of white rot pathogen.

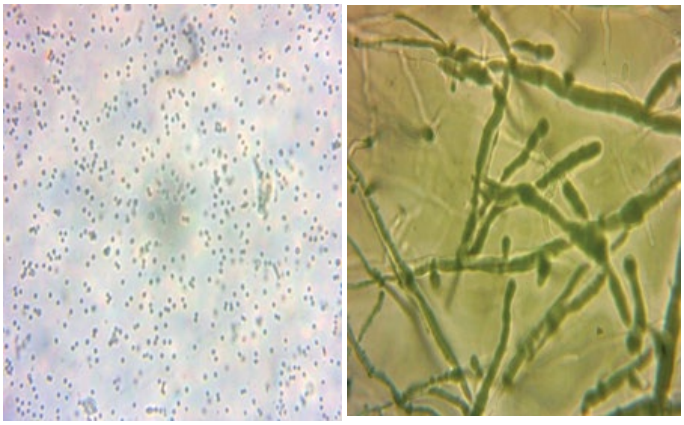
**Figure 4:** White mycelia of the pathogen.

**Figure 5:** Black sclerotia of the pathogen.

## Fungal antagonist

From the total of 38 pure isolates, only 9 isolates inhibited the growth of the pathogen on dual culture test and were isolated and selected for physiological and biochemical characterization.

**Figure 6:** Garlic showing white rot symptoms (A and B) and control (C).



**A-Spores** **B-Mycelia**

**Figure 7:** Microscopic image (40x) of spore and mycelia of *Trichoderma*.

## Morphological characteristics of the trichoderma isolates

Morphologically the isolates were characterized based on their colony color and mycelia growth pattern (Table 1). Among the isolates, 4 (44.4%), 3 (33.3%) and 2 (22.3), white, dark green and green white tuft isolates, respectively. From 4 white isolates 3 of them has no ring, but isolate GUT-8 was with ring. 2 (22.3%) and 1 (11.1%) isolate were whitish and dark green, dark green and green white tuft and white and green white tuft isolated from Denbia, Lay Armacheho, and Gondar Zuria districts in the same order.

Regarding mycelia growth of the isolates, 7 (77.8 %) and 2 (22.2%) of the isolates were raised and flat, respectively. Similarly, the isolates showed variation in colony color and reverse color. Concerning microscopic observation, the isolates were morphologically indistinguishable.

The microscopic image of mycelia and their spore were also observed (Figure 7). The fungal isolates were identified as Trichoderma species using the identification keys such as growth pattern, growth rate and color as described by Sekhar et al. (2017).

The growth rates of the isolates that were recorded at the second, fifth and seventh day (Table 2) showed the highest growth rate was observed by isolate GUT-6 (9mm per day) followed by GUT-4 and 5 (8mm per day) isolated from Lay Armacheho district, while, the minimum growth rate was found to be 5.8mm per day, isolate GUT-2 from Denbia district. Among the isolates 5 (55.5%) were showed a growth rate of 7mm per day. After a week, all the isolates covered the surface of the plate [46].

## Dual culture assay

The antagonistic isolates showed different inhibition efficiency (Table 3 and Figure 8). Among the 9 isolates tested for dual culture, 5 (55.6 %) isolates significantly inhibited the radial growth of the pathogen (>50%), while 4 (44.4 %) of the isolates showed less inhibition

**Table 1:** *Trichoderma* isolates colony color and growth pattern.

| No. | code | Isolation Site | Colony color | Reverse color | Mycelia growth |
|---|---|---|---|---|---|
| 1 | GUT-1 | DB | Whitish | White | Raised |
| 2 | GUT-2 | DB | White | Yellow | Flat |
| 3 | GUT-3 | DB | Dark Green | Bright green | Raised |
| 4 | GUT-4 | LA | Green White tuft | White | Raised |
| 5 | GUT-5 | LA | Dark green | Dark green | Raised |
| 6 | GUT-6 | LA | Dark Green | Pale green | Raised |
| 7 | GUT-7 | GZ | White | Pale yellow | Flat |
| 8 | GUT-8 | GZ | White with ring | White | Raised |
| 9 | GUT-9 | GZ | Green white tuft | Greenish white | Raised |
| GUT=Gondar university Trichoderma, DB= Denbia, LA= Lay Armacheho, GZ= Gondar Zuria | | | | | |

**Table 2:** Growth of *Trichoderma* isolates (mm).

| No. | Code | Sample site | After 2 days(mm) | After 5 days (mm) | At the 7 days (mm) | Growth/day (mm) |
|---|---|---|---|---|---|---|
| 1 | GUT-1 | DB | 8 | 31 | 45 | 6.2 |
| 2 | GUT-2 | DB | 9 | 29 | 45 | 5.8 |
| 3 | GUT-3 | DB | 19 | 38 | 45 | 7.6 |
| 4 | GUT-4 | LA | 15 | 40 | 45 | 8 |
| 5 | GUT-5 | LA | 18 | 40 | 45 | 8 |
| 6 | GUT-6 | LA | 22 | 45 | 45 | 9 |
| 7 | GUT-7 | GZ | 10 | 30 | 45 | 6 |
| 8 | GUT-8 | GZ | 12 | 36 | 45 | 7.2 |
| 9 | GUT-9 | GZ | 13 | 31 | 45 | 6.2 |
| GUT= Gondar University Trichoderma isolate, DB= Denbia District, LA= Lay Armacheho District, GZ= Gondar Zuria District | | | | | | |

**Table 3:** In vitro mycelia growth inhibition of *Trichoderma* isolates (cm).

| No. | Code | Isolation site | After 3 days (cm) | 5days (cm) | 7days (cm) | 14days (cm) | % of inhibition (%) |
|---|---|---|---|---|---|---|---|
| 1 | GUT-1 | DB | 3 | 3 | 3 | 3 | 42.85 |
| 2 | GUT-2 | DB | 2.9 | 3 | 3 | 3 | 42.85 |
| 3 | GUT-3 | DB | 3.5 | 4.3 | 6.2 | 8 | 88.57 |
| 4 | GUT-4 | LA | 3.7 | 3.7 | 3.7 | 3.7 | 52.85 |
| 5 | GUT-5 | LA | 3.2 | 4.2 | 6 | 8 | 85.71 |
| 6 | GUT-6 | LA | 3.2 | 4.5 | 6.3 | 8 | 90 |
| 7 | GUT-7 | GZ | 2.8 | 2.8 | 2.8 | 2.8 | 40 |
| 8 | GUT-8 | GZ | 3.3 | 3.3 | 3.3 | 3.3 | 47.14 |
| 9 | GUT-9 | GZ | 3.6 | 4 | 4.5 | 5.6 | 64.28 |
| 10 | Control | | 4 | 6 | 7 | 8 | |
| GUT= Gondar University Trichoderma isolate, DB= Denbia District, LA= Lay Armacheho District, GZ= Gondar Zuria District | | | | | | | |

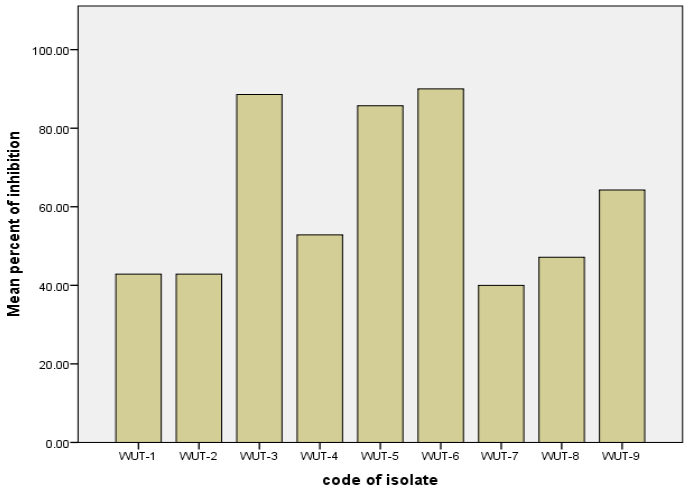



**Figure 8:** The inhibition efficiency of the *Trichoderma* isolates.

of radial growth of the pathogen (<50%). The maximum antagonistic efficiency recorded was 90% (GUT-6), followed by 88.57% (GUT-3) and 85.71% (GUT-5), whereas, the minimum inhibition efficiency recorded was 40% (GUT-7). Generally, 55.5% of the isolates showed better (>50%) inhibition of radial growth of Sclerotium cepivorum mycelia.

The mean value of the inhibition efficiency of Trichoderma isolates was found to be 61.58 % which showed great deviation of the results when compared with the minimum value (40%) and maximum value (90%). The standard deviation also showed 21.15% that indicated great deviation. The sum of mean squares between groups (isolates) recorded 52.58 and using alpha value 0.05, the p-value was 0.000. Since the p-value (0.000) is less than alpha (0.05), there was a significance difference between the means of isolates inhibition efficiency. The total mean was 4.31 0.277 with minimum value 2.7 and maximum value 6.9 and the individual means of isolates with their minimum and maximum value (Table 4).

**Table 4:** Mean min. and max. Value of *Trichoderma* isolates.

| Isolates (treatments) | Isolation site | N | Inhibition (cm) mean | Minimum | Maximum |
|---|---|---|---|---|---|
| GUT-1 | DB | 3 | 3.0 $^{ijgh}$ | 2.9 | 3.2 |
| GUT- 2 | DB | 3 | 3.0 0.05$^{hgij}$ | 2.9 | 3.1 |
| GUT-3 | DB | 3 | 6.2 0.35$^{bac}$ | 5.8 | 6.9 |
| GUT-4 | LA | 3 | 3.7 0.05$^{ef}$ | 3.6 | 3.8 |
| GUT-5 | LA | 3 | 6.0 0.10$^{cba}$ | 5.8 | 6.1 |
| GUT-6 | LA | 3 | 6.3 0.10$^{abc}$ | 6.2 | 6.5 |
| GUT-7 | GZ | 3 | 2.8 0.05$^{ijhg}$ | 2.7 | 2.9 |
| GUT-8 | GZ | 3 | 3.3 0.25$^{ghij}$ | 3 | 3.8 |
| GUT-9 | GZ | 3 | 4.5 0.15$^{d}$ | 4.2 | 4.7 |
| Total | | 27 | 4.31 0.277 | 2.7 | 6.9 |

GUT= Gondar University *Trichoderma* isolate, DB= Denbia District, LA= Lay Armacheho District, GZ= Gondar Zuria District

## Hydrolytic enzyme production of trichoderma

The trichoderma isolates has been showed variation in their ability of hydrolytic enzyme production. There was no record on production of cellulose hence no isolates grew on cellulose containing medium. Among the isolates 7 (77.7%) of them were positive for protease and chitinase production, whereas 2 (22.3%) of the isolates were positive for amylase production.

## Physiological tolerance test

## The effect of temperature on the growth of trichoderma isolates

Regarding temperature tolerance of Trichoderma isolates, no isolate showed growth both above 400 c and below 100 C, whereas, increase in growth of the isolates was observed as incubation temperature increases from 15 to 28 0C. Isolates GUT-6, GUT-5 and GUT-4 exhibited the highest growth characteristic while isolates GUT-2 and GUT-7 showed the lowest growth characteristic at 28 0C.

## The effect of pH on the growth of trichoderma isolates

The Trichoderma isolates grown at pH range of 4 – 9 and showed variation in their growth (Figure 9). The maximum number of isolates showed high growth at pH=6.5 followed by 5 and8 and minimum at 9. Among the isolates GUT-1, 2, 6 and 8 showed more than 1cm growth both at pH of 4 and 5. GUT-2 and 6 were showed higher growth than any other isolates at all pH levels. The highest growth of the isolates recorded at pH of 6.5 and they showed decreasing when goes towards basic pH.

## The effect of salinity on the growth of trichoderma isolates

Concerning salinity tolerance of trichoderma isolates, all of the isolates 9 (100%) were grew at salinity concentration range of 1-5% (Figure 10). Among the isolates 7 (77.7%) and 4 (44.4%) isolates were grown at salinity concentration of 6% and 7%, respectively. There was no isolate showing growth at 8% NaCl concentration [47].

## Antibiotics resistance of trichoderma

Trichoderma isolates were tested for their resistance against some antibiotics including chloramphenicol, erythromycin, tetracycline and neomycin. All 9 (100%) trchoderma isolates were grown over all the tested antibiotics.

## Heavy metal tolerance for trichodrma

All Trichoderma isolates 9 (100%) were showed positive for lead and Zink tolerance test. Among the isolates 7 (77.7%), 8 (88.8%) and

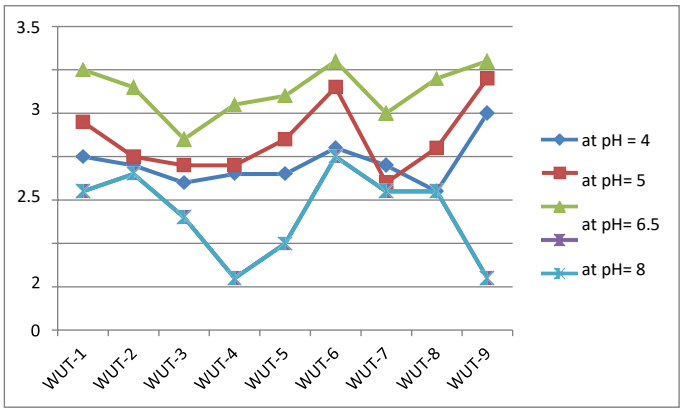



**Figure 9:** pH tolerance of *Trichoderma* isolates.

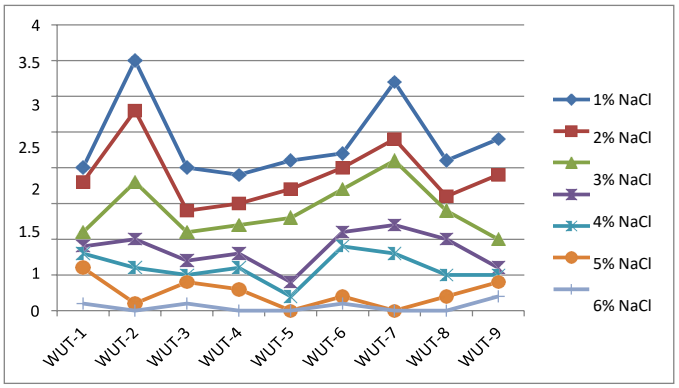



**Figure 10:** Salinity tolerance of *Trichoderma* isolates.

2 (22.3%) isolates were resistant for nickel, mercury and chromium, respectively (Figure 11).

## Rhizosphere bacterial antagonists

From the total of 40 rhizosphere bacterial isolates (15 from Lay Armacheho district, 15 from Gondar Zuria district and 10 from Denbia district) selected based on their colony morphology and cultural characteristics, only 6 isolates showed a significant antifungal activity on dual culture test against white rot of garlic.

## Colony morphology and cultural characteristics of bacterial isolates

The colony morphology including color, elevation, and margin and cell shape was presented (Table 5). The Gram reaction test showed that

**Table 5:** Colony characteristics of bacterial isolates.

| No. | Code | isolati on site | Colony color | Elevati on | Margin | Cell shape | Gram's reaction | % of inhibition |
|---|---|---|---|---|---|---|---|---|
| **1** | GUR-1 | DB | White | raised | smooth | Rod | + | 51.16 |
| **2** | GUR-3 | GZ | Cream | flat | smooth | Spiral | + | 13.95 |
| **3** | GUR-4 | GZ | Cream | raised | smooth | Rod | + | 37.2 |
| **4** | GUR-6 | LA | White | raise | rough | Spherical | + | 30.23 |
| **5** | GUR-7 | LA | Whitish | flat | smooth | Spiral | + | 27.9 |
| **6** | GUR-12 | LA | White | raised | smooth | Rod | + | |
| GUR= Gondar University Rhisozphere bacteria, DB= Denbia District, LA= Lay Armacheho Dstrict, GZ= Gondar Zuria District, += positive | | | | | | | | |

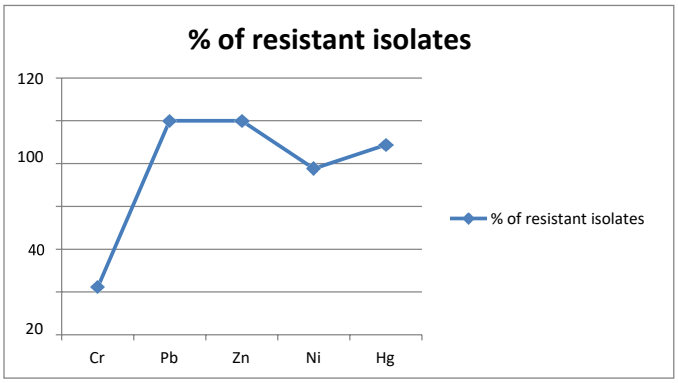



**Figure 11:** Heavy metal resistance of *Trichoderma* isolates.

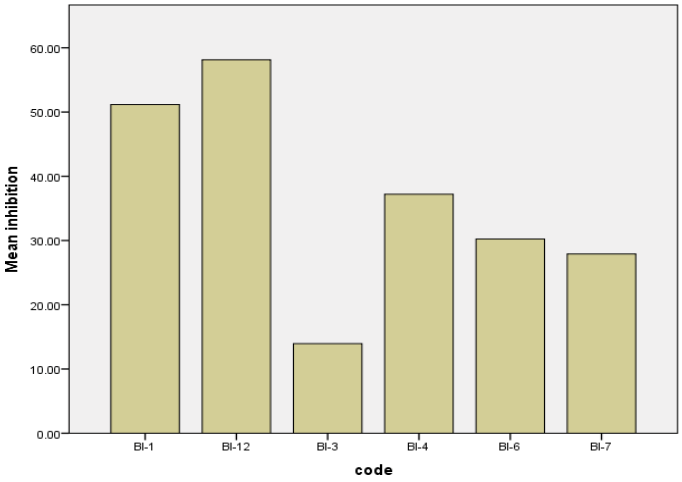



**Figure 12:** The inhibition efficiency of bacterial isolates.

all isolates were Gram positive. 4(66.7 %) and 2(33.3 %) were white and creamy, respectively. Similarly, 4(66.7 %) were raised and 2(33.3 %) were flat. Regarding margin of the isolates, 5 (83.3 %) of the isolates were smooth and 1(16.7 %) were rough (Figure 12).

## Gram reaction of rhizosphere bacterial isolates

All the 6 (100 %) rhizosphere bacteria isolates were Gram positive with different cell shape. Concerning the isolates cell shape, 3(50 %), 2(33.3 %) and 1(16.7 %) were rod, spiral and spherical in the same order.

## Antifungal activity of the rhizosphere bacterial isolates

As shown in Fig bacteria isolates inhibited the growth of the pathogen. The isolates GUR-12 (58%) and GUR-1 (51%) produce the highest inhibition of mycelia growth while other isolates were the least

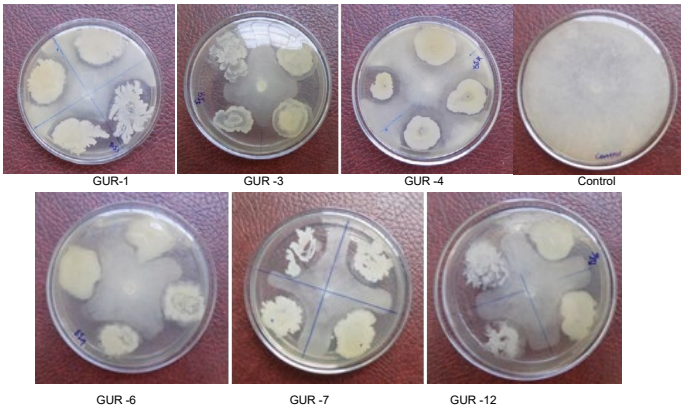



**Figure 13:** Bacterial isolates inhibition in dual culture assay.

effective isolates that inhibited below 50% inhibition when compared to the control (Figure 13).

The mean value of the inhibition efficiency of the rhizosphere bacterial isolates was 36.42% indicating that the average total efficiency was very low.

The analysis of variance of the means showed a p-value=0.000, which is less than alpha value (0.05) and hence there was a significance difference of the isolates mean values. The total mean of the isolates was 2.73 0.15 with minimum value of 1.6 and maximum value of 3.8. (Figure 14).

## Hydrolytic Enzyme production in rhizosphere bacteria

The rhizosphere bacterial isolates tested showed variation only in the production cellulase enzymes production (Figure 15). All the rhizosphere bacterial isolates 6 (100%) were positive for amylase, protease and chitinase production and 4 (66.66%) isolates were positive for cellulase production. More than half of the isolates were positive in all hydrolytic enzyme production tests even though there were 2 isolates that showed low level of performance in cellulose enzyme production. 4 (66.66%) of the isolates produced all the tested hydrolytic enzymes.

## Ammonia and hydrogen cyanide production

The isolates potential to produce ammonia and hydrogen cyanide as bioactive compounds showed no variation (Figure 16). All the isolates 6 (100%) were positive for production of ammonia and hydrogen cyanide [48].

## Biochemical characteristics of rhizosphere bacterial isolates

The isolates were biochemically characterized for some tests (Figure 17). Among the isolates 4(66.66%) and 5(83.33%) were positive for catalase and oxidase production, respectively. On the other hand, all of the 6(100%) isolates utilized starch.

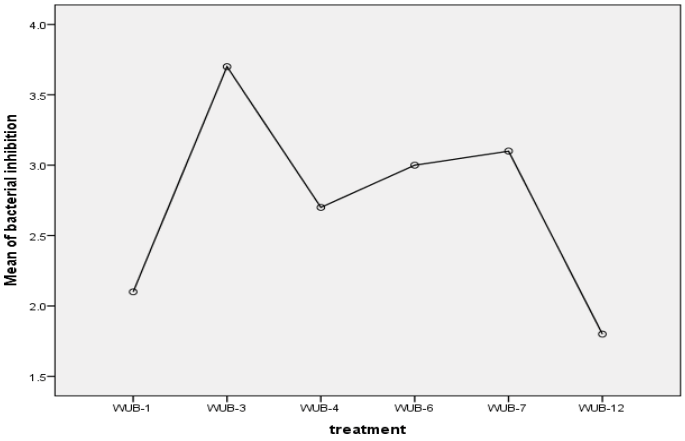



**Figure 14:** Means plot for each individual bacterial isolate.

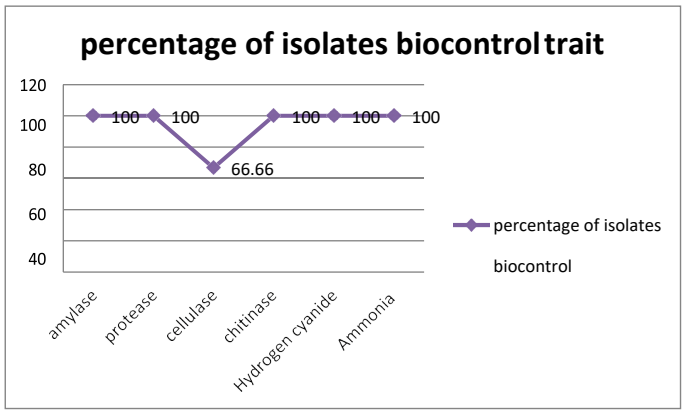



**Figure 15:** Bio control characterization of rhizosphere bacterial isolates.

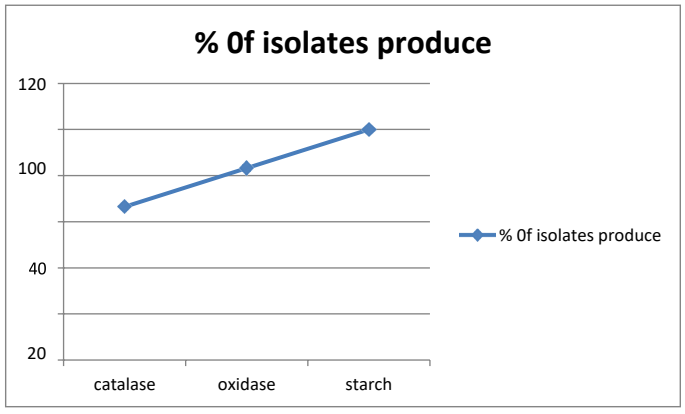



**Figure 16:** Biochemical test efficiency of rhizosphere bacteria isolates.

## Carbohydrate utilization of rhizosphere bacterial isolates

All the rhizosphere bacterial isolates were able to catabolized variety of carbon sources. All carbohydrates were utilized by the isolates at a range of 77.7-100% and only 1 (16.6 %) of the rhizosphere bacterial isolate utilized all the tested carbohydrates. The remaining isolates utilized at a range of 77.7-88.8% indicating the slight variability in utilizing carbohydrate sources. Glucose, fructose, sucrose, maltose and starch were utilized by all 6 (100%) of the isolates, galactose was consumed by 5 (83.3%0 isolates and the remaining lactose, dextrose and cellulose were metabolized by 4 (66.6%) isolates.

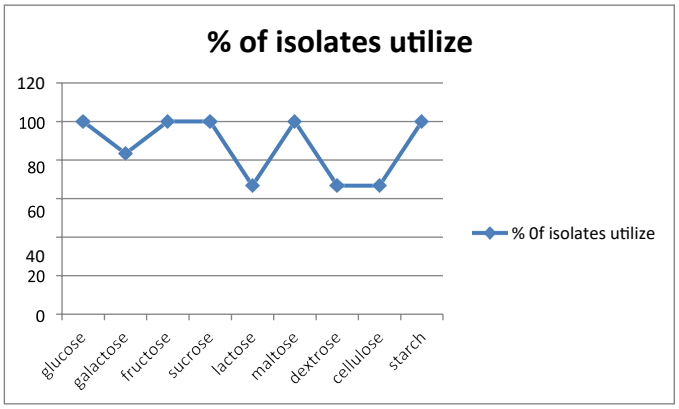



**Figure 17:** Carbohydrate utilization of rhizosphere bacteria isolates.

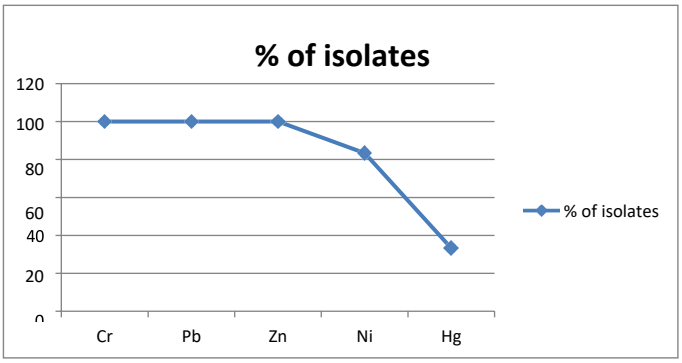



**Figure 18:** heavy metal tolerance of rhizosphere isolates.

## Amino acid utilization of rhizosphere bacterial isolates

Rhizosphere bacterial isolates were able to utilize different amino acid substrate as source of nitrogen. All 6 (100%) Isolates utilized all the given amino acid sources.

## Physiological stress tolerance of rhizosphere bacterial isolates

### pH tolerance

Regarding pH tolerance, all the rhizosphere bacterial isolates displayed maximum growth at nearly neutral pH ranging from 6-8. 2 (33.3%) of the isolates GUR-3 and 7 grew at the pH range of 4-10 were the most tolerant isolates both in acidic and basic medium from Gondar Zuria and Lay Armacheho districts collection site, respectively. Isolates, GUR-4 and 6 from Gondar Zuria and Lay Armacheho districts also showed moderate tolerances at the pH range of 4-9.

### Temperature tolerance

All the isolates showed best growth at the temperature range of 20oC-40°C, there were no growth of isolate at the temperatures greater than 40°C and less than 10. GUR-3 and GUR-7 isolates from Gondar Zuria and Lay Armacheho districts grows at the temperature range of 20 - 40°C, where the remaining 4 (66.6%) isolates grew at the temperature range of 10-40°C.

### Salinity tolerance

Concerning to salt tolerance, the rhizosphere bacterial isolates showed variation in their growth on the growth medium containing different concentration of salt (NaCl). All rhizosphere bacterial isolates

displayed growth at the range of 1-4% salt concentration and isolates growth declined when as the concentration of salt increased from 4% to 7%. All the isolates grew in the salinity concentration range from 1-7%. No growth recorded at 8% salt (NaCl) concentration.

### Heavy metal tolerance

The rhizosphere bacterial isolates showed slight differences in their tolerance to the tested heavy metals (Figure 18). All (100%) of the isolates were found to be tolerant to chromium, lead and zinc whereas, 5 (83.3%) and 2 (33.3%) showed growth on the medium containing nickel and mercury, respectively [49].

### Antibiotics tolerance

Among the rhizosphere bacterial isolates there was no variation in antibiotics tolerance test. All the 6 (100%) isolates were tolerant to (µg ml-1) conc. (30) erythromycin, conc. (20), tetracycline, conc. (20) neomycin and conc. (40) chloramphenicol.

## Discussion

The present study showed the antifungal activity of Trichoderma and rhizosphere bacteria against the pathogen Sclerotium cepivorum. Through the dual culture technique, it was observed that all 9 trichodermal and 6 rhizosphere bacterial isolates had varying level of inhibition on the pathogen mycelial growth. As reported by Harrison and Stewart (1988), 6 isolates of Trichoderma were seen to colonize the surface of Sclerotium cepivorum, similarly among the 9 isolates of trichoderma 3 isolates colonize the entire culture medium surface including the pathogen mycelium. These trichodermal antagonists out grew the pathogen in the Petridis, this can imply as Cicero et al. (2016) hyperparasitism is an action mechanism of these isolates against Sclerotium cepivorum. The result might be a confirmation on usefulness of evaluating different trichodermal isolates antagonistic activity for their mycoparasitic activity and preparation of bio pesticides.

The trichoderma isolates exhibited their antagonism by successful competition with the pathogen at mycelial growth efficiency range of 40 to 90%. This result is in accordance with previous reports on trichoderma isolates inhibition efficiency on Sclerotium cepivorum mycelial growth at a range of 44.5 to 93.3% by Harrison and Stewart (1988), and 53.27 to 82.7% by (Ibarra et al., 2010). Halabial and Kalaivani (2014) also reported the trichoderma inhibition efficiency against M grisea at range of 80 to 100%. Similarly, Hussain et al. (2017) reported all the bio agents assessed showed fungistatic action and significantly inhibited the mycelia growth of Sclerotium cepivorum Berk. The present study indicated that the highest percentage of mycelia growth inhibition was 90% followed by 88.57% and 85.71% which was similarly reported 100% followed by 64.58% and 63.54% (Hussain et al., 2017).

According to Ibarra et al. (2010), antagonists with over 70% inhibition of pathogen mycelia growth considered as effective antagonists. Based on this among the 9 trichodermal isolates 3 (33.3%) of the isolates were considered as effective antagonists. Sagarika et al. (2017) describe the Trichoderma antagonists with inhibition efficiency higher than 40% as a better biological control agent. According to this description the 9 trichodermal isolates indicates higher inhibition (>40%) efficiency, therefore all the isolates can be considered as a better biological control agent.

The rhizosphere bacteria isolates were inhibited the growth of Sclerotium cepivorum mycelia at arrange between 13.95 and 58.13%. As compared with the report by Cicero et al. (2016) in which the 8 bacterial isolates inhibition efficiency range between 42 and 50.2%, 2 (33.4%) and 4 (66.6%) of isolates were seen with better and lower inhibition efficiency than the report, respectively. The rhizosphere bacterial isolate GUR-12 was observed higher inhibition efficiency of 58.13% on Sclerotium cepivorum. This result was nearly similar with the higher inhibition efficiency of B. subtilis (60%) against M. grisea rice fungal pathogen (Hamdial and Kalaivani, 2014).

As reported by Brimner and Boland (2003), antagonists have the ability to penetrate into the hyphae cell wall of the fungi which is attributed to the production of enzymes that catalyze the breakdown of the fungal cell wall. Therefore, in order to determine enzyme activity of the antagonists', isolates were grown on the medium containing respective nutrients as a source of energy. Among the 9 trichodermal isolates only 2 (22.3%) and 7 (77.7%) isolates gives positive result for amylase and protease activity, however, Cimkem (2009) reported only 2 and 3 isolates gives positive result for amylase and protease activity out of 7 Trichodermal isolates, respectively. In contrary to the same author, all the 7 trichoderma isolates utilize cellulose, all the 9 trichodermal isolates were not grown on cellulose containing medium [50].

Several reports have indicated biocontrol efficiency of Trichoderma may differ in regions due to various agro climatic conditions (Harrison and Stewart, 1998). All of the isolates were subjected to physiological tolerance tests to determine their biocontrol ability at different degree of stresses. Growth increment of the isolates from 15 to 28±2 0C was in parallel with the report by Anuradha et al. (2014) trichoderma isolates growth rate were seen increased when the temperature increases (20 to 300C). Similar to the present study findings were also reported that none of Trichoderma sp grew at or above 400C (Sharma et al., 2005). The influence of pH on mycelial growth of Trichoderma clearly indicates acidic pH is preferable than basic pH. This growth of Trichoderma better in acidic condition is supported by the studies of Limon et al (2004), acidic pH favored fungal growth than alkaline. The growth of isolates on wide range of physiological stresses made them efficient antagonists for biocontrol of the pathogen [51].

## Conclusion and Recommendation

### Conclusion

This mycoparasitic efficiency indicates the efficiency of *Trichoderma* biological control ability over the pathogen. This research shows that 3 out of 9 *Trichoderma* isolates tested in vitro can be considered as effective antagonists against Sclerotium cepivorum based on their colonization percentage that exceeds 70%. An attempt has been made to grow different species at varying pH temperature and salinity in order to reveal all the relevant parameters. These isolates are promising antagonists and will be included in more comprehensive future research of their antagonistic effect against Sclerotium cepivorum. Therefore, in the study area, this preliminary study can serve as a primary step in developing a biocontrol against the white rot pathogen to control the devastative disease which causes high yield loss in garlic production. The precise benefits and consequences of the present findings open several avenues for future research in the field of biocontrol and bio pesticide production.

### Recommendation

Based on the findings of this present study, the presence of white rot pathogen is confirmed in the study area. Therefore, spread of Sclerotium cepivorum Berk in the garlic fields should be controlled using management strategies to reduce destructive damage of the disease. Therefore, the following recommendations are given:

- Focus should be given on avoidance by not introducing the pathogen into afield through the movement of soil, materials and garlic cloves.
- Planting only clean stock of garlic from known origins that have no history of white rot
- Cultural practices such as long-term crop rotation schedule to reduce inoculum and disease distribution and sanitation is effective control
- Isolates GUT-6, 3 and 5 showed higher inhibition efficiency (>70%) against the mycelial growth of Sclerotium cepivorum and with good physiological tolerance are recommended to be a candidate for green house investigation
- Similar study needs to be conducted to evaluate the efficiency of the isolates at greenhouse level.
- Extensive research efforts are required to improve management of white rot.

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