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Biocontrol Potential of *Trichoderma* sp. and Rhizosphere Bacteria from Infected Garlic (*Allium sativum* L.): A Promising Study

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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.*

I. INTRODUCTION

According to Mitra *et al.* (2018), plant fungal diseases are a major concern in agriculture and food production globally. Developing countries experience approximately 50% crop losses due to plant diseases, with fungal diseases accounting for one third of these damages. Bastakoti*et al.* (2017) stated that managing plant fungal diseases incurs significant costs in crop production. However, the environmental impact and food residue concerns associated with fungicides have led to the deregistration of several products. As a result, there has been a growing interest in the biological control of plant diseases, aiming to develop economically safe, long-lasting, and effective methods (Philip *et al.*, 2017). The utilization of biocontrol agents has proven to be both eco-friendly and efficient against various plant pathogens (Chemeda Dilbo *et al.*, 2015), particularly species of the *Trichoderma* genus (Naher *et al.*, 2014; Philip *et al.*, 2017).

Regarding garlic, it is a widely cultivated *Allium* vegetable crop of high economic value that necessitates proper production and management practices (Diriba Shiferaw, 2016). Despite its significance and increased cultivation, garlic faces challenges from biotic and abiotic stresses, which impact its yield and quality (Jaleel *et al.*, 2007; Cheruth*et al.*, 2008). Among the 66 diseases attacking *Allium* crops worldwide, white rot disease caused by *Sclerotium cepivorum*Berk is particularly destructive, resulting in substantial losses in onion and garlic production (Coley-Smith, 1987). Consequently, researchers worldwide have been exploring potential microorganisms for the biological control of white rot, aiming to expand the arsenal of disease control tools and reduce reliance on fungicides (Clarkson *et al.*, 2002; Stewart and McLean, 2007).

Biological control involves the use of a Biocontrol Agent (BCA), typically a fungus, bacterium, or virus, or a combination thereof, to suppress diseases in plants or soil. BCAs offer the advantage of high specificity towards pathogens, minimizing harm to non-target species. Numerous effective BCAs for fungal and bacterial diseases in crops have been identified in recent years, with some already in commercial production (Philip *et al.*, 2017). The use of BCAs has gained significant attention due to their ability to suppress various plant diseases and the potential for integration with other control methods (Mitra *et al.*, 2018). Additionally, there is a growing demand for safer, alternative, and effective control agents (Hussain *et al.*, 2017).

Allium crops, such as onion (*Alliumcepa* L.) and garlic (*Allium sativum* L.), are essential vegetable crops widely used as condiments in Ethiopian cuisine. These crops belong to the Alliaceae family and are considered the most significant vegetables and spice crops cultivated on a large

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scale in Ethiopia, both during the dry and rainy seasons (Rubatzky and Yamaguchi, 1997; Mohammed *et al.*, 2014). Garlic production in Ethiopia has shown significant growth, expanding from 6,042 hectares in 2001/02 to 21,258 hectares in 2012/13. This increase resulted in a total production increment from 79,421 tons to 222,548 tons of bulbs. However, despite the growth in production, the productivity of onion and garlic has been hindered by various biotic and abiotic stresses, leading to low yields in Ethiopia (Mohammed *et al.*, 2014).

One of the primary limiting factors for onion and garlic production in Ethiopia is the white rot disease, which causes the breaking of floral stalks and subsequently reduces bulb yield and seed production (Mohammed *et al.*, 2014). Extensive research efforts have been made to understand the distribution of white rot disease in different regions of Ethiopia. Mohammed *et al.* (2014) reviewed the distribution, incidence, and prevalence of garlic white rot in major growing districts of Southeast 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). However, the presence and distribution of white rot in the eastern part of the Amhara region have not yet been assessed.

The use of *Trichoderma* as a biological control agent for white rot disease not only provides a safe solution for farmers and consumers but also contributes to environmental sustainability. Therefore, the present study aims to fill the knowledge gap by assessing the distribution of white rot in the study area and exploring the potential of isolating, characterizing, and in vitro testing the most effective *Trichoderma* species for controlling white rot disease in garlic.

II. MATERIAL AND METHODS

A. Description of the Study Area

This study was conducted at the Microbiology laboratory of the University of Gondar. The research samples were collected from three districts in the Central Gondar Zone: Lay Armacheho, Denbia, and Gondar Zuria.

Lay Armacheho district, a part of the Central Gondar Administrative Zone, spans an area of 129,272 hectares. The district's altitude ranges from 980 to 2820 meters above sea level, and it experiences an average annual rainfall of 1223mm-1700mm. The annual maximum and minimum temperatures in the district are 38°C and 10°C, respectively. The district's agro-ecology is comprised of highland (7%), midland (65%), and lowland (32%) areas, with claylome (25%), vertisoli (8%), and red brown (45%) being the prevalent soil types. Lay Armacheho is bordered by Tachi Armacheho to the north, Gondar town to the south, Chilga to the west, and Wogera districts to the east. The major crops grown in the district include teff, wheat, finger millet, and maize (LDAO, 2015).

Denbia district, also located in the Central Gondar Administrative Zone, covers an area of 148,968 hectares. The district's altitude ranges from 1750 to 2100 meters above sea level. Denbia is bordered by Lake Tana to the south, Takusa to the southwest, Chilga to the west, Lay Armacheho to the north, and Gondar Zuria district to the east. The administrative town of Kolla Diba is situated 35 km away from Gondar town. The district comprises four small urban centers, including Kolla Diba, Aynba, Chuahet, and Robet towns, along with rural Kebeles and peasant associations. The district's topography consists of plains (87%), mountains (8%), plateaus (2.8%), and water-covered areas (2.2%), with clay (65%) and clay lome (35%) being the predominant soil types. The district experiences summer rainfall, with a mean annual rainfall of 1600mm and a mean annual temperature of 20°C. Approximately 64% of the district's land is arable or cultivable (49118 ha), with an additional 25% under irrigation, 6% used as pasture, 4% designated as forest or shrub land, and the remaining 1% considered degraded or other. This district is located adjacent to Lake Tana, covering an area of about 287 square kilometers, which is prone to regular and extensive flooding. The major crops cultivated in Denbia district, in order of importance, are teff, sorghum, finger millet, and maize (DDAO, 2015).

Gondar Zuria district, another district within the Central Gondar Administrative Zone, spans an area of 114,983 hectares. The district's altitude ranges from 1107 to 3022 meters above sea level, and it receives an average annual rainfall between 950mm-1035mm. The maximum and minimum annual temperatures in the Woreda are 33°C and 27°C, respectively. The administrative town of Maksegnete is located 42 km away from Gondar town. The district comprises four small urban centers, including Maksegnete, Teda, Enfranze, and Degoma towns, along with rural Kebeles and peasant associations. Agriculture is the primary source of income for the farmers in the area. The major crops grown in the district include teff, maize, sorghum, and barley (Mulugeta &Achenef, 2015).

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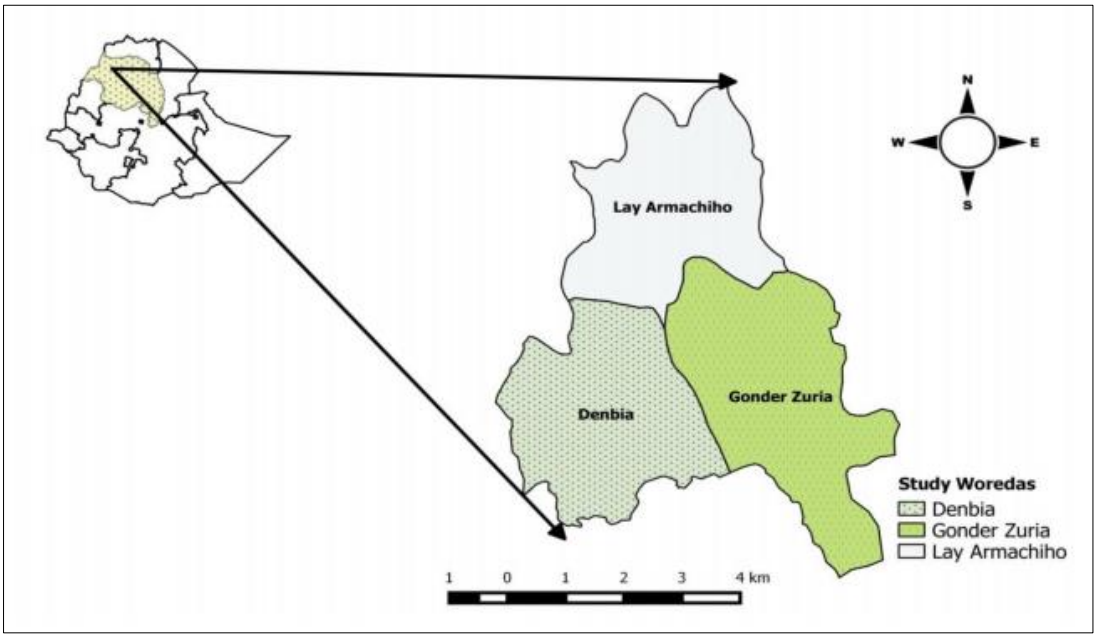

Fig. 1: Map of the study area (using GIS version 9.2 software)

B. Sample Collection

Samples for the isolation of antagonists and pathogens were obtained from soil and garlic plants in the garlic cultivation areas of three districts in Central Gondar (Denbia, Lay Armacheho, and Gondar Zuria). Soil samples were collected from garlic-grown soil, while infected garlic plants were used to isolate the pathogen. The collection process involved carefully gathering the samples and placing them in separate clean polyethylene bags (Somasagaren and Hoben, 1984).

C. Isolation of the Pathogenic Agent (Sclerotiumcepivorum)

To isolate the fungal pathogens from the diseased garlic plants, the symptomatic parts of the stem and bulbs were carefully cut. These parts were then surface sterilized using a 4% sodium hypochlorite solution for 5-10 minutes, followed by rinsing with sterile distilled water as described by Krishna *et al.* (2016). The sterilized samples were placed on potato dextrose agar (PDA) plates, which were prepared using the following ingredients per liter: potato infusion (200 g), dextrose (20 g), agar (15 g), with a pH of 5.6±0.2, as mentioned by Nikan *et al.* (2018). The plates were then incubated at 28 ± 2°C for a period of 3-5 days.

The fungal pathogens that grew on the media were purified using the repeated hyphal tip culturing technique, following the method described by Elsheshtawi*et al.* (2014). The purified isolates were identified using standard identification keys. Colonies on PDA were described as white, typically with a relatively even layer of aerial mycelium, dichotomously branched hyphal tips, black spherical sclerotia, and the mycelium appeared as septate white hyphae, according to Barnett and Hunter (1998).The purified pathogenic agents were stored in a refrigerator at 4°C for further diagnostic tests.

➢ *Confirmation Test*

- *Preparations of Pathogeninoculum*

To obtain a fungal mass of the infected garlic isolate *Sclerotium cepivorum*for soil infestation in laboratory experiments, the isolate was grown on a sand-barley medium following the method outlined by Abd El-Moity (1976).

The medium was prepared by combining 50 g of wheat grains, 50 g of sand, and 40 ml of water. The mixture was then placed in glass bottles with a capacity of 500 ml and sealed with cotton plugs. The bottles containing the medium were sterilized at a temperature of 121 °C for a duration of 30 minutes using an autoclave.

After sterilization, a 5 mm mycelial disk of *Sclerotium cepivorum*was inoculated onto the autoclaved medium. The inoculated bottles were then incubated at a temperature of 18±2 °C for a period of 5 weeks, following the incubation conditions described by Elshahawy*et al.* (2019).

- *Pathogenicity Test*

The trial took place in greenhouses where pots were used. Each pot contained 3 kg of soil. Prior to planting, the soil was contaminated with *Sclerotium cepivorum*at a rate of 2% by weight, two weeks in advance. The garlic cloves were then treated with 70% ethanol for five minutes to ensure surface sterilization, followed by three rinses with sterilized distilled water. The seeds were planted in sterilized soil that was already infected with the pathogen, and this was done in 10-cm-diameter pots.

The pots designated as controls were not infected with the pathogen, as stated in the studies by Zewide*et al.* (2007) and Elshahawy*et al.* (2019). In order to confirm the involvement of the isolated fungus in the occurrence of the disease and the appearance of symptoms, a pathogenicity test, known as the Koch postulate, was conducted. The

observed symptoms included yellowing and wilting of the leaves, the presence of snow-white mycelia on the surface of the bulbs, the formation of small rounded sclerotia, and damage to the root system in the newly grown garlic plants. These symptoms were recorded, and the pathogen was subsequently re-isolated to confirm its presence, as described by Mahdizadehnaraghi*et al.* (2015).

D. Isolation of Bio Control Agent, Trichodermaspecies and other Rhizosphere Bacteria

➢ *Isolation and Purification of Trichoderma*

To isolate *Trichoderma* species, we followed the serial dilution technique. From each solution, 1 ml was taken from dilutions ranging from 10-4 to 10-7, and then inoculated onto Potato Dextrose Agar (PDA) plates. The composition of PDA is as follows: Potato infusion (200 g/L), Dextrose (20 g/L), Agar (15 g/L), and the pH was adjusted to 5.6±0.2 (Rifai, 1969; Watanabe, 1994). The plates were incubated at a temperature of 28 ± 2°C for a period of 3-5 days. Daily observations were made on the culture plates, and based on the distinct colony morphology, individual colonies were isolated onto new plates for purification. The identification of *Trichoderma* spp. growth was carried out using the methods described by Barnett and Hunter (1998), and the identification was further confirmed using a taxonomic key specific to the genus *Trichoderma*. This involved examining the mode of mycelial growth, color changes in the medium, secretion of yellow pigment into the agar, and the presence of loosely or compactly tufted mycelia and distinct concentric rings for each isolate (Rifai, 1969; Watanabe, 1994). All the isolated strains were stored on PDA slants at a temperature of 4°C for short-term storage, and to prevent any unwanted contaminants, the PDA was supplemented with 100 µg/mL chloramphenicol (Ramazan *et al.*, 2015).

➢ *Isolation of Rhizosphere Bacteria and Purification*

Rhizosphere bacteria were isolated from the rhizosphere soil of healthy garlic plants collected from three districts in North Gondar. The serial dilution technique was used for isolation. Approximately 1 gram of rhizosphere soil was homogenized with 9 mL of sterilized distilled water and shaken for 30 minutes. The resulting suspensions were then serially diluted until a dilution of 10-5 was achieved using sterile water, following the method described by Han *et al.* (2015). Next, 0.1 mL of the diluted soil suspension was plated onto respective medium plates containing nutrient agar medium (NA) with the following composition: Peptone (5 g/L), Sodium chloride (5 g/L), Yeast extract (1.5 g/L), Beef extract (1.5 g/L), and Agar (20 g/L). To suppress fungal growth, the medium was supplemented with 100 µg/mL of cycloheximide, as described by Somasagaren and Hobben (1994). The plates were then incubated at a temperature of 28 ± 2°C for a period of 3-5 days. Based on the color and colony morphology, single and visually distinct colonies were selected and purified through repeated re-streaking until pure isolates were obtained. The pure isolates were then incubated at 28 ± 2°C for an additional 3-5 days**.**

E. 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).

➢ *Dual Culture Test for Antagonistic Fungal Isolates*

To assess the antagonist activities of the *Trichoderma* isolates against the pathogen, a dual culture technique was employed. Mycelia discs measuring 5mm in diameter, obtained using a crock borer, were taken from 7-day-old pure cultures of the pathogen. These discs were placed on Potato Dextrose Agar (PDA) plates, positioned 1 cm away from the edge. Similarly, mycelia discs of the same size from the *Trichoderma* isolate were placed on the opposite edge of the Petri plate. Control plates were inoculated only with the pathogen disc. All plates were then incubated at a temperature of 25 ± 2°C for a period of 7 days, as described by Krishna *et al.* (2016). The diameter of radial growth of the targeted fungal pathogens was measured after the 1st, 2nd, 3rd, and 4th days of incubation at two locations from the center of the test plate, and the average diameter was calculated. Finally, the percentage of inhibition of the average radial growth was determined by using the following formula in relation to the growth of the controls (Srijina*et al.*, 2017).

$$L = \left(C - \frac{T}{C} \right) 100$$

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*

To initiate the experiment, a loop full of the pure rhizosphere bacteria broth culture, which had been incubated for 48 hours, was equidistantly spot inoculated on the edges of Potato Dextrose Agar (PDA) plates that had been amended with 0.5% sucrose. The plates were then incubated at a temperature of 28 ± 2°C for another 48 hours, as mentioned in the study by Mussa Adal *et al.* (2018). After the 48-hour incubation period, a 5mm fungal pathogen disc was placed at the center of the plate. The control plate, on the other hand, only contained the pathogen's mycelia disc. Both the experimental and control plates were incubated at a temperature of 28 ± 2°C for 5-7 days. The presence or absence of inhibition zones was recorded. The growth diameter of the pathogen, which refers to the distance between the point of placement of the fungal disc and the actively growing edges of the fungus, was measured using the method described by Amini *et al.* (2012). The percentage inhibition of fungal growth was then calculated by measuring the distance of the clear zone between the bacterial colony and the fungus, applying the formula outlined by Han *et al.* (2015).

Inhibition of growth (%) = ((1TD×) / CD)100

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.

F. Designation of the Antagonists

The purified *Trichoderma* isolates obtained from the study were designated as GUT (Gondar University *Trichoderma* isolates), followed by consecutive numbers representing each isolate. Similarly, the purified rhizosphere bacteria isolates were designated as GUR (Gondar University Rhizosphere bacteria isolates), also followed by consecutive numbers representing each isolate.

G. Characterization of Bacterial Antagonist

➢ *Cultural Characterization of the Rhizosphere Bacterial Isolates*

The isolates underwent examination to assess their colony and cell morphology. Various morphological characteristics, such as size, shape, margins, color, and consistency of the colony, as well as cell shape and arrangement, were analyzed following the established protocols outlined by Aneja (2003) and Cappuccino and Sherman (2010).

➢ *Gram Reaction Test*

To determine the Gram reaction type of the rhizosphere bacterial isolates, the KOH method (Buck, 1982) was employed. A clean microscope slide was used, onto which a drop of 3% KOH was placed. Rhizosphere bacterial isolates were then picked using a loop, dipped into the KOH, and thoroughly mixed for one minute. Following this, the mixtures were lifted with the inoculating loop, about 1 cm from the slide. The presence or absence of noticeable stringiness (viscosity) was recorded as an indicator of Gram-negative and Gram-positive bacteria, respectively, as referenced in (Mussa Adal *et al.*, 2018).

➢ *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).
- **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).
- **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 ± 2ºC 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 wererecorded.

➢ *Hydrolytic Enzyme Production of Antagonistic Rhizosphere Bacteria*

- **Amylase Production:** In order to assess the amylase enzyme activity of the rhizosphere bacterial cultures, a starch hydrolysis test was conducted on starch agar plates, following the method described by Sharman (2010). Pure isolated colonies were streaked onto the plates, with starch serving as the sole carbon source. After incubation at 37ºC for 24-48 hours, the plates were flooded with Gram's iodine, resulting in the formation of a deep blue colored starch-iodine complex. The presence of a clear zone in the agar, indicating degradation of starch, was recorded as the criterion for detecting and screening amylase-producing isolates, as stated by Vaidya and Rathore (2015).
- **Protease Production:** The rhizosphere bacterial isolates, having been grown for 48 hours, were subjected to a protease production test by cultivating them on Skim milk agar. The Skim milk agar was prepared by adding 10g/ml of skim milk powder and 15g/ml of agar, following the method described by Simbert and Krieg (1994). The plates were then incubated at a temperature of 28±20°C for a period of 3-5 days. The ability of the bacterial isolates to clear the skim milk suspension in the agar was considered as evidence of protease secretion. Non-inoculated plates were used as a control, as referenced in the study conducted by Chantawannakul*et al.* (2002).
- **Cellulase Production:** To assess the cellulase activity of the rhizosphere isolates, which were 48 hours old, spot inoculation was performed on Carboxymethyl cellulase (CMC) agar medium with yeast extract plates. The composition of the medium included $NaNO_3$ (2g/L), $K2PO_4$ (1g/L), $MgSO_4$ (0.5g/L), KCl (0.5g/L), CMC sodium salt (2g/L), peptone (0.2g/L), and agar (17g/L), as outlined by Kasana*et al.* (2008). Following inoculation, the plates were incubated at a temperature of 28±20°C for a period of 3-5 days. The presence of a clear zone surrounding the bacterial colonies was recorded as an indicator of cellulase production, in accordance with the findings reported by Samanta *et al.* (1989).
- **Chitinase Production:** The chitinase production potential of the rhizosphere bacteria isolates was determined by cultivating them on chitin agar medium. The composition of the medium consisted of chitin (4g/L), $MgSO_4.7H_2O$ (0.5g/L), K_2HPO_4 (0.7g/L), KH_2PO_4 (0.3g/L), $FeSO_4.7H_2O$ (0.01g/L), $MnCl_2$ (0.001g/L), NaCl (0.3g/L), yeast extract (0.2g/L), and agar (20g/L), following the method described by

Renwick *et al.* (1991). Spot inoculation of the bacterial cells (106 ml-1) was performed on the chitin medium, and the plates were incubated at a temperature of 28±20°C for 3-5 days. The formation of a clear zone around the bacterial colonies was recorded as a positive test for chitinase activity, as reported by Nisa *et al.* (2010).

➢ *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 production 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).

➢ *Ammonia Production*

Each isolate was 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*

The carbon utilization of the isolates was evaluated using a basal medium supplemented with a 10% (w/v) concentration of one of the eight carbohydrates: starch, dextrin, lactose, sucrose, galactose, maltose, and glucose. To reduce the yeast extract to 0.05 g/l, the method developed by Somasegaren and Hoben (1994) was followed. The basal medium comprised KH2PO4 (1g/l), K_2HPO_4 (1g/l), $FeCl_3.6H_2O$ (0.01g/l), $MgSO_4.7H_2O$ (0.2g/l), $CaCl_2$ (0.1g/l), and agar (15g/l), with the addition of 1g/l of mannitol as a supplement.

Similarly, the ability of the isolates to utilize different nitrogen sources was tested using the same basal medium, but this time supplemented with one of the following four amino acids at a concentration of 0.5g/l: L-alanine, L-arginine, L-asparagine, and L-glycine. This testing method was based on the work of Amarger*et al.* (1997). The plates were incubated at a temperature of 28 ± 2°C for a duration of 3-5 days, and the presence or.

H. Characterization of Fungal Antagonist

➢ *Hydrolytic Enzyme Production of Trichodermaisolates*

- **Amylase Activity:** To study the production of the amylase enzyme, the mycelial disc of a trichodema isolate was placed onto a medium containing glucose, yeast extract, peptone, agar, soluble starch, and distilled water. After being incubated at a temperature of 28 ± 2ºC for 48 hours, the plates were flooded with a solution of 1% iodine in 2% potassium iodide. The presence of a clear zone surrounding the colony indicated a positive amylase activity (Maria, 2005).
- **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±2^{0}C 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 28^{0}C 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).

- **Chitinase Production:** Chitinase production potential of *Trichoderma*isolates were determined by growing the isolates on chitin agar medium containing (g L^{-1}) chitin (4), $MgSO_4.7H_2O$ (0.5), K_2HPO_4 (0.7), KH_2PO_4 (0.3), $FeSO_4.7H_2O$ (0.01), $MnCl_2$ (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±2^{0}C for 3-5 days. The clear zone formed around colonies was recorded as a positive test for chitinase activity (Nisa *et al.*, 2010).

I. Physiological Tolerance test for Antagonists, Trichodermaand 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 intriplicates.

➢ *pH, Salinity and Temperature Tolerance Test*

The isolates were cultivated to assess their ability to grow at various pH levels (4, 5, 8, 9, 9, 10) and different concentrations of NaCl (1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%). Additionally, their resistance to different incubation temperatures (5°C, 10°C, 15°C, 35°C, 40°C, and 45°C) as mentioned in a study by Bernal and Graham (2001) and Mussa Adal et al. (2018) was determined. The incubation was carried out at a temperature of 28±2°C for a period of 3-5 days.

➢ *Antibiotic Resistance Test*

To assess the inherent antibiotic resistance (IAR) of the *Trichoderma* and rhizosphere bacterial isolates, they were cultured on solid media. The *Trichoderma* isolate was inoculated on Potato Dextrose Agar (PDA), while the rhizosphere bacteria were inoculated on Nutrient Agar (NA). These media were supplemented with antibiotics at

specific concentrations (µg/ml): Chloramphenicol (40), Erythromycin (30), Neomycin (20), and Tetracycline (30), as indicated in the study conducted by 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"sdextroseagarataconcentrationof100ppm.Thecult urewasinoculatedand following incubation the plates were observed for fungal growth. The mycelial growth was recorded (Kathiresan and Manivannan,2006).

➢ *Heavy Metal Tolerance Test for Rhizosphere Bacteria*

The resistance of the isolates to heavy metals, including Ni, Hg, Pb, Zn, and Cr, was determined using the agar dilution method. Nutrient agar plates were prepared with different soluble heavy metal salts at concentrations of 100 µg/ml. The plates were then inoculated with the isolates and incubated for a period of 3 days at room temperature. The presence of bacterial growth on the plates indicated tolerance to the respective heavy metals, and the results were recorded as per the study conducted by Kumar *et al.* (2012).

J. Data Analysis

Data from each experimental treatment were collected daily at specific time points throughout the duration of the experiment. The experiments were conducted in triplicate, and the average data obtained from each replicate were used for subsequent calculations. The results of the in vitro antagonistic efficiency test were analyzed using a one-way analysis of variance (ANOVA). To compare and contrast the experimental treatments against their respective controls, Duncan's multiple range test (DMRT) was employed. The statistical analysis was performed using SPSS ver. 20, with a significance level set at $P < 0.05$. The mean, maximum, and minimum values of the individual results were also analyzed.

III. RESULT

A. Isolation of the Pathogen

After isolating and growing the fungus from the infected garlic on PDA media, the morphological (Fig.1) and microscopic examination (Fig.2) appearance of the pathogen indicated that the fungal pathogen resembled white and were similar to the morphology of white rotpathogen.

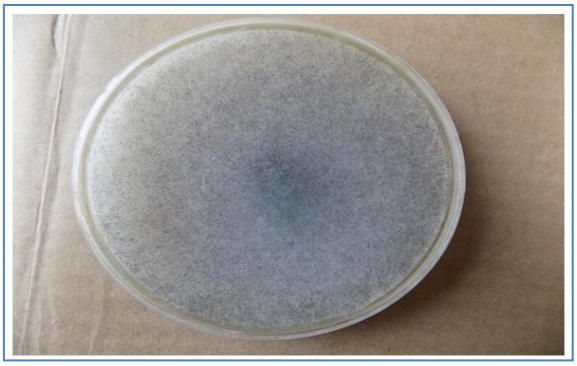

Fig. 1: The Isolated Pathogen on PDA

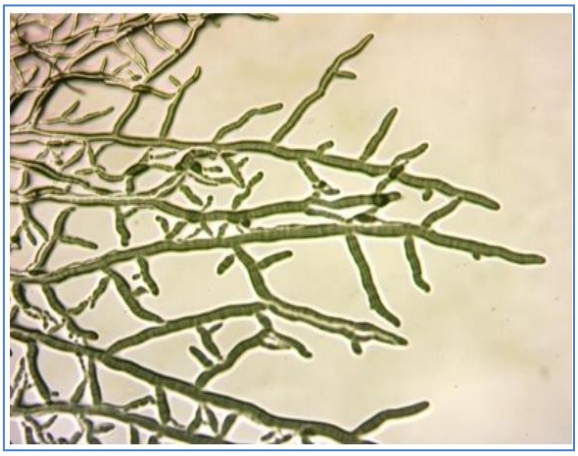

Fig. 2: Microscopic Image of White Rot Pathogen

➢ *Confirmation test of the Pathogen*

As described in Lupien*set al.* (2013) the characteristic symptoms and signs such as dying and dead leaves, cortical rot of roots, the presence of white mycelium on bulbs (Fig.3) and the presence of many small sclerotia (Fig.4) in the outer scale of basal plant confirmed white rot pathogen, *Sclerotumcepivorum*. 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 (Fig.5 and Appendix.11). The pathogen again re-isolated from the infected plant for confirmation and it was similar with the originalisolates.

Fig. 3: White Mycelia of the Pathogen

Fig. 4: Black Sclerotia of the Pathogen

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A B C

Fig. 5: Garlic Showing White Rot Symptoms (A and B) and control (C)

B. 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.

➢ *Morphological Characteristics of the Trichoderma Isolates*

Morphologically the isolates were characterized based on their colony color and mycelia growth pattern (Table.1 and Appendix.12). 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 Zuriadistricts in the same order.

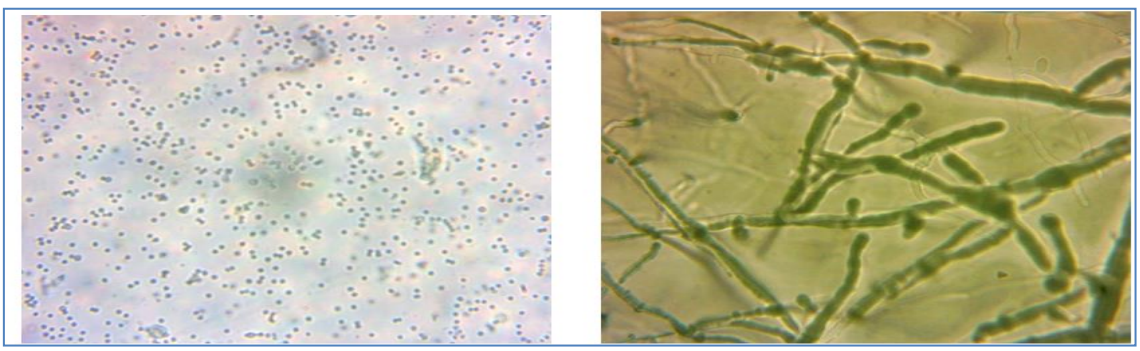
Table 1: *Trichoderma* Isolates Colony Color and Growth Pattern

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

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 (Fig.6). 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).



A-Spores B-Mycelia

Fig. 6: Microscopic Image (40x) of Spore and Mycelia of *Trichoderma*

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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 theplate.

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

➢ *Dual Culture Assay*

The antagonistic isolates showed varying degrees of inhibition efficiency, as observed in Table 3 and Figure 8. Among the 9 isolates tested in a dual culture, 5 (55.6%) isolates demonstrated a significant inhibition of the pathogen's radial growth, exceeding 50%. In contrast, 4 (44.4%) isolates showed a lesser inhibition, with radial growth being below 50%. The highest recorded antagonistic efficiency was 90% for isolate GUT-6, followed by 88.57% for GUT-3 and 85.71% for GUT-5, while the lowest inhibition efficiency was 40% for GUT-7. Overall, 55.5% of the isolates displayed a better inhibition of the radial growth of *Sclerotium cepivorum*mycelia, surpassing the 50% threshold.

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)	% ofinhibition (%)
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 Trichodermaisolate, DB= Denbia District, LA= Lay Armacheho District, GZ= Gondar Zuria District

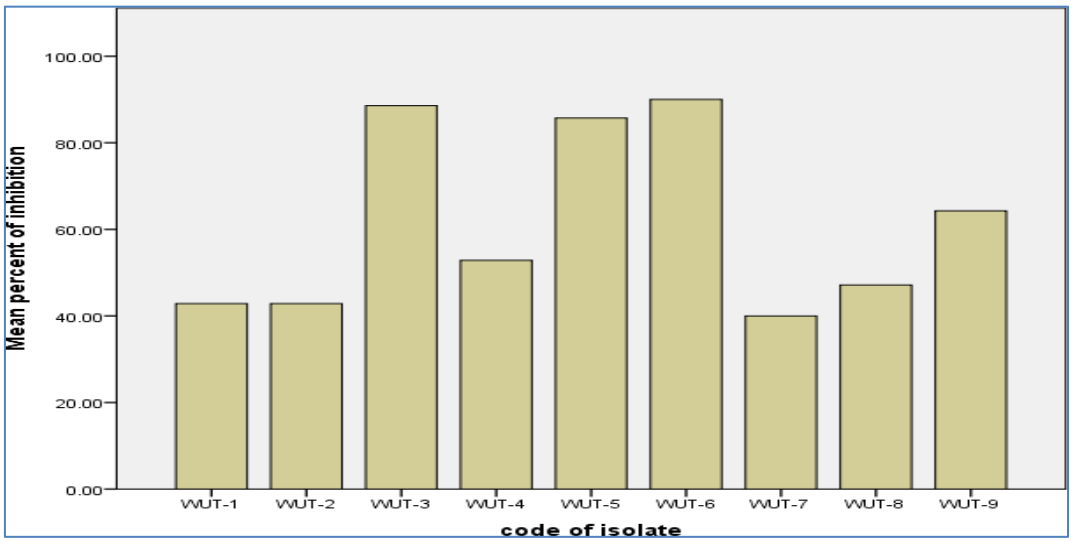

Fig. 7: The inhibition Efficiency of the Trichoderma Isolates

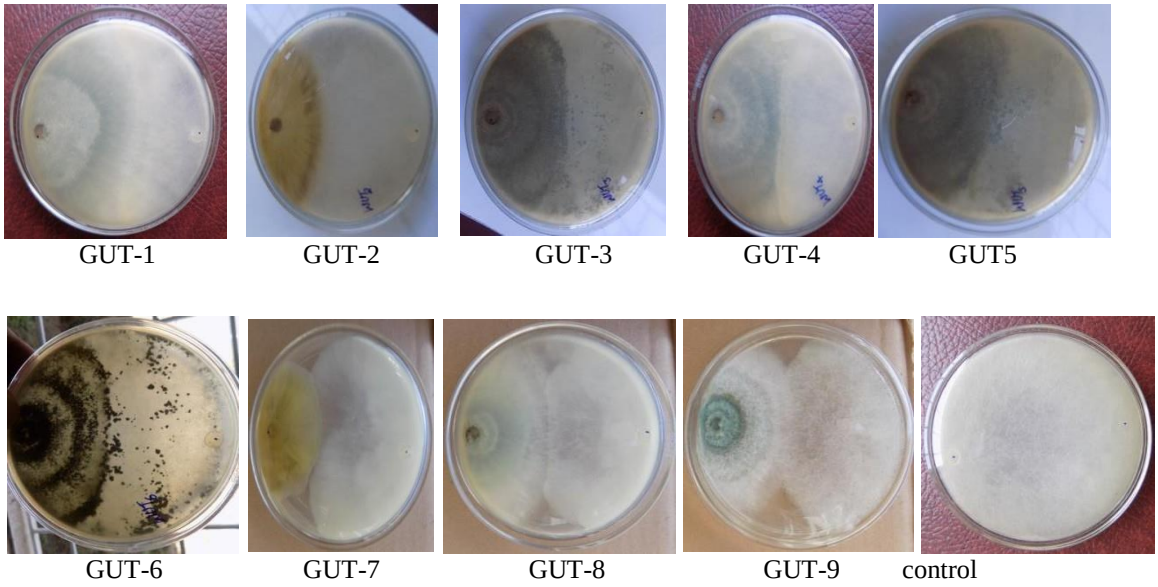

Fig. 8: Trichoderma Isolates Dual Culture Assay

Theme an 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	$3.00.05^{hgij}$	2.9	3.1
GUT-3	DB	3	$6.20.35^{bac}$	5.8	6.9
GUT-4	LA	3	$3.70.05^{ef}$	3.6	3.8
GUT-5	LA	3	$6.00.10^{cba}$	5.8	6.1
GUT-6	LA	3	$6.30.10^{abc}$	6.2	6.5
GUT-7	GZ	3	$2.80.05^{ijhg}$	2.7	2.9
GUT-8	GZ	3	$3.30.25^{ghij}$	3.0	3.8
GUT-9	GZ	3	$4.50.15^{d}$	4.2	4.7
Total		27	4.310.277	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 (Fig.9 and Appendix.1). 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.

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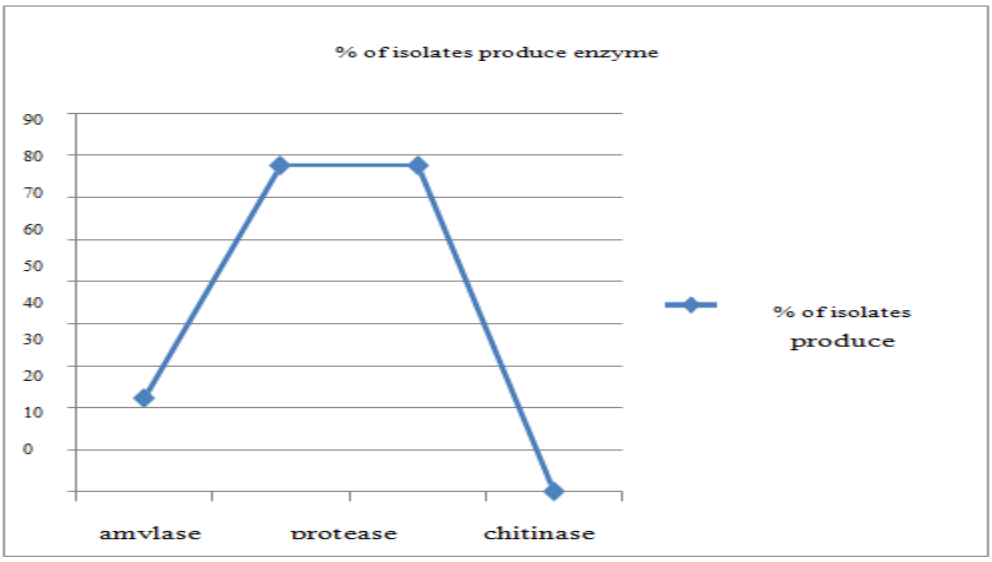

Fig. 9: Enzymatic activity of Trichoderma Isolates

➢ *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 40^0 c and below 10^0 C, whereas, increase in growth of the isolates was observed as incubation temperature increases from 15 to 28 ^{0}C. Isolates GUT-6, GUT-5 andGUT-4 exhibited the highest growth characteristic while isolates GUT-2 and GUT-7 showed the lowest growth characteristic at 28^{0}C.

- *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 (Fig.10 and appendix 2.2). 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 basicpH.

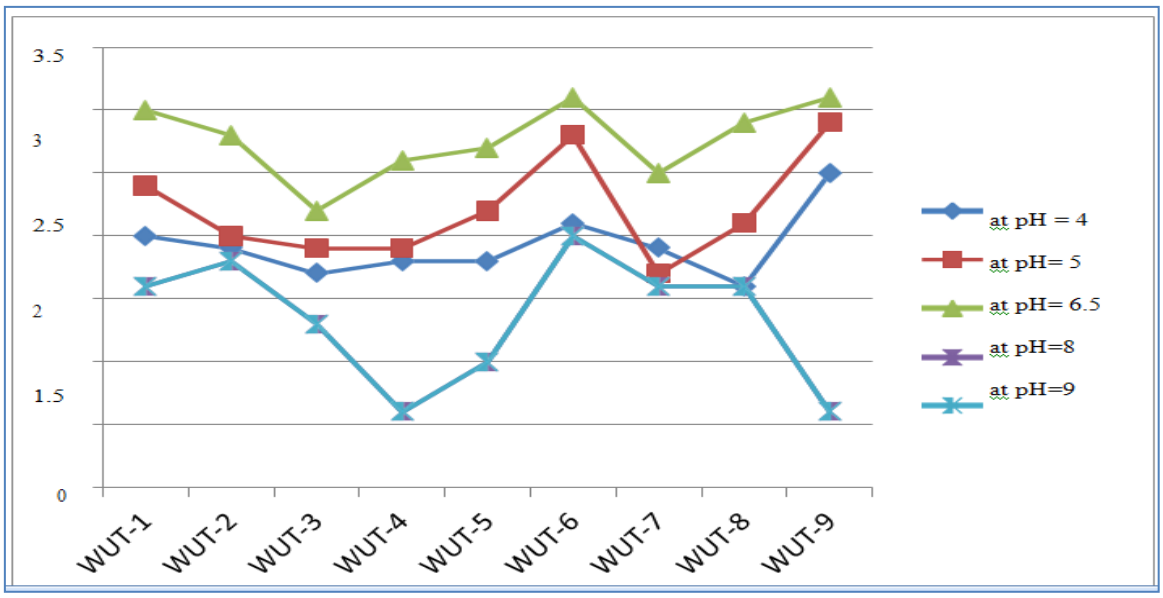

Fig.10: pH Tolerance of *Trichoderma* Isolates

- 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% (Fig.11 and appendix.2.1). 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.

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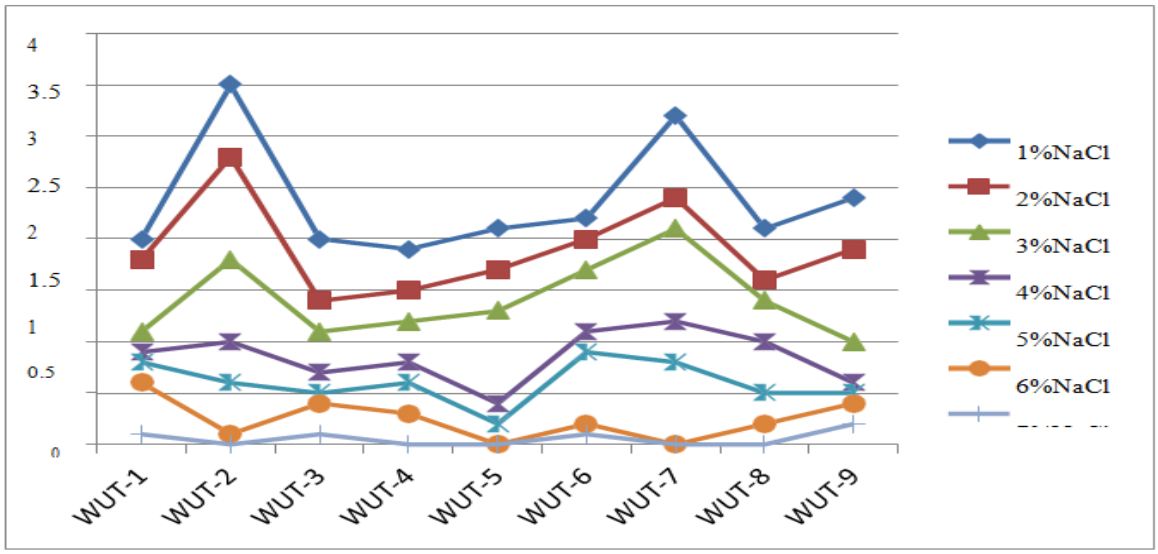

Fig. 11: Salinity Tolerance of *Trichoderma* Isolates

➢ *Antibiotics resistance of Trichoderma*

Trichoderma Isolates were tested for their resistance against some antibiotics including chloramphenicol, erythromycin, tetracycline and neomycin. All 9 (100%) *Trichoderma* isolates were grown over all the tested antibiotics (Appendix. 4).

➢ *Heavy Metal Tolerance for Trichoderma*

All *Trichoderma* Isolates 9 (100%) were showed positive for lead and Zink tolerance test (Fig.12 and appendix.3). Among the isolates 7 (77.7%), 8 (88.8%) and 2 (22.3%) isolates were resistant for nickel, mercury and chromium, respectively.

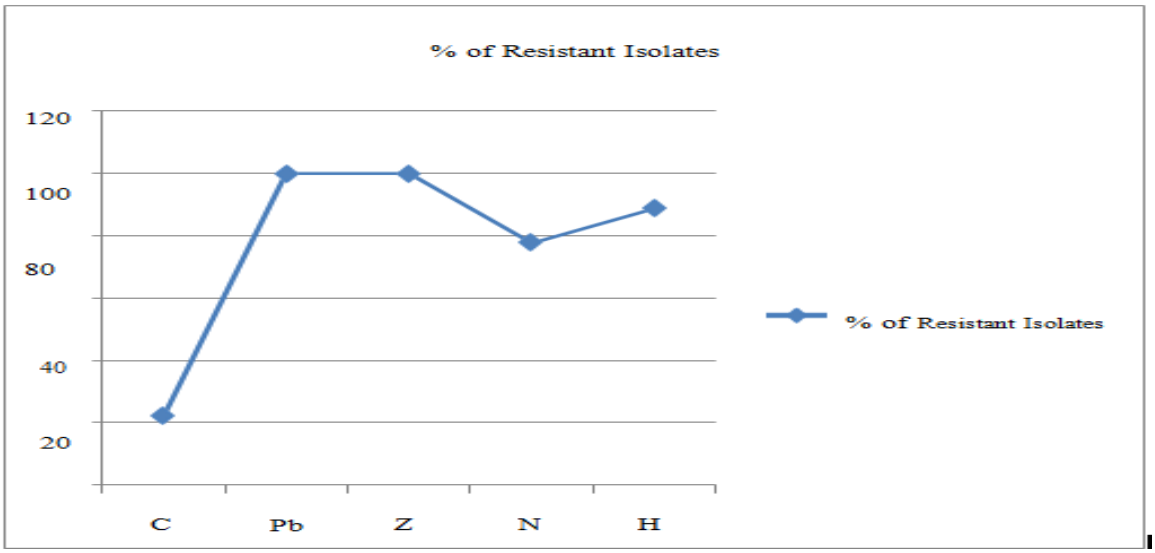

Fig. 12: Heavy Metal Resistance of *Trichoderma* Isolates

C. 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 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.

➢ *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.

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Table 5: Colony Characteristics of Bacterial Isolates

No.	Code	Isolation Site	Colony Color	Elevation	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	+	37.20
4	GUR-6	LA	White	raise	rough	Spherical	+	30.23
5	GUR-7	LA	Whitish	flat	smooth	Spiral	+	27.90
6	GUR-12	LA	White	raised	smooth	Rod	+	

GUR= Gondar University Rhisozphere bacteria, DB= Denbia District, LA= Lay ArmachehoDstrict, GZ= Gondar Zuria District, += positive

➢ *Antifungal Activity of the Rhizosphere Bacterial Isolates*

As shown in Fig. 14 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 effective isolates that inhibited below 50% inhibition when compared to the control.

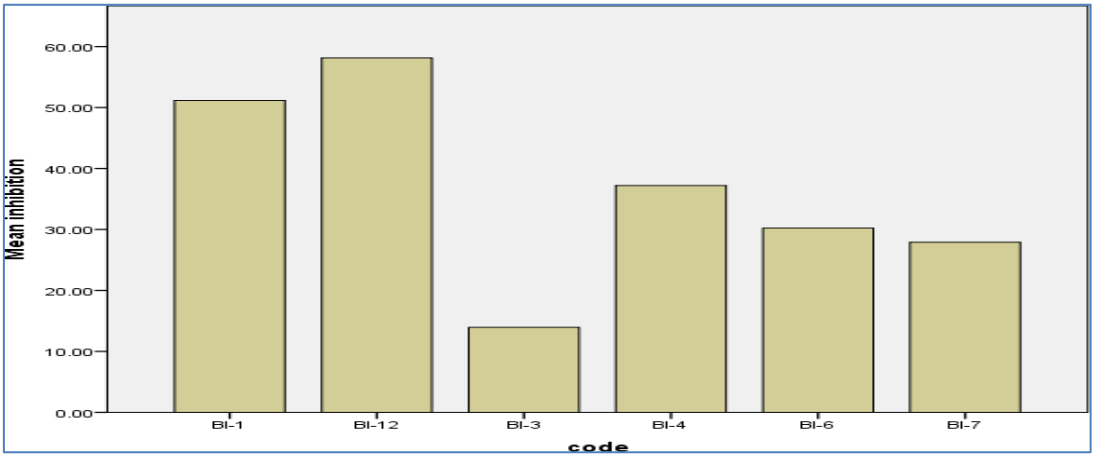
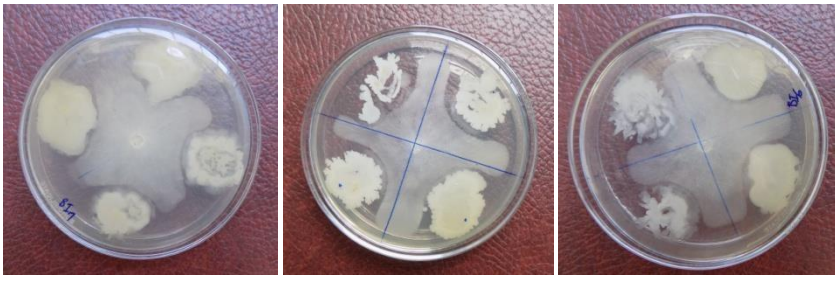


Fig. 13: The Inhibition Efficiency of Bacterial Isolates

GUR-1 GUR-3 GUR -4 Control



GUR-6 GUR -7 GUR -12

Fig. 14: Bacterial Isolates Inhibition in Dual Culture Assay

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

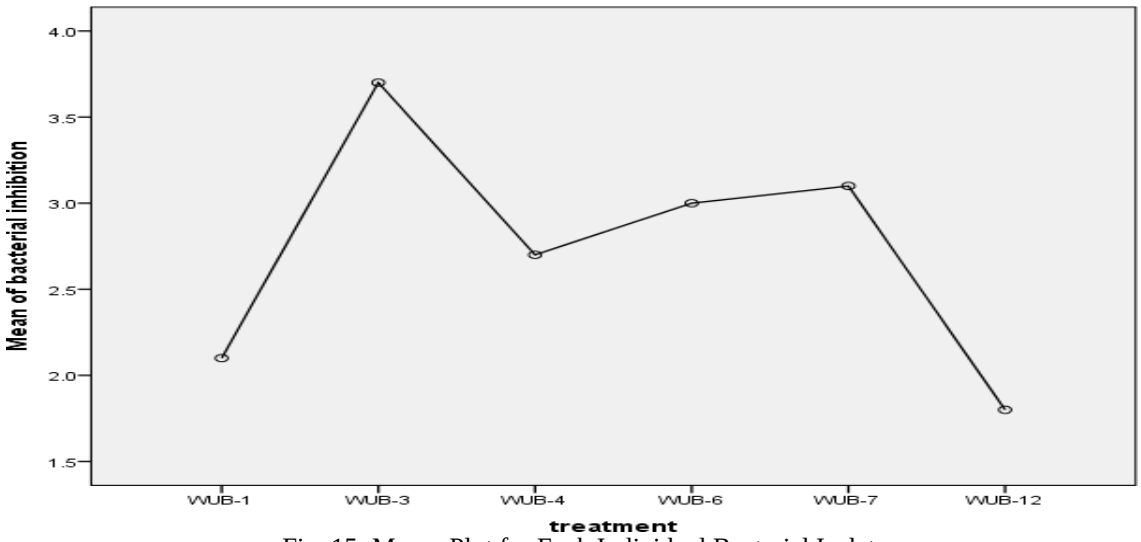


Fig. 15: Means Plot for Each Individual Bacterial Isolate

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. (Fig.15).

➢ *Hydrolytic Enzyme Production in Rhizosphere Bacteria*

The rhizosphere bacterial isolates tested showed variation only in the production cellulase enzymes production (Figure.16 and appendix.7). 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 hydrolyticenzymes.

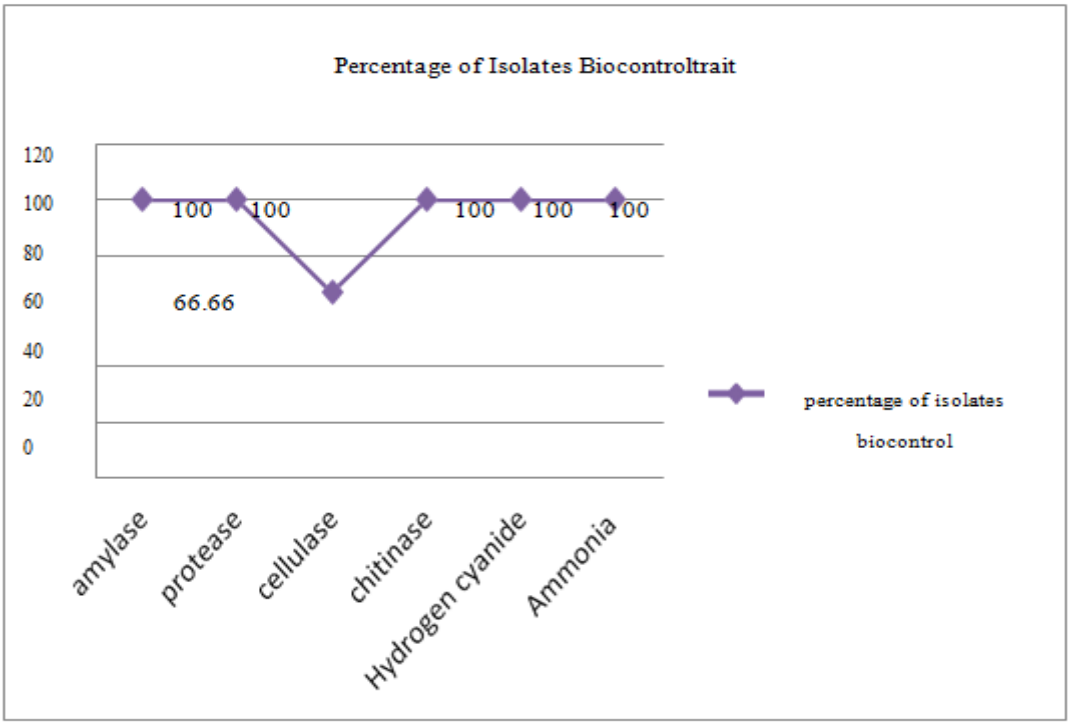


Fig. 16: Bio Control Characterization of Rhizosphere Bacterial Isolates

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➢ *Ammonia and Hydrogen Cyanide Production*

The isolates potential to produce ammonia and hydrogen cyanide as bioactive compounds showed no variation (Figure. 16 and appendix.5). All the isolates 6 (100%) were positive for production of ammonia and hydrogen cyanide.

➢ *Biochemical Characteristics of Rhizosphere Bacterial Isolates*

The isolates were biochemically characterized for some tests (Figure.17 and appendix 5). 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.

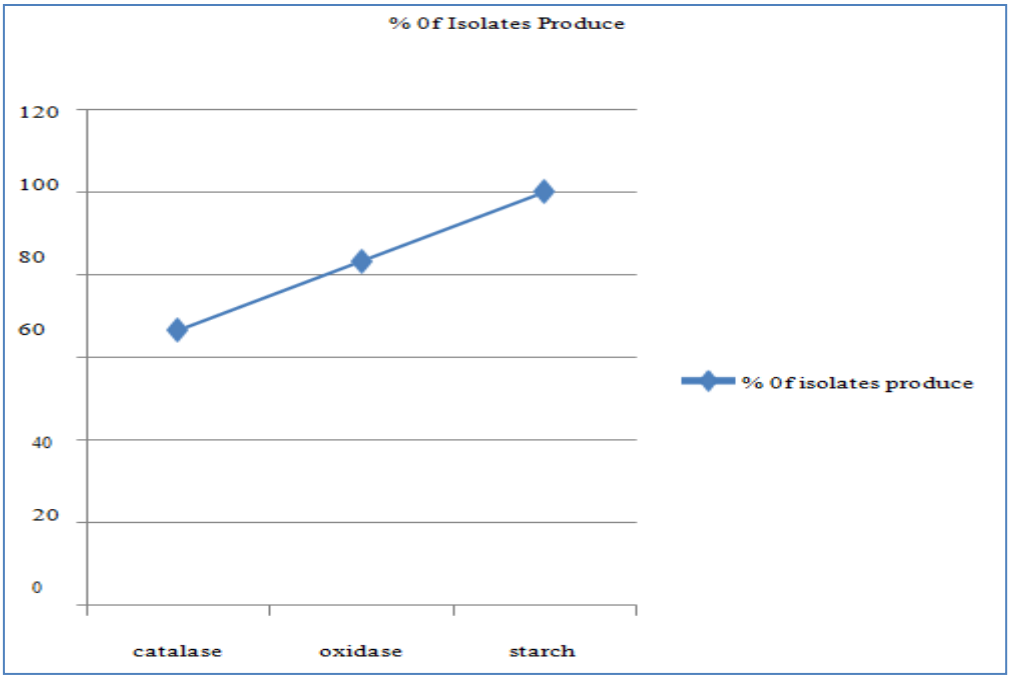

Fig. 17 Biochemical test efficiency of rhizosphere bacteria isolates

➢ *Carbohydrate Utilization of Rhizosphere bacterial Isolates*

All the rhizosphere bacterial isolates were able to catabolized variety of carbon sources (Fig.18 and appendix.6.1). 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 (Figure.18). 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.

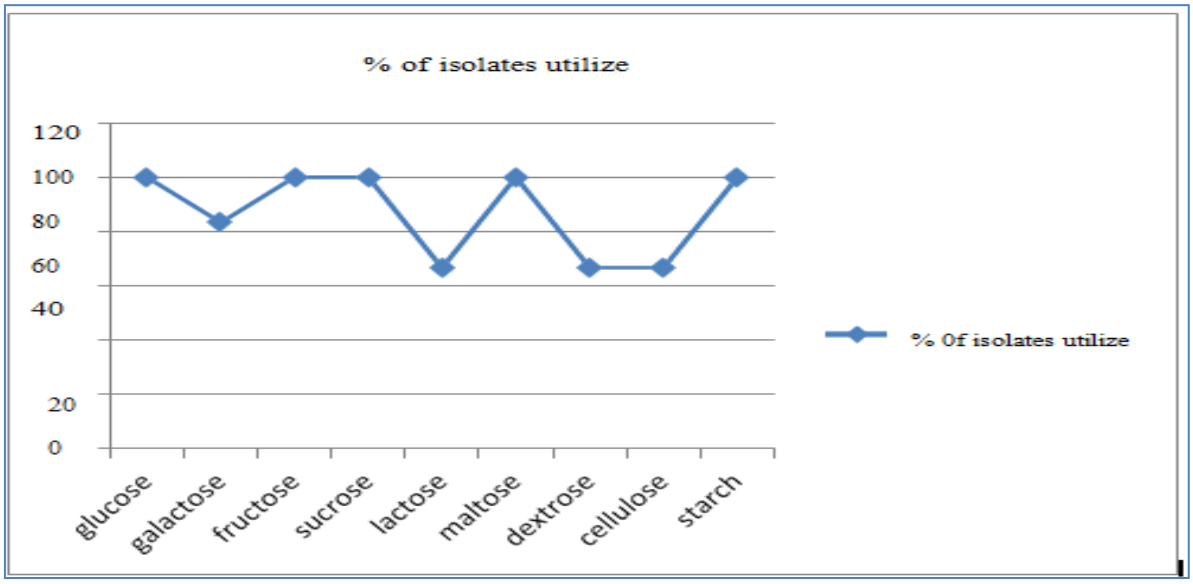

Fig.18: Carbohydrate Utilization of Rhizosphere Bacteria Isolates

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➢ *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 (Appendix.6.2).

➢ *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 (appendix.8).

- *Temperature Tolerance*

All the isolates showed best growth at the temperature range of 20ºC-40ºC, there was no growth of isolate at the temperatures greater than 40ºC and less than 10. GUR-3 and GUR-7 isolates from Gondar Zuria and Lay Armacheho districts grows at the temperature range of 20 – 40ºC, where the remaining 4 (66.6%) isolates grew at the temperature range of 10 – 40ºC. (appendix.8).

- *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(appendix.8).

- *Heavy Metal Tolerance*

The rhizosphere bacterial isolates showed slight differences in their tolerance to the tested heavy metals (Figurte.19 and appendix.9). 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.

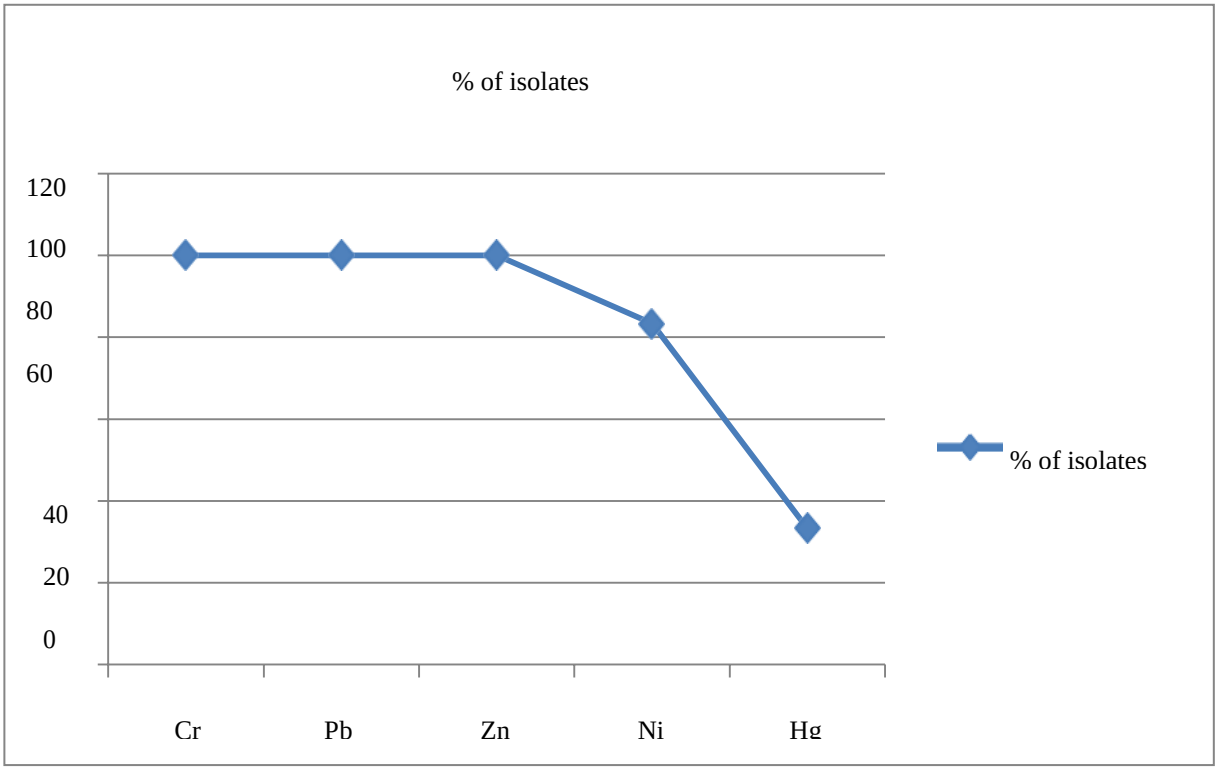

Fig. 19: Heavy metal tolerance of rhizosphere isolates

- *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 (Appendix.10).

IV. 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

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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 microparasitic activity and preparation of bio pesticides.

The antagonistic nature of the *Trichoderma* isolates was evident through their successful competition with the pathogen, resulting in a range of mycelial growth inhibition efficiency from 40% to 90%. This finding is consistent with previous reports by Harrison and Stewart (1988), who observed inhibition efficiencies of *Trichoderma* isolates on *Sclerotium cepivorum*mycelial growth ranging from 44.5% to 93.3%, and by Ibarra *et al.* (2010), who reported inhibition efficiencies ranging from 53.27% to 82.7%. In a study by Halabial and Kalaivani (2014), *Trichoderma* exhibited inhibition efficiencies against M. grisea ranging from 80% to 100%. Similarly, Hussain *et al.* (2017) found that all the assessed bioagents exhibited fungistatic action and significantly inhibited the mycelial growth of *Sclerotium cepivorum*Berk. In the present study, the highest percentage of mycelial growth inhibition was 90%, followed by 88.57% and 85.71%, which is consistent with the findings of Hussain *et al.* (2017) who reported inhibition percentages of 100%, 64.58%, and 63.54%.

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 containingmedium.

Various studies have highlighted the potential differences in the biocontrol efficiency of *Trichoderma* across different regions, which can be attributed to variations in agro-climatic conditions (Harrison and Stewart, 1998). In order to assess their biocontrol capabilities under different stress levels, all isolates underwent physiological tolerance tests. The growth increment of the isolates between temperatures of 15 to 28±2 0C aligned with previous reports by Anuradha *et al.* (2014), which indicated an increased growth rate of *Trichoderma* isolates with rising temperatures (from 20 to 300C). Consistent with our findings, Sharma *et al.* (2005) also reported that no *Trichoderma* species grew at or above 400C. Furthermore, the influence of pH on the mycelial growth of *Trichoderma* revealed a clear preference for acidic conditions over basic pH. This observation aligns with the studies of Limon *et al.* (2004), which demonstrated that acidic pH favored fungal growth compared to alkaline conditions. The ability of the isolates to thrive under a wide range of physiological stresses highlights their potential as effective antagonists for the biocontrol of the targeted pathogen.

V. CONCLUSION AND RECOMMENDATION

A. Conclusion

This microparasitic 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.

B. 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 whiterot
- Cultural practices such as long-term crop rotation schedule to reduce inoculum and disease distribution and sanitation is effectivecontrol
- 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 houseinvestigation
- Similar study needs to be conducted to evaluate the efficiency of the isolates at greenhouselevel.
- Extensive research efforts are required to improve management of whiterot.

REFERENCES

[1]. Abd El-Moity TH. 1976. Studies on the biological control of white rot disease of onion. MSc Dissertation, *Faculty of Agric Menofia University* Egypt, p 122.

[2]. Adams, P.B. and Johnston, S.A. 1983. Factors affecting efficacy of metham applied through sprinkler irrigation for control of *Allium* white rot. *Plant Dis*. 67:978-980.

[3]. Agrawal T. and Kotasthane A.S. 2012. Chitinolytic assay of indigenous *Trichoderma*isolates collected from different geographical locations of Chhattisgarh in Central India. *Springerplus*.1:73.

[4]. Ahemad, M. and Khan, M. S. 2008. Ecological assessment of biotoxicity of pesticides towards plant growth promoting activities of pea (*Pisum sativum*)-specific *Rhizobium* sp. Strain MRP1. *Emir. J. Food Agric*. 24(4): 334-343.

[5]. Alemu, H. 1998. Farming Systems in Awabel and Machakal Woredas. Constraints and possible interventions through research and extension. Amhara National Regional State/Sida Co- operation in Rural Development. Bahir-Dar, Ethiopia.

[6]. Amarger, N., Macheret, V. and Laguerre, G. 1997. *Rhizobium gallicum*sp. Nov. and *Rhizobium giardinii*sp. Nov. from *Phaseolus vulgaris. Int. Syst. Bactriol*. **47**: 996-1006.

[7]. Amin M, Tadele S, Thangavel S. 2014. White rot (Sclerotium cepivorum-Berk) an aggressive pest of onion and galic in Ethiopia: An overview. *Journal of Agricultural Biotechnology and Sustainable Development.* 6: 6-15.

[8]. Aneja K.R. 2003. Experiment In Plant Pathology, Microbiology and Biotechnology. New Age International (p) Ltd., Publishers, New Delhi p:274-275.

[9]. Anuradha Singh, Mohammad Shahid, Mukesh Srivastava, Sonika Pandey, Antima Sharma and Vipul Kumar. 2014. Optimal Physical Parameters for Growth of *Trichoderma* Species at Varying pH, Temperature and Agitation, Singh *et al.*, Virol Mycol, 3(1).

[10]. Ararsa L, Thangavel S. 2013. Evaluation of Arbuscular Mycorrhizal Fungi and *Trichoderma*Species for the Control of Onion White Rot (*Sclerotium cepivorum*Berk) J. *Plant Pathol.Microbiol*.4:159.

[11]. Backhouse D, Stewart A. 1987. Anatomy and histochemistry of resting and germinating sclerotia of *Sclerotium cepivorum*. *Transactions of the British Mycological Society* 89:561-567.

[12]. BakkerM.,P.Bom.,F.J.J.B.KindtKeurentjes.,I.Vander sluis.,L.C.VanLoonanddeBoer.,P.A.H.M.2003.Contr olofFusariumwiltofradishbycombining*Pseudomonas putida* strains that have different disease- suppressive mechanisms. *Phytopathology* 93:626-632.

[13]. Banks E, Edgington LV. 1989. Effect of integrated control practices on the Onion white rot pathogenin organic soil.*Candan J. Plant Pathol*.11:268-272.

[14]. Barnett H.L., Hunter B. 1998. Illustrated Genera of Imperfect Fungi.4th ed. American Phytopathological Society, St. Paul Minnesota, USA, p218.

[15]. Benítez T, Rincón AM, Limón MC, Codón AC. 2004. Biocontrol mechanisms of *Trichoderma*strains.*Int. Microbiol.* 7:249-260

[16]. Bernal, G. and Graham, P. H. 2001. Diversity in the rhizobia associated with *Phaseolus vulgaris* in Ecuadore and comparisons with Mexican bean rhizobia. *Can. J. Microbiol*.**47**:526- 534.

[17]. Bisby GR. 1939. *Trichoderma*viride Pers. ex Fries and notes on Hypocrea.*Trans. Br. Mycol.Soc.* 23(2):149-168.

[18]. Bissett J. 1984. A revision of the genus *Trichoderma*: 1. Section Longibrachiatum, new section. Can. J. Bot. 62:924-931.

[19]. Błaszczyk L, Siwulski M, Sobieralski K, Lisiecka J, Jędryczka M. 2014. *Trichoderma* spp. application and prospects for use in organic farming and industry. J. *Plant. Protect*. Res. 54(4).

[20]. Bloemberg, G. V., and Lugtenberg, B.J. 2001. Molecular basis of plant growth promotion and biocontrol by rhizobacteria. *Curr.Opin.PlantBiol.* 4: 343–350.

[21]. Brimner, T. A. and Greg, J. Boland. 2003. A review of the non-target effects of fungi used to biologically control plant diseases, *Agriculture, Ecosystems and Environment*. 100: 3-16.

[22]. Buck, J. D. 1982. Nonstaining (KOH) Method for Determination of Gram Reactions of Marine Bacteria.*Appl. Environ. Microbiol.***44**(4):992-993.

[23]. Budi S. W, D van Tuinen, C Arnould, E Dumas-Gaudot, V Gianinazzi-Pearson and S Gianinazzi. 2000. Hydrolytic enzyme activity of *Paenibacillus*sp. strain B2 and effects of the antagonistic bacterium on cell integrity of two soil-borne pathogenic fungi. Applied Soil Ecology.15(2):191-199.

[24]. CACC (Central Agricultural Census Commission). 2002. Report on the preliminary result of area, production and yield of temporary crops (Meher season private peasant holdings). Part II. Ethiopian Agricultural sample Enumeration, (2001/2002). Federal Democratic Republic of Ethiopia, Central Statistical Authority, AddisAbaba.

[25]. Cappuccino, J.C. and N. Sherma. 1992. Microbiology: A Laboratory Manual. Wesley Pub. Co. New York, USA.
[26]. Cappuccino, J.C. and N. Sherman. 2010. Microbiology: A Laboratory Manual. Wesley Pub. "Screening of plant growth promoting rhizobacteria to promote early soybean growth". *Soil Science Society of American Journal* 63(1999):1670-1680.
[27]. Chantawannakul P, Oncharoen A, Klanbut K, Chukeatirote E, Lumyong S. 2002. Characterization of protease of *Bacillus subtilis* strain 38 isolated from traditionally fermented soybean in northenthailand. Science Asia 28: 241-245.
[28]. Cheesbrough, M. 2006. Microbiological tests: District laboratory practice in tropical countries, 2nd ed., Cambridge University press, p313-327.
[29]. Chemeda Dilbo, Melaku Alemu, Alemu Lencho and Tariku Hunduma. 2015. Integrated Management of Garlic White Rot (*Sclerotiumcepivorum*Berk) Using Some Fungicides and Antifungal *Trichoderma*Species. *J Plant PatholMicrob. 6(1):*2157-7471.
[30]. Cheruth, A.J., Gopi, R., Sankar, B., Gomathinayagam, M., & Panneerselvam, R. 2008. Differential responses in water use efficiency in two varieties of Catharanthus roseus under drought stress. *Comptesrendus, Biologies*, 331(1): 42-47.
[31]. Chet I, Inbar J, Hadar I. 1997. Fungal antagonists and mycoparasites. In: Wicklow DT, Söderström B (eds) The Mycota IV: Environmental and microbial relationships. *Springer-Verlag, Berlin.* p165-184.
[32]. Chet I, Inbar J. 1994. Biological control of fungal pathogens. Appl. Biochem. Biotechnol. 48:37- 43
[33]. Chet, I., rdentlich, A., Shapira, R. and Oppenheim, A. 1990. Mechanisms of biocontrol of soil- borne plant pathogens by Rhizobacteria. *Plant Soil* 129:85-92.
[34]. Cícero Augusto, Guimarães Fuga, Everaldo Antônio Lopes, Bruno Sérgio Vieira, and Walter Vieira da Cunha. 2016. Efficiency and compatibility of *Trichoderma*spp. and *Bacillus* spp. isolates on the inhibition of *Sclerotium cepivorum. Jaboticabal*44(4):526–531.
[35]. CiDBem Kucuk, MerifKinvanc, 2009. In vitro activity of strains of *Trichodermaharzianum. Turk. J.Biol.*, 28(9): 111-115.
[36]. Clarkson JP, Mead A, Payne T, Whipps JM. 2002. Selection of fungal biological control agents of *Sclerotium cepivorumfor*control of white rot by sclerotial degradation in a UK soil. *Plant Pathol*. 51:735-745.
[37]. Clarkson JP, Mead A, Payne T, Whipps JM. 2004. Effects of environmental factors and *Sclerotium cepivorum*isolate on sclerotial degradation and biological control of white rot by *Trichoderma. Plant Pathol*. 53:353-362.
[38]. Coley-Smith JR, King JE. 1969. The production by species of *Allium* of alkyl sulphides and their effect on germination of sclerotia of *Sclerotium cepivorum*Berk. *Annals Applied Biology* 64:289-301.
[39]. Coley-Smith JR. 1987. *Sclerotium cepivorum*Berk.in European Handbook of Plant Diseases, (eds I. M. Smith, J. Dunez D. H., Phillips R. A. Lelliot and S. A. Archer) Blackwell Scientific Publications,p446-447.
[40]. Coley-Smith, J.R. 1959. Studies of the biology of *Sclerotium cepivorum*Berk. III Host range; persistence and viability of sclerotia. *Annals of Applied Biology* 47: 511-518.
[41]. Coley-Smith, JR. 1960. Studies of the biology of *Sclerotium cepivorum*Berk. IV. Germination of sclerotia. *Annals Applied Biology* 48:8-28.
[42]. Compant, S. 2005. Use of plant growth-promoting bacteria for bio control of plant diseases: principles, mechanisms of action, and future prospects. *Applied Environmental Microbiology*, 71:4951-4959.
[43]. Cook R.J., Baker K.F. 1988. The Nature and Practice of Biological Control of Plant Pathogens. American Phytopathological Society, St. Paul Minnesota, USA, p539.
[44]. Crowe FJ, Hall DH, Greathead AS, Baghott KG. 1980a. Inoculum density of *Sclerotium cepivorum*and the incidence of white rot of onion and garlic. *Phytopathology* 70:6469.
[45]. Crowe FJ, Hall DH. 1980. Soil temperature and moisture effects on sclerotium germination and infection of onion seedlings by *Sclerotium cepivorum*. *Phytopathology* 70:74-78.
[46]. Crowe, F., Darnell, T., Thornton, M., Davis, M., Mcgrath, D., Koepsell, P., Redondo, E. and Laborde, J. 1993. White rot control studies show promise of better future. Onion World 9:22-25.
[47]. CSA (Central Statistical Agency). 2012. Report on area and production of crops (Meher season private peasant holdings). Volume IV, Part III. Ethiopian Agricultural Sample Survey, (2012/13). Federal Democratic Republic of Ethiopia, Central Statistical Authority, Addis Ababa. Statistical Bulletin 446. p19-29.
[48]. de Souza JT, Weller DM, and Raaijmakers JM. 2003. Frequency, diversity and activity of 2, 4-diacetylphloroglucinol producing fluorescent Pseudomonas spp. in Dutch take-all decline soils. *Phytopathology*. 93:54–63.
[49]. Deresse D. 2010. Antibacterial effect of garlic (*Allium* sativum) on Staphyloccus aureus: An in vitro study. Asian Journal of Medical Sciences. 10: 62-65.
[50]. Dilbo C, Alemu M, Lencho A, Hunduma T. 2015. Integrated management of garlic white rot (*Sclerotium cepivorum*Berk) using some fungicides and antifungal *Trichoderma* species. *J Plant PatholMicrob.* 6:251.
[51]. Dingley JM. 1957. Life history studies in the genus *Hypocrea*Fr. Trans. R. Soc. N. Z. 84:689- 693.
[52]. Diriba, S. 2016. Review of management strategies of constraints in garlic (*Allium sativum* L.) Production. *Journal of Agricultural Sciences*. 11: 186-207.
[53]. Diriba-Shiferaw, G., Nigussie-Dechassa, R., Woldetsadik, K., Tabor, G, and Sharma, J.J. 2015. Effect of nitrogen, Phosphorus, and Sulphur Fertilizers on Growth, Yield, and Economic Returns Of Garlic (*Allium Sativum* L.). *African Journal of Agricultural Research*. 4(2): 10–22.

[54]. Domsch KH, Gams W, Anderson T-H. 1980. Compendium of soil fungi. Academic Press, London. 2nd edition updated by W. Gams, 2006. Centraal bureau voor Schimmel cultures,Utrecht.
[55]. Druzhinina IS, Seidl-Seiboth V, Herrera-Estrella A, Horwitz BA, Kenerley CM, Monte E, Mukherjee PK, Zeilinger S, Grigoriev IV. and Kubicek CP. 2011. *Trichoderma*: the genomics of opportunistic success. *Nat. Rev. Microbiol.* 9:749-759
[56]. Earnshaw, D.M., Mcdonald, M.R., Boland, G.J., 2000. Interactions among isolates and mycelial compatibility groups of *Sclerotium cepivorum*and cultivars of onion (*Allium cepa*). Can. J. Plant Pathol. 22:387-391.
[57]. EIAR-DZARC (Ethiopian Institute of Agricultural research-Debre Zeit Agricultural Research Centre). 1995. Garlic Production System. Ethiopia Agricultural Research Organization, Debre Zeit Research Center.
[58]. El-Hassan SA, Gowen SR, Barbara Pembroke. 2012. Use of *Trichoderma hamatum* for biocontrol of lentil vascular wilt disease: efficacy, mechanisms of interaction and future prospects. *J. Plant. Prot. Res.* 53(1).
[59]. Elshahawy I. E., Nehal Saied, F. Abd-El-Kareem and A. Morsy. 2019. Effect of inoculum density of *Stromatiniacepivora*on the amount of white rot reduced by *Trichoderma*species in garlic. *Bulletin of the National Research Centre.*43:27
[60]. Elshahawy I.E., n.m. Saied, a.a. Morsy. 2017. *Fusarium proliferatum*, the main cause of clove rot during storage, reduces clove germination and causes wilt of established garlic plants. 99(1):5-16.
[61]. ElsheshtawiMohamed , Ashraf A. Hatamleh, Amany M. Saad, and Abdallah M. Elgorban. 2014. Selective *Sclerotium cepivorum*Growth Agar Media and Other Condition Factors Affecting. *Journal of pure and applied microbiology*. **8**(4):2785-2790
[62]. Entwistle AR. 1990. Root diseases, in Onions and Allied Crops: Volume 11 - Agronomy, Biotic Actions, Pathology, and Crop Protection, (eds H. D. Rabinowitch and J. L. Brewster) CRC Press, Inc., Boca Raton, Florida. p103-154.
[63]. Entwistle AR. 1992. Controlling *Allium* white rot (*Sclerotium cepivorum*) without chemicals. *J. Phytoparasitical*. 20:121-125.
[64]. FAO (Food and Agriculture Organization). 2006. Food security in Ethiopia, Agriculture and Consumer protection Department Fekadu, M., and G. Dandena. 2006. Review of the status of vegetable crops Production and marketing in Ethiopia. Uganda *J. Agric*. Sci. 12(2):26-30.
[65]. FAO (Food and Agriculture Organization). 2006. Food Security in Ethiopia, Agriculture and Consumer Protection Department.
[66]. Farr DF, Rossman AY. 2017. Fungal Databases, U.S. National Fungus Collections, ARS, USDA.
[67]. FDRE/CSA (The Federal Democratic Republic of Ethiopia, Central Statistical Agency).
[68]. 2011/12. Area and production of Major crops Statistical Bulletin volume I.
[69]. Fekadu M, Dandena G. (2006). Review of the status of vegetable crops production and marketing in Ethiopia. *Uganda Journal of Agricultural Sciences*. 12: 26-30.
[70]. Fekadu M, Dandena G. 2006. Review of the status of vegetable crops production and marketing in Ethiopia. Uganda *J. Agric. Sci.* 12(2):26-30.
[71]. Food and Agriculture Organization of the United Nations Statistics (FAOSTAT). 2015. Area and production of crops by countries.
[72]. Francisco D.H., Angelica M.P., Gabriel M., Melchor C.S., Raul R., Cristobal N., Francisco C.R. 2011. In vitro antagonist action of *Trichoderma*strains against *Sclerotium sclerotiorum*and *Sclerotium cepivorum*. *American Journal of Agriculture Biology Science* 6 (3): 410– 417.
[73]. Fuga CAG, Lopes EA, Vieira BS. 2012. Etiologia, epidemiologia e controle de doençascausadaspor Sclerotium rolfsii e S. cepivorum. *RevisãoAnual de Patologia de Plantas.* 20:278- 322.
[74]. Fujimori F, Okuda T. 1994. Application of the random amplified polymorphic DNA using the polymerase chain reaction for efficient elimination of duplicate strains in microbial screening. *I. Fungi. J. Antibiot.* (Tokyo). 47:173-182.
[75]. Gajera H, Domadiya R, Patel S, Kapopara M, Golakiya B. (2013). Molecular mechanism of *Trichoderma* as bio-control agents against phytopathogen system. *Curr. Res. Microbiol. Biotechnol*. 1(4):133-142
[76]. Getachew T, Asfaw Z . 2000. Achievement in Shallot and Garlic Research. Ethiopia Agricultural Research Organization, Addis Abeba, Ethiopia.
[77]. Getachew T, Eshetu D, Tebkew D. 2011. Management system of onion and garlic production and productivity technique by using fungicides (in Amharic) Debre Zeit Agricultural Research Centre (2nded.)p29-34.
[78]. Ghisalberti EL, Narbey MJ, Dewan MM, Sivasithamparam K. 1990. Variability among strains of *Trichoderma*harzianum in their ability to reduce take-all and to produce pyrones. *Plant.Soil*. 121:287-291.
[79]. Glick, B.R. 1995. The enhancement of plant growth by free living bacteria. *Can. J. Microbiol*.**41**:109-117.
[80]. Haas D, Defago G. 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. Natra Rev Microb. doi:10.1038/nrmicro1129.
[81]. HaLAia Ali1 and Kalaivani Nadarajah. 2014. Evaluating the efficacy of *Trichoderma*spp and *Bacillus substilis*as biocontrol agents against *Magnaporthe grisea* in rice. *AJCS* 8(9):1324-1335
[82]. Han J, Shim H, Shin J, Kim KS. 2015. Antagonistic activities of Bacillus spp. Strains isolated from tidal flat sediment towards anthracnose pathogens *Colletotrichum acutatum*and *C. gloeosporioides*in South Korea. *Plant Pathol J* 31:165–175.
[83]. Handelsman J, Stab EV. 1996. Biocontrol of soil borne plant pathogens. *Plant C* 8:1855–1869
[84]. Harman GE, Howell CR, Viterbo A, Chet I, Lorito M. 2004. *Trichoderma*species opportunistic, avirulent plant symbionts. *Nat. Rev. Microbiol*.

2(1):43-56.
[85]. Harrison YA, Stewart A. 1988. Selection of fungal antagonists for biological control of onion white rot in New Zealand. *N Z J Exp Agric*. 16(3):249–256.
[86]. Hermosa R, Viterbo A, Chet I, Monte E. 2012. Plant-beneficial effects of *Trichoderma*and of its genes. *Microbiology*. 158:17-25.
[87]. Heydari A., Pessarakli M. 2010. A review on biological control of fungal plant pathogens using microbial antagonists. *Journal of Biology Science*. 10 (4): 272–290.
[88]. Hussain, W.A., Elzaawely, A.A., El Sheery, N.I., Ismail, A.A., El-Zahaby, H.M., 2017. Biological control of onion white rot disease caused by *Sclerotium cepivorum*. *EBSS* 1:101–107.
[89]. Ibarra-Medina, V.A., Ferrera-Cerrato, R., Alarcón, A., Lara-Hernández, M.E., & Valdez- Carrasco, J.M. 2010. Isolation and screening of *Trichoderma*strains antagonistic to *Sclerotinia sclerotiorum*and *Sclerotinia minor*. *Revista Mexicana de Micologia*, 31:53- 63.
[90]. Jaleel, C.A., Manivannan, P., Sankar, B., Kishorekumar, A., Gopi, R., Somasundaram, R., & Panneerselvam, R. 2007. Water deficit stress mitigation by calcium chloride in Catharanthus roseus; effects on oxidative stress, proline metabolism and indole alkaloid accumulation. *Colloids Surf. B: Biointerfaces*, 60:110–116.
[91]. Kakvan N, Heydari A, Zamanizadeh HR, Rezaee S, Naraghi L. 2013. Development of new bioformulations using *Trichoderma*and Talaromyces fungal antagonists for biological control of sugar beet damping-off disease. *Crop Prot.* 53:80–84.
[92]. Kasana, R. C., Salwan, R., Dhar, H., Dutt, S. and Gulati, A. 2008.A Rapid and Easy Method for the Detection of Microbial Cellulases on Agar Plates Using Gram's Iodine. *Current Microbiol*.**57:**503–507.
[93]. Kathiresan K., Manivannan S. 2006. Amylase production by *Penicillium fellutanum*isolated from mangrove rhizosphere soil. African Journal of biotechnology.5(10).
[94]. Kaur, J. 2014. Screening of microbes for their carbohydrates utilization. *Current Research in Biotechnology*. 7(1):22-31.
[95]. Kennedy, A.C. 2005. Rhizosphere (with discussion). In: *Principles and applications of soil microbiology*, 2nd edn. Pearson, Prentice Hall, Upper Saddle River, *NJ*. p242–262.
[96]. Keusgen M 2002. Health and *Allium*s, In H.D. Rabinowitch and L. Currah, eds. *Allium* crop science: Recent advances. CAB International, Walling ford, UK.
[97]. Khalid S. Abdel-latei. 2017. *Trichoderma*as biological control weapon against soil borne plant pathogens. *African Journal of Biotechnology.*16(50):2299-2306
[98]. Khiyami M.A., Omar M.R., Abd-Elsalam K., El-Hady Aly A. 2014. *Bacillus*-based biological control of cotton seedling disease complex. Journal of Plant Protection Research 54(4): 340–348.
[99]. Kirk PM, Cannon PF, Minter DW, Satalpers JA. 2010. 10 ed. Dictionary of the Fungi.
[100]. Wllingford, CABI. p784.
[101]. Kloepper J. W., Beauchamp R.N. 1992. Plant growth promotion mediated by rhizosphere bacterial colonizers. Kluwer Acadamic publishers, Dordrecht, Boston. p315-326.
[102]. Kloepper JW, Lifshitz R, Zablotowicz RM. 1989. Free-living bacterial inocula for enhancing crop productity. Trends Biotechnol.7:39–43.
[103]. Kobayashi DY, Reedy RM, Bick JA, Oudemans PV. 2002. Characterization of chitinase gene from *Stenotrophomonas maltophilia*strain 34S1 and its involvement in biological control. *Appl Environ Microbiol*68(3):1047–1054
[104]. Krishna M. S. R., Sharmila T., Raaga K., Sri Deepthi R. and Aswini A. 2016. *In vitro* antifungal activity of *Trichoderma*strains on pathogenic fungi inciting hot pepper *(Capsicum annuum L.). J. Chem. Pharm. Res*. 8(4):425-430
[105]. Kumar S. 2013. *Trichoderma*: a biological weapon for managing plant diseases and promoting sustainability. *Int. J. Agric. Sci. Vet. Med*.p106-121.
[106]. Kumar, V., Gupta, P. and Dwivedi, S. 2012. Bio-efficacy of fly-ash based *Trichoderma*formulations against damping-off and root-rot diseases in tomato (*Lycopersicum esculentum*). Indian Phytopathology, 65(4):404-405.
[107]. Laila Naher, Umi Kalsom Yusuf, Ahmad Ismail and Kausar Hossain. 2014. *Trichoderma*spp.: A biocontrol agent for sustainable management of plant diseases. *Pak. J. Bot*. 46(4): 1489-1493
[108]. Landa, B.B., Hervas, A., Bethiol, W. and Jimenez-Diaz, R.M. 1997. Antagonistic activity of bacteria from the chickpea rhizosphere against *Fusarium oxysporum*f.sp.ciceris. *Phytoparasitica*.**25:**305–318.
[109]. Leggett ME, Rahe JE. 1985. Factors affecting the survival of sclerotia of *Sclerotium cepivorum*
[110]. in the Fraser Valley of British Columbia. *Ann. Appl. Biol.* 106:255-263.
[111]. Lemma, D., and Herath, E. 1994. Agronomic Studies on *Allium*: Horticultural Research and Development in Ethiopia. 1-3 December 1992. Institute of Agricultural research and food and Agricultural Organization. Addis Ababa, Ethiopia p139-145.
[112]. Leuchtmann A, Petrini O, Samuels GJ. 1996. Isozyme subgroups in *Trichoderma*section Longibrachiatum. *Mycologia*88:384-394.
[113]. Limón MC, Chacón MR, Mejías R, Delgado-Jarana J, Rincón AM, *et al.* 2004. Increased antifungal and chitinase specific activities of *Trichodermaharzianum*CECT 2413 by addition of a cellulose binding domain. *Appl MicrobiolBiotechnol*. 64: 675-685.
[114]. Lo CT, Nelson EB, Hayes CK, Harman GE. 1996. Ecological studies of transformed *Trichoderma*harzianum strain 1295-22 in the rhizosphere and on the phylloplane of creeping bent grass. *Phytopathology*. 88: 129-136.
[115]. Lucy M, Reed E, Glick BR. 2004. Applications of free living plant growth-promoting rhizobacteria. Antonie Van Leeuwenhoek: *Int J Gen Mol Microbiol*. 86:1–25.

[116]. Mahdizadehnaraghi, R., Heydari, A., Zamanizadeh, H.R., Rezaee, S. & Nikan J. 2015. Biological control of garlic (*Allium*) white rot disease using antagonistic fungi-based bioformulations. *Journal of Plant Protection Research*. 55(2):136–141.
[117]. Massart S, Jijakli HM. 2007. Use of molecular techniques to elucidate the mechanisms of action of biocontrol agents: A review. J. Microbiol. Methods 69:229-241.
[118]. Massola Jr. NS, Jesus Jr, Kimati H. 2016. Doenças do alho e cebola. In: Kimati H, Amorim L, Bergamin Filho A, Camargo LEA, Rezende JAM (Ed.). 5ed. *Manual de Fitopatologia: doenças das plantascultivadas. Piracicaba, Ceres p*. 53-65.
[119]. Mathiyazhagan S, Kavitha K, Nakkeerans S, Chandrasekar MK, Renukadevi P, Krishnamoorthy AS, Fernando GZB. 2004. PGPR mediated management of stem blight of *Phyllanthus amarus*(Schum and Thonn) caused by *Corynesporacassiicola*(Berk and Curt) wei. Archives *Phytopathol Plant Prot* 37:183–199.
[120]. Mclean KL, Stewart A. 2000. Application strategies for control of onion white rot by fungal antagonists. *New Zealand Journal of Crop and Horticultural Science 28:115-122*.
[121]. Mengistu H. 1994. Research on vegetables disease in Ethiopia. In: Herath E, Dessalegne L (eds.) Proceedings of the Second National Horticultural Workshop of Ethiopia, 1-3 December 1994. IAR, Addis Ababa, Ethiopia. p209-215.
[122]. Metcalf D.A., Dennis J.J.C., Wilson C.R. 2004. Effect of inoculum density of *Sclerotium cepivorum*on the ability of *Trichodermakoningii*to suppress white rot of onion. Plant Disease. 88 (3): 287–291.
[123]. Meyer W, Morawetz R, Börner T, Kubicek CP. 1992. The use of DNA fingerprints analysis in the classification of some species of the *Trichoderma*aggregate. *Curr.Genet*. 21:27-30.
[124]. Miller, H. J. 1990. Notes on the behavior of fluorescent pseudomonads in rhizosphere studies. Symbiosis **9:**377–381.
[125]. Mitra Aboutorabi. 2018. A review on the biological control of plant diseases using various microorganisms. *Journal of Research in Medical and Dental Science*. 6(4): 30-35
[126]. Mohammed Amin, Shiberu Tadele and Thangavel Selvaraj. 2014. White rot (*Sclerotium cepivorum*Berk) an aggressive pest of onion and garlic in Ethiopia: An overview. *Journal of Agricultural Biotechnology and Sustainable Development.* 6(1): 6-15.
[127]. Mukesh Srivastava, Mohammad Shahid, Sonika Pandey, Vipul Kumar, Anuradha Singh, Shubha Trivedi, Y. K. Srivastava and Shivram. 2015. *Trichoderma*: A scientific approach against soil borne pathogens. *African journal of microbiology research.* 9(50):2377-2384
[128]. Mussa Adal Mohammed, Tadege Million, Assefa Fassil. 2018. Rhizospheric bacterial isolates of grass pea (*Lathyrus sativus* L.) endowed with multiple plant growth promoting traits. *Journal of applied microbiology*. 126 :6.
[129]. Muthumeenakshi S, Mills PR, Brown AE, Seaby DA. 1994. Intraspecific molecular variation among *Trichoderma*harzianumisolates colonizing mushroom compost in the British Isles. *Microbiology* 140:769-777
[130]. Naher L, Yusuf U, Ismail A, Hossain k. 2014. *Trichoderma*SPP: A biocontrol agent for sustainable management of plant diseases. *Pak. J. Bot*. 46(4):1489-1493.
[131]. Nandakumar R, Babu S, Viswanathan R, Sheela J, Raguchander T, Samiyappan R. 2001. A new bio-formulation containing plant growth promoting rhizobacterial mixture for the management of sheath blight and enhanced grain yield in rice. *Biocontrol.* 46: 493–510.
[132]. Naraghi L., Heydari A., Rezaee S., Razavi M. 2013. Study on some antagonistic mechanisms of *Talaromyces flavus* against *Verticillium dahliae*and *Verticillium albo-atrum*, the causal agents of wilt disease in several important crops. *Biocontrol in Plant Protection* 1(1): 13– 28.
[133]. Neilands, J.B. 1995. Siderophores: structure and function of microbial iron transport compounds. *J.Biol.Chem*. 270:26723-26726.
[134]. Nevalainen H, Penttilä M. 1995. Molecular biology of cellulolytic fungi. In: The Mycota II. Genetics and Biotechnolog. Edt. U. Kuck. *Springer Verlag, Berlin*, p303-319.
[135]. Nikan Jafar, Asghar Heydari, Laleh Naraghi, Amir Arjmandian and Razak Mahdizadehnaraghi. 2018. Application of some fungal bioformulations for controlling garlic white rot disease in the field conditions. *Biocontrol in Plant Protection*. 5(2): 75-83**.**
[136]. Nisa Rachmania Mubarik, Mahagiani Irni, Santoso Suegung and Anindyputry Amaryllis. 2010. Chitinolytic Bacteria Isolated from Chili Rhizosphere: Chitinase Characterization and Its Application as Biocontrol for Whitefly (*Bemisiatabaci*Genn.). *American Journal of Agricultural and Biological science*. 5(4).
[137]. Okon Y, Labandera-Gonzales CA. 1994. Agronomic applications of *azospirillum*: An evaluation of 20 years worldwide field inoculation**.** *Soil. Biol. Biochem*. 26: 1591–1601.
[138]. Okuda T, Fujiwara A, Fujiwara M. 1982. Correlation between species of *Trichoderma*and production patterns of isonitrile antibiotics. *Agric. Biol. Chem*. 46:1811-1822.
[139]. Ongena M, Daayf F, Jacques P, Thonart P, Benhamou N, Paulitz TC, Cornelis P, Koedam N, Belanger RR. 1999. Protection of cucumber against Pythium root rot by fluorescent pseudomonads: predominant role of induced resistance over siderophores and antibiosis. *Plant Pathol*48:66–76
[140]. Pal KK, KVB Tilak, AK Saxena, R Dey,S Singh. 2001. Suppression of maize root disease caused by *Macrophominaphaseolina, Fusarium moniliforme*and *Fusarium graminearum*by plant growth-promotingrhizobacteria.
[141]. Pal, K.K., Tilak, K.V., Saxena, A.K., Dey, R., and

Singh, C.S. 2000. Antifungal characteristics of a fluorescent *Pseudomonas* strain involved in the biological control of *Rhizoctonia solani*. *Microbiol.Res.* 155,233–242.

[142]. Pandya JR, Sabalpara AN, ChaGZa SK. 2011. *Trichoderma*: a particular weapon for biological control of Phytopathogens. *J. Agric. Technol*. 7(5):1187-1191.

[143]. Philip A. O'Brien. 2017. Biological control of plant diseases. Australasian Plant Pathology. https://www.researchgate.net/publication/315594677

[144]. Prados-Ligero AM, Bascon-Fernandez J, Calvet-pinos C, Corpas-Hervias C, Ruiz AL, Melero- vara JM, MJBU. 2002. Effect of different soil and clove treatments in the control of white rot of onion. *J. Appl. Biol*. 140:247:253.

[145]. Raaijmakers JM, Vlami M, de Souza JT. 2002. Antibiotic production by bacterial biocontrol agent. *Anton van Leeuwenhoek* 81:537–547.

[146]. Ramamoorthy V, Viswanathan R, Raguchander T, Prakasam V, Samayapan R. 2001. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. *Crop Prot.* 20:1–11

[147]. Ramazan GÜMRAL1, Aylin DÖĞEN, Mehmet Macit İLKİT. 2015. Comparison of the contamination rates of culture media used for isolation and identification of dermatophytes. *Turkish Journal of Medical Sciences*. 45: 587-592.

[148]. Rifai MA. 1969. A revision of the genus *Trichoderma*.*Mycol. Pap*. 116:1-56.

[149]. Rodrguez, H. and Fraga, R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol. Adv.* 17:319-339.

[150]. Rubatzky VE, Yamaguchi M. 1997. World vegetable, In Chapman and hall, (eds.) Principles, production and nutritive values, 2nd edition. International Thomson publishing New York. USA. P. 843.

[151]. Sadfi N, Cherif M, Fliss I, Boudabbous A, Antoun H. 2001. Evaluation of bacterial isolates from salty soils and *Bacillus thuringiensis* strains for the biocontrol of *Fusarium* dry rot of potato tubers. *J Plant Pathol*. 83:101–118.

[152]. Sadykova VS, Kurakov AV, Kuvarina AE, Rogozhin EA. 2015. Antimicrobial Activity of Fungi Strains of *Trichoderma*from Middle Siberia. *Appl. Biochem. Microbiol*. 51:355-361.

[153]. Sagarika Kannangara1, R. M. G. C. S. Dharmarathna1 and D. L. Jayarathna. 2017. Isolation, Identification and Characterization of *Trichoderma*Species as a Potential Biocontrol Agent against *Ceratocystis paradoxa The Journal of Agricultural Sciences Vol. 12, No. 1, January 2017. p51-62.*

[154]. Samanta, R., Pal, D. and Semi, S.P. 1989. Production of hydrolases by N-fixing microorganisms. Biochemistry and Physiology Plant. 185: 75–81.

[155]. Samuels GJ, Petrini O, Manguin S. 1994. Morphological and macromolecular characterization of Hypocreaschweinitzii and its *Trichoderma anamorph*. *Mycologia*86:421-435.

[156]. Schwartz HF, Mohan SK. 1995. Compendium of onion and garlic diseases.*American Phytopathology Society Press, St. Paul*. p 54.

[157]. Seema Dua, S. S. Sindhu. 2012. Effectiveness of Rhizosphere Bacteria for Control of Root Rot Disease and Improving Plant Growth of Wheat (*Triticum aestivum* L). *Journal of Microbiology Research*. 2(2): 26-35.

[158]. Sethi Neeraj , Kaura Sushila, Dilbaghi Neeraj , Parle Milind and Pal Minakshi. 2014. Garlic: A Pungent Wonder from Nature. *Int. Res. J. Pharm*. 5 (7).

[159]. Sharifi R., Ahmadzadeh M., Talebi-Jahromi K. 2010. Pyoverdine production in *Pseudomonas fluorescens* UTPF5 and its association with suppression of common bean damping off caused by *Rhizoctonia solani*(Kühn). *Journal of Plant Protection Research.* 50(1):72– 78.

[160]. Sharma RA. 2012. A brief review on mechanism of *Trichoderma*fungus use as biological control agents. *Int. J. Innov. Bio-Sci*. 2:200-210.

[161]. Sharma, K.D, Chen W., Muehlbauer F.J. 2005. Genetics of chickpea resistance to five races of Fusarium wilt and a concise set of race differentials for *Fusarium oxysporum f. sp. ciceris. Plant Disease*.89:385-390.

[162]. Silva HSA, de Silva RR, Macagnan D, de Almeda Halfeld-Viera B, Pereira MCB, Mounteer A. 2004. Rhizobacterial Induction of systemic resistance in tomato plants: nonspecific protection and increase in enzyme activities. *Biol Control.* 29:288–295.

[163]. Simbert R.M and N.R. Krieg. 1994. Phenotypic characterization. In: *Methods for general and molecular bacteriology* American Society for Microbiology, Washington DC. *5*: 611– 654.

[164]. Sindhu S, Sunita Suneja, AK Goel, N Parmar, KR Dadarwal 1997. Plant growth promoting effects of *Pseudomonas* sp. on coinoculation with *Mesorhizobium*sp. *Cicer* strain under sterile and "wilt sick" soil conditions. *Applied soil ecology*. 9(1):57-64.

[165]. Siyoum Zeray and Yesuf Mohammed. 2013**.** Searching and Evaluating of Cost Effective Management Options of Garlic White rot (*Sclerotium cepivorum*Berk) in Tigray, Northern Ethiopia. *J Plant PatholMicrob*. 4:7

[166]. Somasegaren P, Hoben HJ. 1984. Methods in legume rhizobium technology. University of Hawaii NifTAL Project and MIRCEN Department of Agronomy and Soil Science. USAID, USA

[167]. Somasegaren, P. and Hoben, H. J. 1994. Hand book for rhizobia. Methods in legume rhizobium technology. Springer – verlag, New York. p1-441.

[168]. Srijana Bastakoti, Shiva Belbase, Shrinkhala Manandhar, Charu Arjyal. 2017. *Trichoderma*species as Biocontrol Agent against Soil Borne Fungal Pathogens. *Nepal Journal of Biotechnology*. 5(1): 39-45

[169]. Srivastava M, Shahid M, Pandey S, Singh A, Kumar V, Gupta S, Maurya M. 2014**b**. *Trichoderma*Genome to Genomics: A Review. J. Data Mining in Genomics Proteomics. 5(3):1000172.

ISSN No:-2456-2165

[170]. Srivastava M, Shahid M, Pandey S, Singh A, Kumar V, Gupta S, Maurya M. 2014. *Trichoderma*Genome to Genomics: A Review. *J. Data Mining in Genomics Proteomics.* 5(3):1000172.
[171]. Stewart A, McLean KL. 2007. Biological controls of onion white rot. Chapter 6 in Biological control of plant diseases. *The Haworth Press, Inc*. p123-148.
[172]. Tamire Z, Chemeda F, Parshotum PK, Sakhuja PK, Ahmed S. 2007. Association of white rot (Sclerotium cepivorum) of garlic with environmental factors and cultural practices in the north Shewa highlands of Ethiopia. *J Crop Prot.* 26: 1566–1577.
[173]. Toppo, S.R., Naik, U.C. 2015. Isolation and Characterization of Bacterial Antagonist to Plant Pathogenic Fungi *(Fusarium* Spp.) from Agro Based Area of Bilaspur. *International Journal of Research Studies in Biosciences (IJRSB). p6-14*
[174]. Ulacio-Osorio D, Zavaleta-Mejía E, Martínez-Garza A, Pedroza-Sandoval A. 2006. Strategies for management of *Sclerotium cepivorum*Berk in garlic. *Journal of Plant Pathology* 88: 253-261.
[175]. Vaidya Soumya and Rathore Pragya. 2015. Isolation, screening and characterization of amylase producing bacteria from soil of potato dump sites from different regions of madhya Pradesh. Sanghvi Institute of Management and Science, Indore, India.
[176]. Valdir Lourenço JR, Bruno Sérgio Vieira, Everaldo Antônio Lopes, Oscar N. Villalta. 2018. Etiology, epidemiology, and management of white rot on onion and garlic: current knowledge and future directions for Brazil. *Jaboticabal*.46(3): 241–256
[177]. Valle RV, Aguilar MMM. 2004. Persistencia de esclerocios de *Sclerotium cepivorum*Berk.Emsuelosinfestados de Aguascalientes y Zacatecas, México.*Revista Mexicana de Fitopatologia*22:143-146.
[178]. Van L, Bakker L C, and pieterse C M J. 1998. Systemicesistance induced by rhizosphere bacteria Annu. Rev. *Phytopathol*. 36:453-483.
[179]. Vinale F, Sivasithamparamb K, Ghisalbertic EL, Marraa R, Woo SL, Loritoa M. 2008.
[180]. *Trichoderma*–plant–pathogen interactions. *Soil. Biol. Biochem*. 40:1-10.
[181]. Vinale F, Sivasithamparam K, Ghisalberti EL, Woo SL, Nigro M, Marra R, *et al.* 2014. *Trichoderma* Secondary Metabolites Active on Plants and Fungal Pathogens. *Mycol. J*. 8:127-139.
[182]. Wandersman C, Delepelaire P. 2004. Bacterial iron sources: from siderophores to hemophores. Annu Rev Microbiol 58:611–47.
[183]. Watanabe T. 1994. Pictorial Atlas of Soil and Seed Fungi, Morphologies of Cultured Fungi and Key to Species. Boca Raton: CRC Press.
[184]. Webster J, Rifai MA. 1968. Culture studies on Hypocrea and *Trichoderma*IV.*Hypocrea pilulifera* sp. nov. Trans. Br. *Mycol. Soc*. 51:511-514.
[185]. Weindling R. 1932. *Trichoderma*lignorum as a parasite of other fungi. *Phytopathology* 22:837.
[186]. Weldemariam Seifu, Tsion Yemane, Senayt Bedada and Tariku Alemu. 2017. Evaluation of Different Mulching Practices on Garlic (*Allium sativum* L.) Growth Parameters under Irrigated Condition in Fiche, North Shoa Ethiopia. Journal of Biology, Agriculture and Healthcare. 7(9),
[187]. Weller DM, Raaijmakers JM, McSpadden Gardener BB, Thomashow LS. 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annual Review of Phytopathology. 40:309-48.
[188]. Whipps, J.M. 2001. Microbial interactions and biocontrol in the rhizosphere. *J. Exp. Biol.* **52**: 487-511.
[189]. Willets HJ, Bullock S. 1992. Development biology of sclerotia. *Mycological Research* 96:801- 816.
[190]. Xu X., Harrington TC, Gleason ML, Baltzer JC. 2010. Phylogenetic placement of plant pathogenic Sclerotium species among teleomorph genera. *Mycologia*102:337-346.
[191]. Zeray S, Mohammed Y. 2013. Searching and evaluating of cost effective management options of garlic white rot (*Sclerotium cepivorum*Berk) in Tigray, Northern Ethiopia. J. *Plant Pathol. Microb*.4: 189.
[192]. Zeray S. 2011. Distribution and Management of Garlic White Rot in Northern Ethiopia: Distribution, Significance and Management of Garlic White Rot in Northern Ethiopia, Ambo University, Ambo Ethiopia.
[193]. Zewide T, Fininsa C, Sakhuja PK, Ahmed S. 2007. Association of white rot (*Sclerotium cepivorum*) of garlic with environmental factors and cultural practices in the North Shewa highlands of Ethiopia. *Crop Protect*. 26:1566-1573
[194]. Zewide T, Fininsa C, Sakhuja PK. 2007. Management of white rot (*Sclerotium cepivorum*) of garlic using fungicides in Ethiopia. Crop Prot 26:856–866
[195]. Zhou T, Paulitz TC. 1994. Induced resistance in the biocontrol of *Pythium aphanidermatum* by Pseudomonas spp.