
**College of Natural Sciences**

**Department of Biology**

# Biocontrol of *Trichoderma* isolates against *Phytopthora infestans* and evaluating the growth of tomatoes *(lycopersicon esculentum)*

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# Dedication

This M.Sc. Thesis is dedicated to my beloved family and my beloved parents Mr. Jacob Both Dol, Mrs. Mary Nyakot Tap, for their unlimited affection and effort in the success of my research work.

# Declaration

By my signature below, I declare and affirm that this Thesis is my original work. I have followed all ethical principles of scholarship in the preparation, data collection, data analysis and completion of this Thesis. All scholarly matters that have been included in the Thesis have been given recognition through citations. I affirm that I have cited and referenced all sources used in this document. Every serious effort has been made to avoid any plagiarism in the preparation of this Thesis.

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## Biographical sketch

The author was born in Dimma district, Gambella Regional State on 17 June 1993 G.C. His parent Mr. Jacob Both Dol and Mrs. Mary Nyakot Tap are living in Gambella town. All the family are a Protestant, Christian and belong Nuer ethnic group. He has married and become father of son and daughter. He attended his primary and high school education at Dalkoch School and Gambella Secondary and Preparatory School in Gambella town, respectively.

After successfully passing the Ethiopian Schools Leaving Certificate Examination (ESLCE), he joined Haramaya University in 2012/13 G.C and graduated with Bachelor of Science Degree in Biology in July 2015. Following his graduation, he was employed as a teacher at Winy-diew Secondary and Preparatory School in Makuey Woreda, Nuer Nation Zone from 2015 G. C by Gambella Regional State Education Office. After serving in the school for two years, he got opportunity to study Master of Science Degree in Programs at Jimma University in 2017/18 G.C for promotion to his educational level and earned M.Sc. Degree in Biology (Applied microbiology).

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# List of abbreviations and acronyms

**ABC:** ATP-binding cassette

**ANOVA:** Analysis of variance

**BCAs:** Biological control agents

**CA:** Carrot agar

**CSA:** Central statistical agency

**CZA:** Czapexdox agar

**DS:** Disease severity

**DMRT:** Duncan's Multiple Range Test

**DNA:** Deoxyribonucleic acid

**GLM:** General linear model

**ISR:** Induced systemic resistance

**PPE:** Personal protective equipment

**PR-proteins:** Pathogenesis-related proteins

**SAS:** Statistical analysis software

**SD:** Standard deviation

# Abstract

*Tomato (lycopersicon esculentum) is one of the most important vegetable crops belongs to the family solanaceae ranking 8$^{th}$ in annual national production in Ethiopia. In spite of this, major pests like, Phytopthora infestans, affect the production of tomatoes around the world. To achieve profitable yields economically, producers intensify chemical treatments to control plant diseases but indicate health risks to the consumers. As result, application of biological antagonists against tomatoes disease reduces incidence and severity of the pathogen. Therefore, the aim of the study is to assess the antagonistic potential of isolates of Trichoderma against the Phytopthora infestans on the growth and yield of tomato plants. The 40 diseased plants samples were collected from Dedo and Goma districts and analyzed in Jimma university. All the data were subjected to one-way ANOVA at $p \leq 0.05$. From 36 isolates, 18 were identified to be Phytopthora infestans and from 103 isolates obtained from soil, 10 were Trichoderma spp. In dual culture assay, the 3 days percentage of inhibition of pathogen by Trichoderma-Ju-TGDa-2, Ju-TGDb-3, Ju-TDWc-2 and Ju-TGDb-2 varied from 10.2 to 15%, 38% to 43.4% in 6 days and increased to 58 to 61.4% in 9 days. The non-volatile compounds of Trichoderma-Ju-TGDb-3, Ju-TDWc-2, Ju-TGDa-2, Ju-TGDb-2 and Ju-TGDb-1 inhibited with 63.9%, 60.9%, 57.7%, 44.4% and 24%, respectively. The volatile compounds of the Trichoderma-Ju-TGDb-2, Ju-TDWc-2, Ju-TGDb-3 and Ju-TGDa-2 inhibition varied between 42.5 to 2.5 in 3 days, 63.5 to 34.7% in 5 days and 64.1 to 35.8% in 6 days. In greenhouse, the shoot length, root length, fresh and dry weights and yields of tomato plants were 78.7 cm, 28.21 cm, 104.11 g, 28.44 g, 15.96 g respectively. It is concluded that the Trichoderma isolates Ju-TGDa-2, Ju-TGDb-3 and Ju-TDWc-2 are the most promising biocontrol agent against the late blight as well as enhance the growth and reduce the relative percent yield loss of tomato plants. It should be recommended that the potential isolates should be further evaluated for their safety and the protocol of administration need to be developed for field application by smallholder farmers.*



**Keywords:** *Antagonism; Biocontrol agents; late blight; non-volatile compounds; Volatile compounds.*

# 1. Introduction

Tomato *(Lycopersicon esculentum)* is one of the most economically important edible and nutritious vegetable crops in the world that belongs to the family *Solanaceae* second to potato (Mutschler *et al.,* 2006; Canene-adams *et al.,* 2005). It is the most widely cultivated and lucrative vegetable and ranking 8$^{th}$ in annual national production in Ethiopia (Derbew *et al.,* 2012; Arah, 2015). It is produced both during the rainy and dry seasons under supplemental irrigation (Tsedeke, 2007). The fruit supplies several nutrients of high nutritional values in human diet. Tomato supplies more than 90% of the vitamin C by fruits and vegetables and products contain lycopene, which is a naturally occurring phytochemical that gives fruits and vegetables a red color, and flavonoids (Pereira *et al.,* 2017). Its production is steadily increasing in the last ten years worldwide. The current world tomato production reached to be more than 163.4 million ton cultivated on more than 4.6 million ha of land (FAO, 2016). Under this circumstance, the total area under tomato production reaches 9,767.78 hector and in main cropping season production is estimated to be over 913,013.42 ton with the average productivity of 93.47 t ha-1 (CSA, 2016).

In spite of its huge potential, the production and productivity of tomato in the Ethiopia is very low. This may be due to lack of improved varieties, poor and traditional agronomic practices and high disease and insect pest incidences. Moreover, especially in humid and sub-humid areas of the country, tomato production is not common during the rainy season mainly due to high incidence of late blight caused by *Phytophthora infestans* (Sanoubar & Barbanti, 2017; Worku & Sahe, 2018). To achieve profitable yields economically, producers intensify chemical inputs to control diseases. The synthetic chemical inputs significantly influence the quality of tomatoes at harvest and are effective in reducing disease severity as well as improving crop yield (Meaza *et al.,* 2007; Tadesse *et al.,* 2010). However, chemicals pesticides have health risks to the consumers. Their increase and unjustified use affects directly farmers health because they do not use personal protective equipment (PPE) during handling and spraying (Tarla *et al.,* 2013; Meya *et al.,* 2014) and their employment also favored the development of pathogens resistant to fungicides (Suprapta, 2012). Thus, application of biological agents against tomatoes disease reduces incidence and severity of the pathogen and increases the yield and quality of tomato fruits.

Biological control refers to the application of living antagonists directly or their metabolic products to impede or kill undesired microorganisms (Sanae *et al.*, 2007; Zhou *et al.*, 2011). As shown in many studies (De la Cruz-Quiroz *et al.*, 2018), the *Trichoderma* species have been reported to possess antagonistic activities against plant pathogens. It controls pathogens in an indirect way by producing several groups of antibiotics that inhibit the growth of the pathogen (Kumar *et al.*, 2012; Herath, 2015; De la Cruz-Quiroz *et al.*, 2018). Apart from that, there are direct methods showing antagonism against the pathogen, which is called mycoparasitism. *Trichoderma* species can also inhibit or reduce the growth of plant pathogens through competition for space, enzyme substrates, nutrients, and or oxygen (Sanchez *et al.,* 2006). Therefore, this study was carried out with the objective to assess the antagonistic effect of *Trichoderma* isolates against *P. infestans* and enhancement of tomato production both its growth and yields.

## 1.1. Statement of the problem

Tomato production has shown a marked increase since it became the most profitable crop to small-scale farmers compared to other vegetable crops (Nelson, 2008). It is mainly cultivated by smallholder farmers as cash crop in mid- to low-altitude areas of Ethiopia for both local consumption and regional export markets. Its small-scale commercial production is mostly practiced especially in central Rift Valley region of the country (Lemma, 2002; EIAR, 2007). However, the production of tomato has been declining due to the problem related to the infection by late blight caused by *Phytopthora infestans* (Mont.) de Bary, which is the most destructive disease of tomato and potato in many parts of the world (Tsedaley, 2014). The late blight was responsible for the Irish famine in the middle of the 19$^{th}$ century where over one million died and 1.5 million emigrated (Tsedaley, 2014). It was in France where late blight on tomato was first described by Tulasne in 1854 and is one of the most disturbing diseases of tomatoes and potatoes (Drenth *et al.,* 1995) both in temperate and tropical regions. The development of disease is seemed to be favored by other factors such as lack of improved and well performing resistant cultivars, poor fruit setting due to heavy rains and excessively high temperatures. Heavy rain increase the direct contact of plants with pathogen, coupled with other predisposing factors such as favorable temperatures and high relative humidity that makes the environment suitable for the development and spread of late blight which restrict the production of tomato in such areas

(Kelley *et al.,* 2014). In Ethiopia, late blight causes serious loss in yield and quality as well as reduces its marketability values (Getachew, 2017). Nonetheless, tomato yield losses due to the disease were estimated to range between 65-70% and complete crop failures are frequently reported (Adissu, 2011). Similarly, different tomato growing farms of Jimma zone encounter the same problem and the yield losses due to the late blight are attributed to both premature death of foliage, stems and fruits of tomato (Amin *et al.,* 2013). Therefore, the purpose of study is to determine the antagonistic effect of *Trichoderma* isolates against the agent causing late blight of tomato plants *P. infestans*.

## 1.2. Research questions

Therefore, this study was undertaken to answer the following research questions:

- Are potential *Trichoderma* spp. are associated with tomato rhizhosphere?
- Can tomato disease be managed using *Trichoderma* isolates?

## 1.3. Objectives of the study

### 1.3.1. General objective

- To assess the antagonistic effect of biocontrol agents against *Phytopthora infestans* in order to increase the productivity of tomato crop.

### 1.3.2. Specific objectives

- To isolate *Phytopthtora infestans* and biocontrol *Trichoderma* species from diseased plants and rhizosphere soil of tomato plants
- To in vitro test the pathogenicity of *Phytopthtora* isolates on detached tomato leaflets.
- To evaluate *in vitro* antagonism of *Trichoderma* isolates against *Phytopthtora infestans*
- To determine antagonism efficacy of *Trichoderma* isolates against the development of tomato late blight under greenhouse condition
- To determine the yield of plants inoculated with different treatmented

## 1.4. Significance of the study

This study should be important to local community because it will increase awareness to farmer on how to protect their tomato farms from plant pathogens caused by late blight. It also helps for the studying point of anyone to conduct further and detailed research around this topic or for direct intervention of the problem. It may help the other researchers to easily access and use the potential *Trichoderma* isolates. It could also be used as a literature for a future research, which would be conduct around this topic.

# 2. Review of related literatures

## 2.1. The overview of tomato

Tomato *(Lycopersicon esculentum)* is one of the most important vegetables worldwide. World tomato production was about 152.9 million tons of fresh fruit with a value $74.1 billion soil (Tsedeke, 2007; FAO, 2009). As it is a relatively short duration crop and gives a high yield, it is economically attractive and the area under cultivation is increasing daily. It belongs to the Solanaceae family. This family also includes other well- known species, such as potato, tobacco, peppers and eggplant (aubergine) (Naika et al., 2005). Its origin is the Andean zone particularly Peru-Ecuador-Bolivian areas but cultivated tomato originated in Mexico. Tomato is one of the most popular salad vegetables and is taken with great relish (Kelley *et al.,* 2014). The cultivated tomato was brought to Europe by the Spanish conquistadors in the sixteenth century and later introduced from Europe to southern and eastern Asia, Africa and the Middle East. More recently, wild tomato has been distributed into other parts of South America and Mexico. Common names for the tomato are: tomate (Spain, France), tomat (Indonesia), faan ke'e (China), tomati (West Africa), tomatl (Nahuatl), jitomate (Mexico), pomodoro (Italy), nyanya (Swahili).

## 2.2. Tomato production in Ethiopia

Tomato is one of the most important edible and nutritious vegetable crops in Ethiopia. It is cultivated in almost all home gardens and also in the field by the use of rainfall and irrigation for its adaptability to wide range of soil and climate in Ethiopia (Gemechis *et al.,* 2012). It ranks next to potato and sweet potato in respect of vegetable production in the world. It is widely cultivated in tropical, sub-tropical and temperate climates and thus it ranks third in terms of world vegetable production. The leading tomato producing countries are China, United States of America, India, Egypt, Turkey, Iran, Mexico, Brazil and Indonesia (FAO, 2006)

Tomato is grown in many parts of the country in Ethiopia possessing difference agro-ecological conditions. It grows at an altitude between 700 and 2000 meter above sea level, which is characterized by warm and dry days and cooler nights, which are favorable for optimum growth and development. Current productivity under farmers' condition in Ethiopia is 90 q/ha whereas yield up to 400 q/ha is recorded on research plots (Lemma, 2002).

Farmers get lower yield mainly due to diseases, pests and sub-optimal fertilization (Balem, 2008). Small-scale farmers, commercial growers and state farm enterprises grow the crop for its fruits in difference regions. Most intensive production is found in the rift valley, mainly along Awash River valley and the lakes region (Lemma, 2002). Its production ranks foremost among vegetables in the processing industry. The production of this crop mostly serves as source of cash income, supplement to cereal diet by smallholder farmers and raw materials for processing industry in the commercial sector, especially in the rift valley regions of Ethiopia. It has also very high export potential. It is produced both during the rainy and dry seasons under supplemental irrigation (Gemechis *et al.,* 2012).

## 2.3. Importance of tomato

Tomato serves in various raw and processed materials. Fresh tomatoes are key ingredients in all around the world and processed tomatoes are used to make soup, juice and other products. Tomato is one of the most important and fames vegetables products in the country and most of the time tomato production rural community is one of the sources of income generation crop of rural areas (Gemechis *et al.,* 2012). The tomato fruit contains abundant and well-balanced nutrition consisting of minerals (calcium, iron, and phosphorus), vitamins (vitamin A, vitamin C), protein (essential amino acids), sugars, dietary fiber (pectin), citric acid, etc. (Canene-adams *et al.,* 2005). In addition, the red pigment of the lycopene which tomato fruit contains in plenty has attractive interest because the lycopene has high antioxidant ability against oxygen radicals that probably cause cancer, aging, arteriosclerosis, etc. (Canene-adams *et al.,* 2005). Thus, tomato would contribute to our enjoyable diet and good health all over the world (Pereira *et al.*, 2017; Naika *et al.*, 2005).

## 2.5. The pathogen *Phytophthora*

*Phytophthora infestans*was first named *Botrytis infestans* by M. J. Berkeley in the 1840's. In 1876, the pathogen was renamed by Anton de Bary to *Phytophthora infestans* (de Bary, 1876). The name is derived from the Greek: *Phyto* = plant, *phthora* = destroyer. *P. infestans* is a member of the *oomycetes*, a group of organisms sometimes referred to as the "water molds". *Oomycetes* are not true fungi but are more closely related to brown algae. The mycelium is hyaline and coenocytic (few septa), and the nuclei are diploid. Most fungi are

haploid. Eva Sansome, a plant geneticist (Ristaino *et al.*, 2007), first described the diploid life cycle of *Phytophthora*. The family name for this group of organisms is *Peronosporaceae*, which have been assigned to the Kingdom *Stramenopila* of the eukaryotes. *Oomycetes* are no longer considered members of the Kingdom Fungi although they share many biological, ecological, and epidemiological characteristics with fungal plant pathogens (Nelson, 2008).

A recent phylogenetic analysis of the genus *Phytophthora* based on mitochondrial and nuclear DNA sequences several lines of evidence point to the fact that *P. mirabilis, P. ipomoeae,* and *P. infestans* evolved from a common ancestor (Figure 1). Kroon *et al.* (2004) confirmed that *P. ipomoeae* is consistently placed within the Ic clade (Cooke *et al.*, 2000) with *P. infestans, P. phaseoli, and P. mirabilis. P. phaseoli* first diverged from the other members of this clade. Interestingly, whereas *P .infestans* and *P. mirabilis* are heterothallic, *P. ipomoeae* and *P. phaseoli* are homothallic. Kroon *et al.* (2004) consider *P. ipomoeae* and *P. phaseoli* to be secondary homothallics.

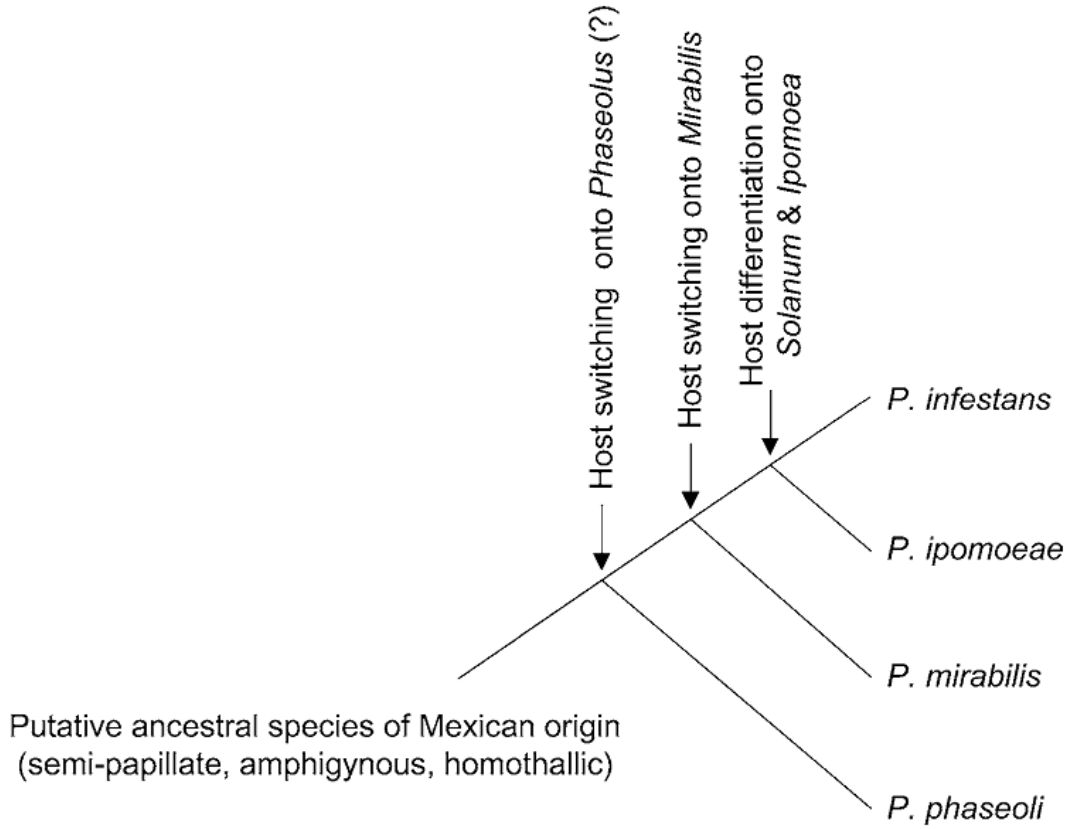


**Figure 1.** Co-evolutionary relationships of the foliar, Mexican *Pytophthora* species related to the potato late blight pathogen *P. infestans* (Grünwald & Flier, 2005)

### 2.5.1. The life cycle and epidemiology of *P. infestans*

#### 2.5.1.1. The life cycle

Because *P. infestans* is a host-specific parasite, it needs plant tissue to grow in. Its reproduces asexually and life cycle usually begins as the sporangia are carried by the wind, land on plant tissue, and release their zoospores (Marcin, 2013).This happens when the conditions are wet and cool and is called indirect germination. These spores are biflagellated—having both a tinsel and whiplash flagellum, which is characteristic of *oomycota* and will swim until they encyst in the host. When conditions are warmer, *P. infestans* infect the plant by direct germination—that is a germ tube form from the sporangium and penetrate the host tissue allowing it to gain nutrients from its host (Schumann & D'Arcy, 2000).

Sexual reproduction can and does occur when both mating types are present. The antheridium and oogonium (the only haploid parts in the life cycle of *P. infestans*) nuclei fuse together (*karyogamy*) when the *antheridium* enters the *oogonium* (Sawant, 2014). They will form a diploid oospores, which develop into a sporangium and the cycle will continue as is would asexually. As a result, the infected plants show signs of small brown or black lesions on the leaves or stems, but soon spread and kill the plant. The growth of sporangia on the surface of the leaves or stems makes is white, which is another warning sign of infection. Similar signs exist for the tomato plants (Schumann & D'Arcy, 2000).

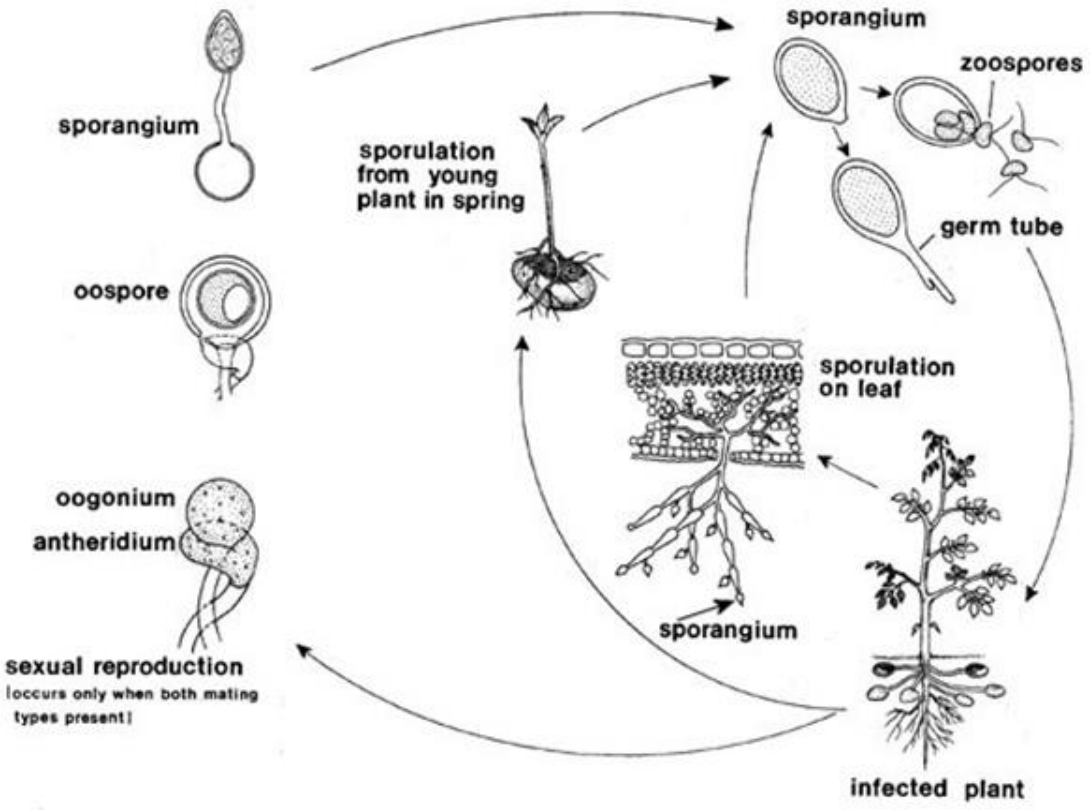


**Figure 2.** The disease cycle of *P.infestans* on potato(Marcin, 2013).

Temperature and moisture are the most important environmental factors affecting late blight development. Sporangia are formed on the lower leaf surfaces (Figure 2) and infected stems (Figure 4) when relative humidity is < 90%. Sporulation can occur from 3-26°C (37-79°F), but the optimum range is 18-22°C (64-72°F). Sporangia germinate directly via a germ tube at 21-26°C (70-79°F). Below 18° C (65°F), sporangia produce 6 to 8 zoospores which require water for swimming (Nelson, 2008).

Each zoospore is capable of initiating an infection, which explains why disease is more severe in cool, wet conditions. Cool nights, warm days, and extended wet conditions from rain and fog can result in late blight epidemics in which entire potato fields are destroyed in less than two weeks. The pathogen can sporulate on infected tubers in poorly controlled storage areas (Figure 2) where conditions are too humid. Condensation produces water droplets on the surface of infected tubers, which may then cause the pathogen to produce sporangia and contaminate neighboring tubers, leading to destruction of the entire pile by soft rot bacteria (Nelson, 2008).

### 2.5.1.2. Epidemiology of *P. infestans*

Hosts of *P. infestans* are limited to members of the *Solanaceae* (nightshade family) that encompasses eggplant, pepper, potato, petunia, tomato and some weedy members of the family, such as hairy nightshade *(Solanum sarrachoides).* Thus, *P. infestans* can survive in infected tomato, and volunteer plants growing from infected tomato during the following growing season can serve as a source of inoculum for new outbreaks of late blight. Sporangia of *P. infestans* can also be carried in wind currents for long distances to initiate disease outbreaks in new geographic areas; late blight is commonly spread to new locations by this means (Becktell *et al.*, 2005).

Leaves and fruits carrying the pathogen are the real carriers and serve as the source of the disease in the subsequent season. The pathogen sporulates on the primary lesions and the sporangia so formed are carried over by wind currents/rain splashes to other plants/fields, thereby setting a chain reaction. Fungal sporangia are also washed down to soil with rain water or dew and infect the new tubers. Sporagiospores produced on infected tomato and sporagiospores can travel through the air, land on infected plants, and if the weather is sufficiently wet, cause new infections. Sporagiospores can also be washed through the soil to infect Solanum crops, which may rot or damage tubers and/or fruits before harvest, or serve as source of inoculum for later spoilage in storage. This organism is well known for its 17 ability to produce millions of sporagiospores from infected plants under the wet weather conditions that favor the disease (Agrios, 2005; Fry, 2008; Kawchuk *et al.*, 2011).

The disease occurs commonly in most tomato and potato growing areas, depending on the presence of the pathogen and cool, wet weather conditions. Sources of *P. infestans* inocula are seed tubers, dumps, volunteers, closely related weed hosts and adjacent plants of potatoes or tomatoes that are affected by the pathogen. Sporagiospores released from infected plants are known to be capable of wind-borne migration over several kilometres. This pathogen can survive in plant debris and initiate the disease in subsequent years. Therefore, leaving crop debris in the field should be avoided and crop rotation exclusive of tomato, potato, eggplant and pepper for three or more years is recommended to reduce the pathogen populations in the field. Survival in infected tubers in the fields and piles or tomato seeds is considered to be the most important primary inoculums sources (Zwankhuisen *et al.*, 2000; Majid *et al.*, 2008).

*Phytophthora infestans* survives poorly in nature apart from its host plants. Under most conditions, the hyphae and sporangia can survive for only brief periods in plant debris or soil, and are generally killed off during frosts or very warm weather. The exceptions involve oospores, and hyphae present within tubers. The persistence of viable pathogen mycelium (or oospores) within tubers, such as those that are left in the ground after the previous year's harvest or left in cull piles, are major problems in disease management. This is because it might left inocula for the next season eather of it or its alternate host, Solanum crops. In particular, volunteer plants sprouting from infected tubers are thought to be a major source of inocula at the start of a growing season (Majid *et al.,* 2008). This can have devastating effects by destroying the entire crops.

Sporagiospores are produced during the night because they are sensitive to light. Cloudiness or heavy wetness following lower temperature favours disease development. Zoospore survival in soil is uncertain; sporangia remain infective to tomato seedlings for 15 - 77 days in different soils. Oospores are able to survive at least one winter in the field under European conditions. The keys to success of *P. infestans* epidemics are abundant sporulation, wind dissemination and long distance migration, persistent, tenacious pathogen and survival potential or ability on potato tubers or tomato plant parts in the field or in the storage (Majid *et al.,* 2008; Kawchuk *et al.,* 2011). Long-distance dispersal of *P. infestans* is associated with human transport of diseased plant material. Short-distance 18 dispersal of sporangia is through wind and rain splash (VegaSanchez *et al.,* 2000) with zoospores contributing to short distance spread of the disease.

### 2.5.2. Host range of disease and it symptoms on tomatoes

Although generally considered to have a limited host range and to be a near biotrophic pathogen, *P. infestans* has been reported to cause infection on a large number of species. Erwin and Ribeiro (1996) listed 89 host species, but more than 25% of these were included because artificial inoculations resulted in lesions. In agriculture, *P. infestans* is mainly limited to *solanaceous* crops, including tomato, nightshade *(Solanum nigrum),* and potato. Sweet potato*(Ipomea batatas)* is not a host for *P. infestans* (Nelson, 2008). The most important diseases of tomato plants caused by *phytophthora* species include Late blight and Buckeye rot. According to Sanoubar and Barbanti (2017), Late blight, usually appear in either young (upper) or old (lower) leaves in mid- or late August. It first appears as pale green water-soaked spots starting at leaf tips

that enlarge rapidly, forming irregular, greenish black blotches (Figure 3) that expand rapidly when leaves are wet or humidity is high. White mold usually develops at the margins of affected areas giving the plant a frost-damaged appearance may be rapidly defoliated when conditions favor the disease. If stems and petioles are infected, brown streaks along the stems will be presented and the areas above these infections wilt and die (Dixon, 1981). Infection of green or ripe fruit produces large, irregularly shaped brown blotches that usually start at the stem. Infected fruits rapidly deteriorate into foul-smelling masses (Nelson, 2008).

The initial symptom of Buckeye rot on the fruit is a grayish green or brown water-soaked spot that usually appears near the blossom end, or at the point of contact between the fruit and soil. The spot further enlarges and develops into a lesion with a characteristic target-like pattern of concentric rings of narrow dark brown and wide light brown bands resembling the markings on a buckeye (Figure 3). Buckeye fruit rot may produce a white, cottony fungal growth on the lesion under moist conditions (Sanoubar and Barbanti, 2017).


**Figure 3.** Late blight symptoms. **Where:A & D:** Leaf blight, **B, C, E & F**: Bukeyerot affecting both green and ripe tomato fruit (Sanoubar & Barbanti, 2017).

## 2.6. Management of tomatoes disease

Effective management of late blight requires a comprehensive approach. Currently different methods are being used and recommended like field sanitation to eliminate all early inoculums, crop rotation to break the disease cycle, proper planting space to allow proper aeration among the plants and proper sun light penetration, tool hygiene solarization to kill *P. infestans* in contaminated soils and other methods all can be mentioned. There is very little worked report on natural enemies of *P. infestans,* and none has been employed in managing this *oomycete.* The two most widely used and arguably, the most effective are host resistance and fungicides (Neshev, 2008).

### 2.6.1. Cultural control

Cultural control involves practices and farming techniques that will help to increase the quality and quantity of the yield and reduce the influence of pests and diseases. It involves manipulation of the environment in non-mechanical ways to control plants pests and diseases. It includes altering farming practices to make the environment unfavorable for the growth of disease pathogens and pests (Ajilogba & Babalola, 2013). It is also the purposeful manipulation of a garden or farm in the growing, planting and cultivation of plants to reduce plant disease, pest damage and numbers of pests. It has been shown that the correct implementation of cultural methods to control soil borne pathogens yields improved soil structure and consequently decreases disease incidence (Neshev, 2008). These methods are mainly preventive and a good knowledge of the nature, behavior and environmental conditions of the growth of the disease agent is very important to controlling the disease development. Some of the methods used in the cultural control of disease on tomato are shown in (Figure 4).

### 2.6.2. Physical control

Physical control like soil solarization and soil disinfection using heating and steam is done by spreading a clear plastic sheet over the soil for several weeks. This helps to trap solar energy, which in turn inhibits soil borne disease, nematodes, insects and many weed seeds. This is usually done during summer when the air temperature is high and there is intense radiation (McGovern, 2015).

Soil disinfection is usually done as a preplanting method. Hot water can be used according to and can keep the soil sterilized for up to three years. Steam can also be used especially in greenhouse conditions (Gullino *et al.*, 2003). Soil disinfection helps to keep the soil sterile and free from disease causing pathogens.


**Figure 4.** Some of the methods used in the cultural control of tomato fungal disease (Worku & Sahe, 2018)

### 2.6.3. Chemical control

Chemical control is the use of chemicals to control plant diseases (fungal, viral, bacterial and nematicidal), pest infestation and weeds. Some of these chemicals include prochloraz, propiconazole, thiabendazole, carbendazim, benomyl, thiophante, fuberidazole and all of the benzimidazoles (Qualhato *et al.*, 2013). The control of the disease by chemical is not satisfactory because generally the disease is controlled by preplant soil fumigation with methyl bromide. In addition toother potential health, safety and environmental risks, methyl bromide was classified as an ozone-depleting compound and has been banned from use (Gullino *et al.*, 2003). It may create imbalances in the microbial community, which may be unfavorable to the activities of the beneficial organisms and may lead to the development of resistant strains of pathogens. These

due to lack of reliable chemical control agents (Grünwald & Flier, 2005). Since the earliest observations of antagonist disease suppressing soil microorganisms more than 70 years ago, plant pathologists have been fascinated by the idea that such microorganisms could be used as environmentally friendly biocontrol agents both in the field and in greenhouses (Jakubikova *et al.,* 2006).

### 2.6.4. Biocontrol of Fungal Pathogens

Biological control of soil borne pathogens has been studied over 80 years, but most of the time it has not been considered commercially feasible. However, interest and research in this area increased steadily. There is a shift toward the important role of biological control in agriculture in the future (Suprapta, 2012).Microorganisms that can grow in the rhizosphere are ideal for use as biocontrol agents, since the rhizosphere provides the front-line defense for root against attack by pathogens (Sawant, 2014). Pathogens encounter antagonism from rhizosphere microorganisms before, during primary infection, and during secondary spread in the roots. In the suppressive soil to pathogens, microbial antagonism of the pathogen is especially great, leading to substantial disease control (Qualhato *et al.,* 2013).

The BCAs possess other traits such as rhizosphere competence, tolerance of fungicides, saprophytic competitive ability, and ability to tolerate high and low temperatures, adaptability to different conditions. They have a good searching ability, host specificity, high reproduction rate, short life cycle, adaptability, well adapted to different stages of life cycle of target host, able to maintain itself after reducing host population (Suprapta, 2012). These traits are useful for good BCA as they help in the establishment of the BCA in a given agro-ecological region. Among the most successfully used biological control agents in agriculture is *Trichoderma* spp. which are one among the BCAs and much of the registered biofungicides worldwide being *Trichoderma* based (Jakubikova *et al.,* 2006).

#### 2.6.4.1. *Trichoderma*

*Trichoderma* species is the soil borne non-phytogenic fungus. They are found in every soil but in the plant roots niche the population is quite high. The genus of *Trichoderma* contains the number of species and strains. The species of *Trichoderma* are filamentous which are widely distributed in nature. The habit of these species are saprophytic, faster growing, and easy to grow and

maintain, produce a high density of conidia which can enlarge their self-life (Jahan *et al.,* 2013). They have rapid growth and development, and produce a large number of enzymes, induced by the presence of phytopathogenic fungi (De la Cruz-Quiroz *et al.,* 2018). Its high tolerance to extreme environmental conditions and habitat, where fungi are the cause of various diseases, makes it an efficient agent of control equally; it can survive in media with high levels of pesticides and other chemicals. Their colonies grow as dark-green, yellow-green, with woolly flappy or appear as concentric ring. All these characteristics depend on the fungus and sometimes of the food such as media (Al-Hazmi & TariqJaveed, 2016).

The proper classification, taxonomy, and nomenclature of fungi are essential while authorities are sometimes in disagreement over taxonomic issues, the frequently changing mycologic nomenclature results from new insights into methods of conidiogenesis, phylogenetic relationships, new species, etc., and more precisely delineates etiologic agents. *Trichoderma* is a genus of fungi in a Family *Hypocreaceae*; Order *Hypocreales*; Class *Sordariomycetes*; Division *Ascomycota* (Kirk *et al.,* 2008). For a long time, it was considered to consist of only one species, *Trichoderma viride,* named for producing green. *Hypocrea* are teleomorphs of *Trichoderma* which themselves have *Hypocrea* as anamorphs (Samuels, 2006).

**Mechanism of action of *Trichoderma* species as bioagents**

*Trichoderma* have been reported as effective biocontrol agents against several soil-borne pathogens demonstrating diverse mode of biological actions to control plant pathogens. Their antagonistic mechanism to phytopathogenic fungi include colonization, antibiosis and direct mycoparasitism, secretion of lytic-enzymes, competition for space and nutrients, and induction of systemic resistance (Rincón *et al.*,2005; Suprapta, 2012; Qualhato *et al.*, 2013). This antagonistic potential serves as the basis for effective biological control applications of different *Trichoderma* strains as an alternative method to chemicals for the control of a wide spectrum of plant pathogens (Jakubikova *et al.*, 2006).

**Antibiosis:** Both volatile and non-volatile antibiotics are known to be produced from *Trichoderma* species (Okigbo & Ikediugwu, 2000; Rincón *et al.,* 2005). Peptaibols (trichorizianines, trichokindins, trichorzins, trichorozins and harzianins), a class of antibiotics, are produced by most species and strains of *Trichoderma.* Peptaibols are thought to act on the

membrane of the target fungus to inhibit membrane-associated enzymes involved in cell wall synthesis (Okigbo & Ikediugwu, 2000). The antibiotics trichodermin, trichodermol, harzianins A and harzianolide are also known to be produced from *T. viride* and other species of *Trichoderma* (Barbaso *et al.,* 2001).

**Lytic enzymes:** Antagonistic fungi exert parasitizing mechanism toward other fungi by applying lytic enzymes (Boch *et al.,* 2002), proteolytic enzymes (Qualhato *et al.,* 2013), ABC transporter membrane pumps (Ullah *et al.,* 2011), diffusible or volatile metabolites, and other secondary metabolites (Qualhato *et al.,* 2013). These lytic enzymes are probably responsible for hyphal lysis through the digestion of major cell wall components. As shown in Table 1, it is believed that these enzymes act synergistically with the antibiotics to inhibit the growth of fungal spore (Srinon *et al.,* 2006). It appears that the weakening of the host cell wall by the enzymes increases the rate of diffusion of the antibiotics through the cell wall.

**Table 1.** The enzymes activity assays of antagonistic fungi on agar plates

| **Antagonistic fungi** | **Enzyme activity assays** | |
|---|---|---|
| | **Cellulase (diameter of clear zone; cm)** | **Hemicellulase (xylanase)** |
| ***Nigrospora sp.*** | 2.04 | + |
| ***Penicillium sp.*** | 4.03 | + |
| ***T. harzianum*** | 1.56 | ++ |
| ***T. hamatum*** | 4.16 | +++ |
| ***T. hamatum*** | 3.58 | +++ |
| ***T. viride*** | 3.47 | ++ |
| ***G. virens*** | 2.88 | ++ |

Source : Secondary(Srinon *et al.,* 2006).

**Mycoparasitism:** Mycoparasitism occurs when one fungus exists in intimate association with another from which it derives some or all of its nutrients while conferring no benefit in return (Qualhato *et al.,* 2013; De la Cruz-Quiroz *et al.,* 2018). *Trichoderma* spp. are best-known mycoparasite species because it attacks a great variety of phytopathogenic fungi that are responsible for most important diseases of major economic importance worldwide (Kumar *et al.,* 2012). The mycoparasitic relationship between *Trichoderma* spp. and its potential host might involve biochemical and physiological interactions that lead the microscopically visible

phenomena of hyphal coiling, aspersorium formation, penetration and cytoplasmic degradation (Suprapta, 2012).

**Competition:** Competition is an indirect effect whereby pathogens are excluded by depletion of food bases or by physical occupation of sites (Suprapta, 2012). The study of Barbaso *et al.* (2001) in the in vitro antagonism of *Trichoderma* species on *Cladosporium herbarum* revealed that the colonies of *Trichoderma* species grew always faster than *C. herbarumin* single or mixed culture. Thus, the rapid growth of *Trichoderma* spp. gives it an important advantage in the competition for space and nutrients with plant pathogenic fungi (Barbaso *et al.,* 2001). In the rhizosphere competition for space as well as nutrients is one of major importance of microbial interaction. Thus, an important attribute of a successful rhizosphere biocontrol agent would be the ability to remain at high population density on the root surface, providing protection of the whole root for the duration of its life (Vargas *et al.,* 2009).

**Induction of host resistance:** Molecules produced by *Trichoderma* and/or its metabolic activity also have potential for promoting plant growth (Suprapta, 2012). Applications of *T. harzianum* to seed or the plant resulted in improved germination, increased plant size, augmented leaf area and weight, greater yields (Youssef *et al.,* 2016). Metabolic changes occur in the root during colonization by *Trichoderma* spp., such as the activation of pathogenesis-related proteins (PR-proteins), which induce in the plant an increased resistance to subsequent attack by numerous microbial pathogens (Malacca *et al.,* 1999). Such stimuli can either induce or condition plant host defenses through biochemical changes that enhance resistance against subsequent infection by a variety of pathogens. Induction of host defenses can be local and/or systemic in nature, depending on the type, source, and amount of stimuli. Induced systemic resistance (ISR) has been used to describe the systemic resistance induced against pathogens by nonpathogenic or plant growth-promoting rhizobacteria (Suprapta, 2012).

# 3. Materials and methods

## 3.1. Description of the study area and period

The study was conducted at Jimma University which is located in Jimma town, Oromiya Regional State. It is the largest city in southwestern Ethiopia located at 352 km southwest of the capital Addis Ababa. It has a GPS coordinate 7°40′N 36°50′E/7.667°N 36.833°E.

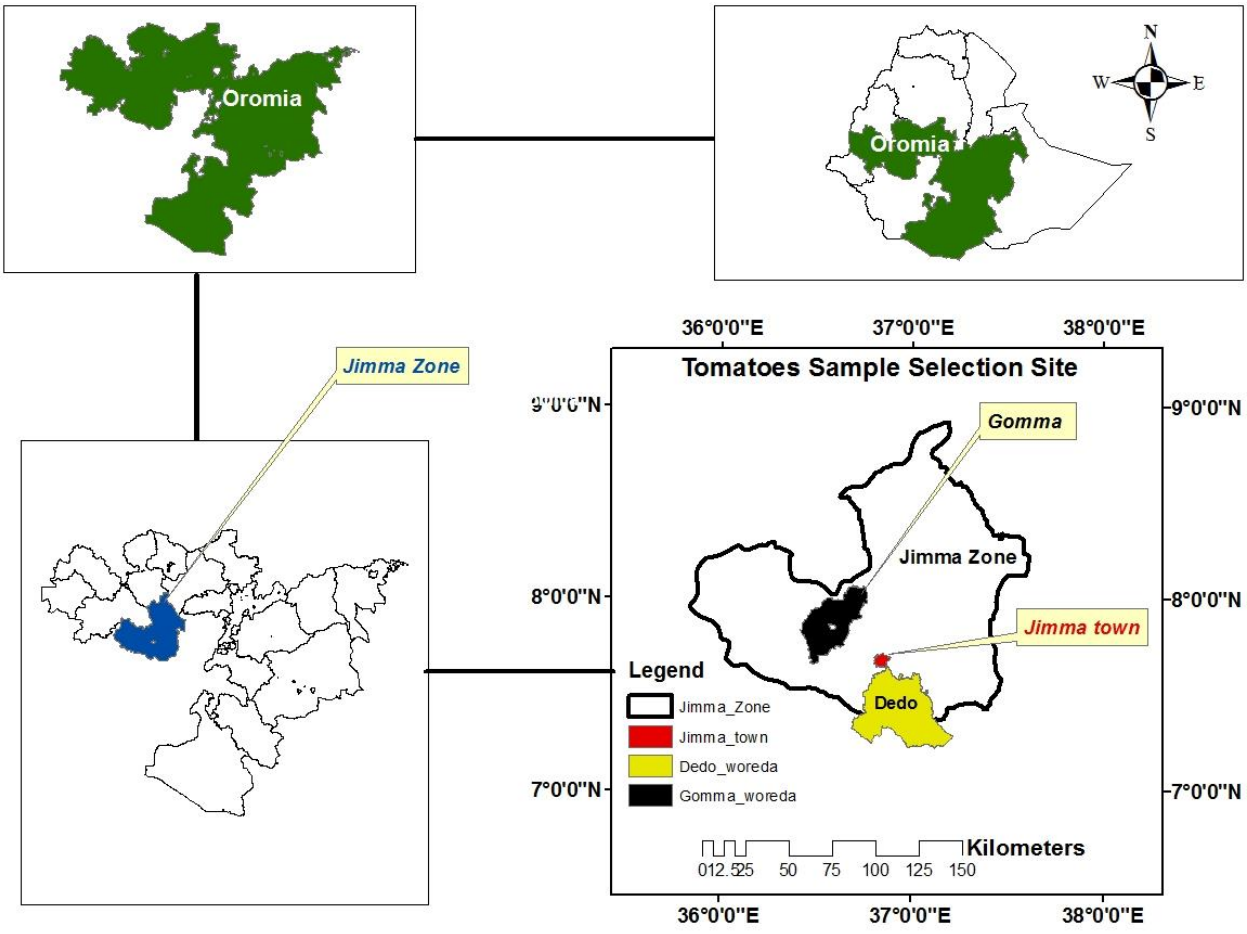


**Figure 5.** Map of study site

Based on the 2007 Census conducted by the Central Statistical Agency (2010), Jimma town has a total population of 120,960, of whom 60,824 were men and 60,136 women. With an area of 50.52 square kilometers, it has a population density of 2,394.30 all are urban inhabitants. A total of 32,191 households were counted in this Zone, which results in an average of 3.76 persons to a household, and 30,016 housing units. The research was carried out between the months of March and October 2019 at the Research and Postgraduate Laboratory, Department of Biology.

Greenhouse activities were conducted at the College of Agriculture and Veterinary Medicine, Jimma University. The tomato variety susceptible to late blight used in this study was the Moneymaker obtained from Melkasa Agricultural Research Center located near Adama town, Ethiopia.

### 3.3. Study design

Cross sectional study and experimental laboratory-based design were used to evaluate the antagonistic effect of *Trichoderma* isolates against the development of late blight caused by *P. infestans* on the growth and yield of tomato plants. The samples were obtained based on non-probability by using purposive sampling technique from local/commercial tomato plantation of Dedo and Goma which are known for large scale production of tomato.

### 3.4. Sample collection and transportation

A total of 40 samples (30 samples taken from tomato plants rhizospheric soil and 10 from plant's organs including leaves, stems and/or fruits infected with *P. infestan*) were collected. From Goma districts, 20 rhizospheric soils and 6 plant samples were collected and 10 soil and 4 plants were collected from Dedo district. For sampling, tomato plants with visible disease symptoms on their leaves or stems were sampled and soil sample were obtained from 10 cm depth of tomato plant rhizosphere using spatula. Each sample was taken and put into their respective polyethylene plastic bag. Thereafter, the samples were transported to Research and Postgraduate Laboratory for immediate analysis. Samples of soil were collected from the same field and pooled as one each making up four big samples.

### 3.5. Sample preparation and incubation

According to Schmitthenner & Bhat (1994), the plant tissues were washed thoroughly to remove any soil or loose plant debris and disinfected with 70% ethanol for 5 sec. The ethanol was rinsed off using sterile distilled water and allowed to dry. After air drying, small pieces were cut from advancing margin of rot or edge of a lesion and placed on carrot agar (Carrots 200.0 g, Distilled water 1000 ml and Agar 20.0 g) amended with chloramphenicol (250 mg). Twenty CA plates each inoculated with 4-5 tissue pieces from different organs of tomato plants were sealed with parafilm. Subsequently, CA plates were inverted for incubation at 18$^{\circ}$C-20$^{\circ}$C until the mycelial

growth was observed. After 3-7 days of growth on CA plates, the fungal mycelium and spores that grew out of the plant tissues were sub-cultured and purified on Potato Dextrose Agar (Potato infusion 200 gm, Dextrose, 20 gm, Agar 20 gm and Distilled water 1 liter) plates amended with chloramphenicol (250 mg).

The method used to isolate *Trichoderma* spp. from soil is a soil dilution technique (Jones & Stewart 1997). Dried and crushed soil samples (10 g) under room temperature in the Laboratory bench were added in 90 ml of sterile water for 10 min then left standing for a further 20 min. A dilution series was made up to $10^{-6}$. Aliquots (0.5 ml) were pipette and spread onto Czapek-dox agar (Sucrose 30.000, Sodium nitrate 2.000, Dipotassium phosphate 1.000, Magnesium sulphate 0.500, Potassium chloride 0.500, Ferrous sulphate 0.010, Agar 15.000 and Final pH 7.3±0.2) plates amended with chloramphenicol (250 mg) then incubated at 25°C for 1 week (Jones & Stewart 1997). Resulting colonies of fungal isolates on $10^{-4}$, $10^{-5}$ and $10^{-6}$ plates were picked and purified on PDA plates and were identified using standard mycological keys. The isolates were maintained on PDA slants and stored in the refrigerator at 4°C for further investigation. All the procedures were done on the sterilized media and glassware.

### 3.6. Isolation of fungal isolates

#### 3.6.1. Pathogen

The pathogenic fungi isolated from tomato plants leaves, stems and fruits were identified based on colony morphology and by the characteristics of sporangium, oospores and pathogenicity tests based on method described by Waterhouse (1963) and Stamps *et al.* (1990). For microscopic examination of *P. infestans*, the mycelia and spores were taken form 21 days old culture of CA plates using needle wire loop and then flooded with lactophenol cotton blue. The mounted slides were seen under the Light compound microscope for observations of spores and hyphae. The length: width of spores structure measurements were made using Piximetre 5.9 for confirmation. The identification of *Phytophthora* was based on the including sporangium morphology, morphology of sexual structures such as antheridia, oogonia and oospores, presence or absence of chlamydospores, morphology of hyphae and common host where the species of *Phytophthora* found.

### 3.6.2. Pathogenicity tests

For virulence tests of isolates, apparently healthy tomato leaflets of susceptible cultivar Moneymaker were taken from the upper third of 6-8 weeks old plants before flowering (Cohen, 2002). A suspension of sporangia prepared from one-week-old CA plates flooded with 1ml sterilized water then scraped with microscopic slide was dropped on abaxial side of each leaflet. The inoculated leaves were placed on the mesh beneath moisturized cloth then incubated at 15-18°C in the dark. After six day, their ability to cause infection was assessed and then the pure isolates obtained were maintained in PDA slants in screwed test tubes at 4°C.

### 3.6.3. Preliminary antagonistic tests and morphology of screened BCAs

The isolates suspected to be *Trichoderma* spp. and standard *Trichoderma*-JUT (obtained from College of Agriculture and Veterinary Medicine) were preliminary tested using dual culture techniques by taking 6 mm fungal agar blocks from the leading margin of each cultures. The agar disks of pathogen-antagonist were placed 4-5cm apart from each other at the center of pre-solidified PDA amended with chloramphenicol (250 mg). Pathogens alone in PDA plate was served as control and all the plates were incubated at temperature of $24^{o}C \pm 4$ for 9 days. Mycelial growth inhibitions of all the plates were measured for 9 days post inoculation. Three replications were done and the percentage of growth inhibition was calculated using the formula (Edington *et al.*, 1971).

$$I= (C-T)/(C\times100)$$

Where **I** is inhibition of radial mycelial growth; **C** is radial growth measurement of the pathogen in control; **T** is radial growth of the pathogen in the presence of *Trichoderma* isolates.

Isolates that show positive mycelial growth inhibition on dual culture medium were identified to it genus level and used for further investigations. From all the screened isolates, the 6mm mycelial discs of young actively growing culture were inoculated at the center of the PDA plates. All PDA plates were incubated at 24 ± 4°C. For morphological characterization of *Trichoderma* isolates, the colony radial growth of isolates were measured and observed for characters including the presence of pigments, green conidia, odor and colony appearance every day for one week (Samuels *et al.*, 2002). The lactophenol cotton blue was used as a stain for characterization

or observations of spore and mycelia under light compound microscope. The length× width of all the isolates' spores measurements were made for confirmation by using Piximetre 5.9.

## 3.7. Other antagonistic tests

The in vitro techniques including non-volatile compounds and volatile activities were carried out in order to evaluate the antagonistic effect of *Trichoderma* isolates against *P. infestans* in laboratory.

### 3.7.1. Non-volatile compounds activities of *Trichoderma*

The effect of non-volatile compounds of the fungal antagonists on the growth of pathogen was studied using the same method described in Dennis & Webster (1971). *Trichoderma* isolates were grown in potato dextrose broth (PDB) at room temperature with daily hand shaking. Metabolites were collected after 14 days, filtered through the Whatmann #1 filter paper, and centrifuged at 2000 rpm for 10 min. The filtrates were amended with molten PDA to make 5%, 10%, 20% and 40% concentration in flask containing molten PDA medium. Solidified PDA amended with *Trchoderma* filtrates were inoculated at the center with 6 mm diameter mycelial disc of *P. infestans.* The plates were incubated in dark at $24^{o}C \pm 4$ for one week. Plates without filtrate served as control. The experiment was replicated four times and percentage growth inhibition was calculated using the formula (Edington *et al.,* 1971).

$$I= (C-T)/(C\times100)$$

Where **I** is inhibition of radial mycelial growth; **C** is radial growth measurement of the pathogen in control; **T** is radial growth of the pathogen in the presence of *Trichoderma* isolates.

### 3.7.2. Volatile compounds activities of *Trichoderma*

The effects of volatile metabolites from *Trichoderma* isolates were tested using same method described in Dennis & Webster (1971). Fungal biocontrols were grown on PDA plates for 48 hrs. After the two days growth of antagonists, the lid of each plates were replaced with the bottom position of a plate dispensed with PDA inoculated with *P. infestans* at the center. The bottom of the plates containing centrally inoculated mycelial disc of *P. infestans* was kept inverted to the plates containing PDA media only which served as control. The pairs of each plate were attached

together with parafilm. An observation on the mycelial growth of the pathogen was recorded after 3-6 days of incubation at $24^{o}C \pm 4$. The colony diameter of the pathogen in the treatment in comparison with that of the control gave growth inhibition percent. The experiment was replicated four times and percentage growth inhibition was calculated using the formula (Edington *et al.*, 1971).

$$I= (C-T)/(C\times100)$$

Where **I** is inhibition of radial mycelial growth; **C** is radial growth measurement of the pathogen in control; **T** is radial growth of the pathogen in the presence of *Trichoderma* isolates.

### 3.8. Pot experiment

Tomato seeds of susceptible variety (moneymaker cultivar) were first washed successively with sterile distilled water and disinfected with 70% ethanol. Thereafter, the seeds were planted/seeded into their respective holes in growth trays for nursery rising. After three weeks, the seedlings rose in growth plastic trays were uprooted gently without damaging the tender roots and then completely dipped into the *Trichoderma* suspensions. Individual *Trichoderma* isolates and *P. infestans* were grown separately on PDA agar for 7 days and on CA agar for 10 days, respectively. From all the plates, the cells were harvested by scrapping colonies with sterile microscopic slide after drenching the plates with 10 ml sterile distilled water and the stock cultures were serially diluted to $10^{-5}$ spore suspensions (Nautial, 1997). The seedlings were transferred into 2 L plastic pots (1 seedling per pot) filled with soil sterilized by autoclaving for one day at $70^{o}$C in dry oven. Planting soil (consisted of top soil, sieved sand and manure in the ratio of 3:2:1). According to Nautial (1997), the sets were sprayed after one week 250, 000 spores/ml of $10^{-5}$ ml spore suspension of *P. infestans* in their respective pots. All the treatments were arranged on a greenhouse bench covering 9.5 m × 2 m in a Randomized Complete Block Design (RCBD) with three replicates for each treatment made up of 11 pots per replicate giving 33 pots (Table 2).


**Figure 6.** Spore suspensions to be inoculated **(A)** and tomato seedlings to be transplanted into pots **(B).**

After the inoculation of pathogen, The disease severity (D.S) was calculated based on the scale and formula by Wokocha, (1990) and the height of the plants such as shoot and root length were

taken viz. time points 1, 2 and 3 using a ruler and the number of leaves were counted physically and recorded for determination of late blight severity. The fresh weights of plants were taken and the dry weights were measured after 24 hrs. of incubation in dry oven adjusted to temperature about 70°C.

**0**=No visible disease symptom

**1**=less than 15% of leaves infected

**2**= 15%-35% of leaves infected

**3**=36%-49% of leaves infected.

**5**=more than 75% of leaves infected

$$\text{D. S} = \frac{\text{Number of unit assessed (affected leaves)in each category}(\boldsymbol{n})}{\text{Maximum numerical value of symptom category (5)}} \times \frac{100}{\text{Total number of assessed unit (all leaves)}}$$

The relative yield loss from each treatment was calculated using the formula suggested by Robert & James (1991):

$$\text{Relative \% yield loss} = \frac{\text{Y}_{\text{bt}} - \text{Y}_{\text{lt}}}{Y_{bt}} \times 100$$

Where, $Y_{bt}$ is the yield of best treatment and $Y_{lt}$ is the yield of lower treatments.

**Table 2.** Greenhouse design using factorial complete randomized block design (CRBD)

| **Block 1** | **Block 2** | **Block 3** |
|---|---|---|
| Mm + T4 + P | Mm + T4 | Mm + T5 |
| Mm + T4 | Mm + T2 + P | Mm + T3 + P |
| Mm + T1 + P | Mm + P | Mm + P |
| Mm + T3 | Mm + T5 + P | Mm + T2 + P |
| Mm + T5 | Mm + T4 + P | Mm + T3 |
| Mm + T1 | Mm + T3 + P | Mm + T1 |
| Mm + T5 + P | Mm + T1 | Mm + T5 + P |
| Mm + T2 +P | Mm + T1 + P | Mm + T1 + P |
| Mm + T2 | Mm + T2 | Mm + T2 |
| Mm + P | Mm + T5 | Mm + T4 + P |
| Mm + T3 + P | Mm + T3 | Mm + T4 |

Mm: moneymaker cultivar of tomato. T1, T2, T3, T4 and T5 are *Trichoderma* speices and P: *P. infestans* Ju-PGD-5

### 3.9. Statistical Analysis

The in vitro (dual culture assay, volatile and non-volatile activities) and greenhouse (late blight severity shoot and root lengths, fresh and dry weight, and marketable fruits yielded) data generated from all experiments were entered and arranged in excel before analysis. All the data were exported and subjected to one way analysis of variance (ANOVA) using the general linear model procedure (GLM) of the SAS 9.1 Statistical Package and the treatment means were calculated by (DMRT) Duncan's Multiple Range Test at $p \leq 0.05$.

# 4. Results

## 4.1. Morphology of Fungal isolates

### 4.1.1. Pathogen

Among 36 isolates obtained from diseased plant tissues, 29 showed typical *Oomycetes* like. Morphologically, they form a white colony and their respective sporangiums (1a) are distinctively semi-papillate and are usually borne terminally (Figure 4). They often produced white, profusely branching aseptate mycelium. They possess semi-papillate, caduceus sporangia with amphigynous antheridia and compound sympodial oospores and their oogonia (1b) were thick smooth walled (Figure 4). Their average sporangial length: Width were measured to be 117.7 × 95.5 and the average oogonial ratio 84.4 × 77.3 (Table 3).

### 4.1.2. Pathogenicity test

From a total isolated (29) suspected tested for their virulent, 18 strains were aggressive and violent on detached leaves.

### 4.1.3. Preliminary antagonistic tests and morphology of screened BCAs

From 102 fungal isolates from soil rhizosphere, 21 suspected to be *Trichoderma* spp. when compared with standard *Trichoderma*-JUT (obtained from College of Agriculture and Veterinary Medicine). After 9 days, 10 and *Trichoderma*-JUT antagonists inhibited the growth of *P. infestans* in a varying degree of inhibitions (Table 3). In three days, growth of pathogen in the presence of antagonists was suppressed by BCAs Ju-TGDb-1 (15.86%), Ju-TGDb-2 (15.1%), Ju-TGDb-3 (13.03%), Ju-TDc-2 (12.18%), Ju-TGDa-2 (10.2%), Ju-TDa-9 (13.3%), JUT (10%), Ju-TDd-4 (12.2%), Ju-TDa-11 (8.78%), Ju-TGDa-14 (6.23%) and Ju-TGDc-3 (.28%). Observation after six days, showed that BCAs Ju-TGDb-2 (43.4%), Ju-TDc-2 (42.7%), Ju-TGDb-3 (42%) and Ju-TGDb-1 (41.6%) inhibit above 40 percent compared to Ju-TGDa-2 (38.5%), Ju-TDa-9 (37.8%), Ju-TGDa-14 (34.2%), JUT (32.6%), Ju-TDd-4 (31.3%), Ju-TGDc-3 (28.2%) and Ju-TDa-11 (26.4%). The growth of BCAs-Ju-TGDb-2 (61.4%), Ju-TGDb-3 (60.5%), Ju-TDc-2 (60.9%) and Ju-TGDb-1 (60.1%) reduced the radial growth of the pathogen more than Ju-TGDa-2 (58%), Ju-TDa-9 (57.5%), Ju-TGDa-14 (55%), JUT (54%), Ju-TDd-4 (53%), Ju-TGDc-3

(51%) but Ju-TDa-11 (49.8%) showed low inhibition against the pathogen (Table 3).

The biocontrol agents' inhibition of radial growth of pathogen varied from 0.28 to 15.86% in 3 days, 26.4% to 43.4% in 6 days and increase to 49.8 to 61.4% in 9 days (Table 3). In the dual culture plates, the growth of *P. infestans* is lower than its growth in the single culture incubated under similar conditions and same durations. In isolate-Ju-TGDb-1, Ju-TGDb-2, Ju-TGDb-3, Ju-TDa-11, Ju-TGDc-3, Ju-TDc-2 and JUT-9 dual culture plates, the contact zone appeared as a curve, with concavity oriented towards pathogen while the mycelia of BCA-Ju-TGDa-2, Ju-TGDa-14, Ju-TDa-9 and Ju-TDd-4 grow over that of pathogen (Figure 7).

**Table 3.** The inhibition of *P. infestans* in the dual culture assay by BCAs

| **Treatments** | **3 days** | | **6 days** | | **9 days** | |
|---|---|---|---|---|---|---|
| | **Mean ± SD** | **%** | **Mean ± SD** | **%** | **Mean ± SD** | **%** |
| **Ju-TGDa-2** | $3.17 \pm .058^{c}$ | 10.2 | $3.37 \pm .058^{b}$ | 38.52 | $3.40 \pm .00^{bc}$ | 58 |
| **Ju-TGDa-14** | $3.31 \pm .138^{c}$ | 6.23 | $3.64 \pm .112^{b}$ | 34.17 | $3.64 \pm .112^{bc}$ | 55 |
| **Ju-TGDb-1** | $2.97 \pm .058^{b}$ | 15.86 | $3.23 \pm .058^{b}$ | 41.59 | $3.23 \pm .058^{b}$ | 60.1 |
| **Ju-TGDb-2** | $3.00 \pm .00^{c}$ | 15.01 | $3.13 \pm .058^{b}$ | 43.4 | $3.13 \pm .058^{c}$ | 61.4 |
| **Ju-TGDb-3** | $3.07 \pm .115^{bc}$ | 13.03 | $3.20 \pm .100^{b}$ | 42 | $3.20 \pm .100^{c}$ | 60.5 |
| **Ju-TDa-9** | $3.06 \pm .211^{bc}$ | 13.3 | $3.44 \pm .09^{b}$ | 37.8 | $3.44 \pm .09^{bc}$ | 57.5 |
| **Ju-TDa-11** | $3.22 \pm .54^{c}$ | 8.78 | $4.07 \pm .058^{bc}$ | 26.4 | $4.07 \pm .058^{bac}$ | 49.8 |
| **Ju-TGDc-3** | $3.52 \pm .058^{a}$ | 0.28 | $3.97 \pm .05^{bc}$ | 28.21 | $3.97 \pm .05^{bac}$ | 51 |
| **Ju-TDc-2** | $3.10 \pm .10^{bc}$ | 12.18 | $3.17 \pm .06^{b}$ | 42.7 | $3.17 \pm .06^{c}$ | 60.9 |
| **Ju-TDd-4** | $3.09 \pm .112^{bc}$ | 12.2 | $3.8 \pm .058^{bc}$ | 31.3 | $3.8 \pm .058^{bac}$ | 53 |
| **JUT-9** | $3.18 \pm .00^{c}$ | 10 | $3.73 \pm .058^{b}$ | 32.6 | $3.73 \pm .058^{bac}$ | 54 |
| **P** | $3.53 \pm .115^{a}$ | | $5.53 \pm .451^{a}$ | | $8.10 \pm .20^{a}$ | |

**Where:** Each value is the mean ± SD of 3 replicate. Ju-TGDa-2, Ju-TGDa-3, Ju-TGDb-1, Ju-TGDb-2, Ju-TGDb-3, Ju-TDc-2, Ju-TDa-9, Ju-TDa-11, Ju-TGDc-3, Ju-TDd-4 and JUT-9 are BCAs isolates and P: *P. infestans*. Mean with the same letter are not significantly different by Duncan's Multiple Range Test *($P < 0.05$).*

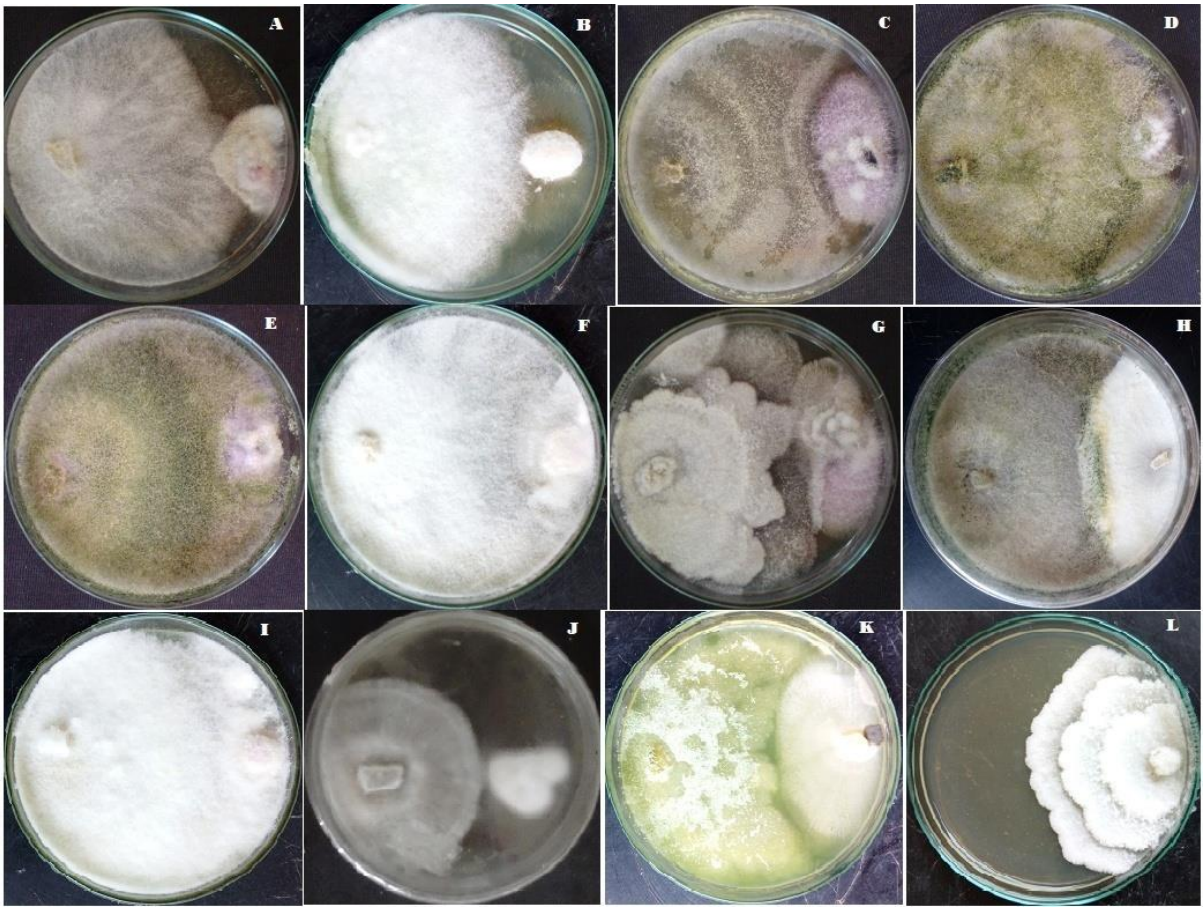


**Figure 7.** Antagonistic ability of five *Trichoderma* isolates on *P. infestans* after 6 days in terms of percentage inhibition of radial growth rate. Where: *Trichoderma*-Ju-TGDa-2 **(A),** Ju-TGDa-3 **(B),** Ju-TGDb-1 **(C),** Ju-TGDb-2 **(D),** Ju-TGDb-3 **(E),** Ju-TDc-2 **(F),** Ju-TDa-9 **(G),** Ju-TDa-11 **(H),** Ju-TGDc-3 **(I),** Ju-TDd-4 **(J)** and JUT-9 **(K)** and P: *P. infestan*-Ju-PGD-5 alone **(L).**

The BCAs Ju-TGDa-2, Ju-TGDb-1, Ju-TGDb-2, Ju-TGDb-3 and Ju-TDc-2 grow very fast on 9 cm PDA plates for only 4 days with mean ± SD of 9 ± .00 (Table 3). The mycelial characteristics of Ju-TGDa-2 and Ju-TGDb-1 were white, Ju-TGDb-2 and Ju-TGDb-3 were green and the isolates Ju-TDc-2 was yellow with white margin. They all possess septate hyaline hypae and the spores of all isolates were green (Figure 8). The average length: width (µm) of Ju-TGDa-2, Ju-TGDb-1, Ju-TGDb-2, Ju-TGDb-3 and Ju-TDc-2 spore was 22.6 × 20.4, 36.7 × 31.0, 29.8 × 25.5, 35.8 × 31.1 and 36.1 × 33.4 respectively (Table 3).

**Table 4.** Morphological characteristic of five screened *Trichoderma* isolates and *P. infestans*

| **Isolates** | **Mycelia** | | **Spore** | | |
|---|---|---|---|---|---|
| | **Color** | **Diameter** | **Types** | **Shape** | **Length: width (µm)** |
| **Ju-TGDa-2** | White | 9 ± .00 | Conidia | Ovoid | 22.6 × 20.4 |
| **Ju-TGDb-1** | White | 9 ± .00 | Conidia | Ovoid | 36.7 × 31.0 |
| **Ju-TGDb-2** | Deep green | 9 ± .00 | Conidia | Ovoid | 29.8 × 25.5 |
| **Ju-TGDb-3** | Green | 9 ± .00 | Conidia | Ovoid | 35.8 × 31.1 |
| **Ju-TDc-2** | Yellow to white | 9 ± .00 | Conidia | Circular | 36.1 × 33.4 |
| **Ju-PGD-5** | White | 8.87 ± .248 | Sporangia | Lemoniform | 117.7 × 95.5 |
| | | | Oogonia | Circular | 84.4 × 77.3 |

**Where:** Ju-TGDa-2, Ju-TGDb-1, Ju-TGDb-2, Ju-TGDb-3, Ju-TDc-2 are *Trichoderma* and Ju-PGD-5 is *P. infestans.*

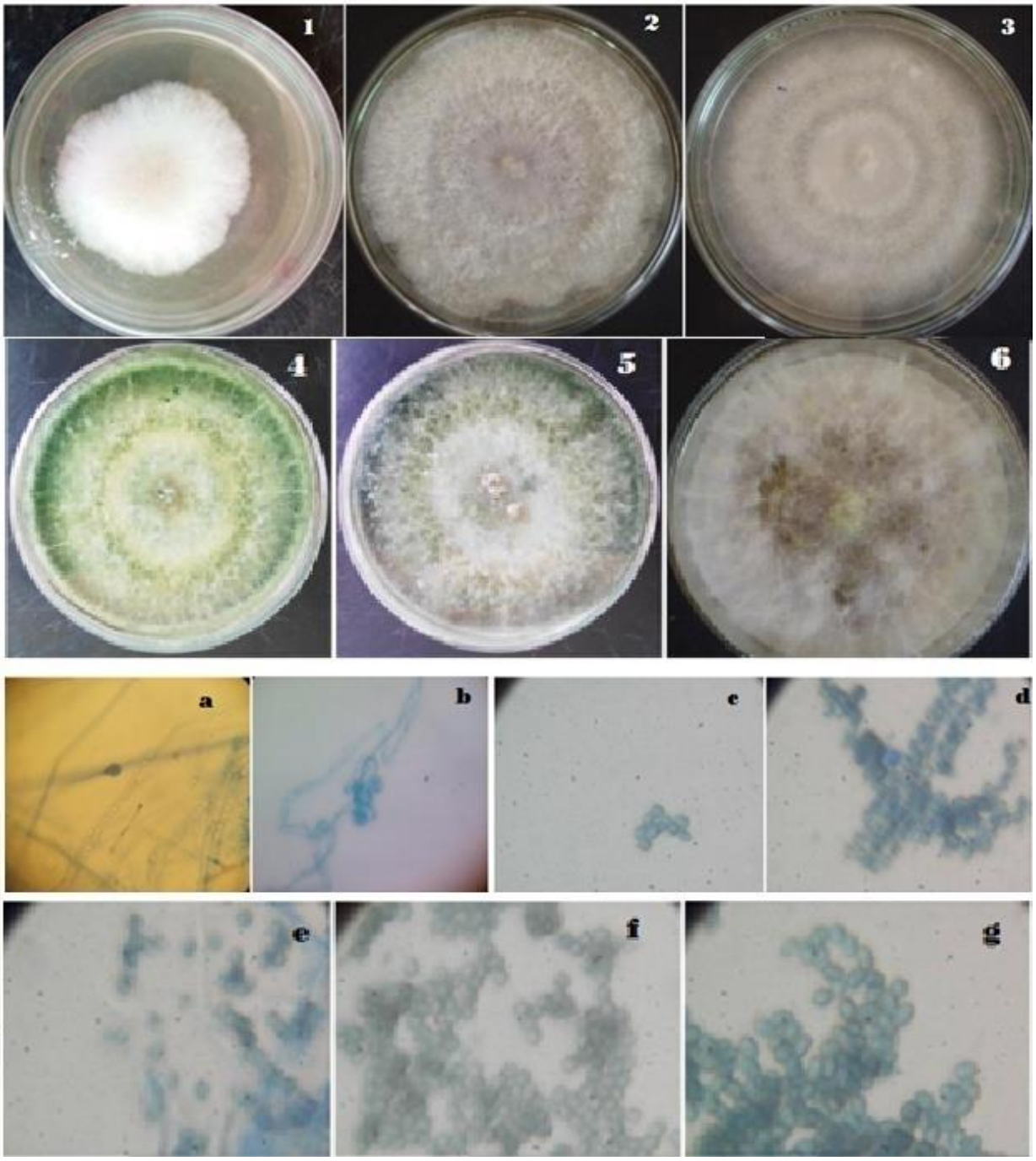


**Figure 8.** The morphology of *P. infestans* and *Trichoderma* isolates on PDA plates and spores. *P. infestans*-Ju-PGD-5 colony **(1)** sporangia **(a)** & oogonia **(b);** *Trichoderma*-Ju-TGDa-2 colony **(2)** & conidia **(c);** Ju-TGDb-1 colony **(3)** & conidia **(d);** Ju-TGDb-2 colony **(4)** & conidia **(e);** Ju-TGDb-3 colony **(5)** & conidia **(f);** and Ju-TDc-2 colony **(6)** & conidia **(g).**

## 4.3. Other vitro antagonistic tests

### 4.3.2. Non-volatiles compounds activities

The activities of non-volatile compounds of 10%, 20% and 40% of potential *Trichoderma* isolates successfully inhibit the extensions of pathogen's mycelia after only 6 days (Table 5). The effective isolates with high inhibitions activities in 10% filtrates were *Trichoderma*-Ju-TGDb-3, Ju-TGDa-2 and Ju-TDc-2 with 44.4%, 43.5% and 41.7%, respectively (Table 5). The 10% filtrate from *Trichoderma*-Ju-TGDb-2 and Ju-TGDb-1 were very low in inhibitions of the mycelial growth of the pathogen with 9.8% and 8% respectively. The 20% filtrates of *Trichoderma* isolates inhibitions on pathogen mycelial growth measured was more effective. The *Trichoderma*-Ju-TGDb-3, Ju-TGDa-2, Ju-TDc-2, Ju-TGDb-2 and Ju-TGDb-1 inhibited the growth of pathogen with 61.5%, 55%, 49.7%, 38.4% and 31%, respectively in their decreasing order (Table 5). In 40% of non-volatile compounds of *Trichoderma* isolates, the growth of pathogen mycelia was much reduced (Table 5). According to their order of inhibition, the *Trichoderma*-Ju-TGDb-3, Ju-TDc-2, Ju-TGDa-2, Ju-TGDb-2 and Ju-TGDb-1 inhibited with 63.9%, 60.9%, 57.7%, 44.4% and 24%, respectively (Table 5).

**Table 5.** The effect of different concentrations of non-volatile compounds of *Trichoderma* isolates on the mycelial growth of *P. infestans* after 6 days

| **Treatments** | **10%** | | **20%** | | **40%** | |
|---|---|---|---|---|---|---|
| | **Mean ± SD** | ***%*** | **Mean ± SD** | ***%*** | **Mean ± SD** | ***%*** |
| **Ju-TGDa-2** | $5.08 \pm .206^{b}$ | 9.8 | $3.47 \pm .096^{c}$ | 38.4 | $3.13 \pm .126^{c}$ | 44.4 |
| **Ju-TGDb-1** | $5.18 \pm .096^{b}$ | 8 | $3.88 \pm .126^{b}$ | 31 | $4.28 \pm .096^{c}$ | 24 |
| **Ju-TGDb-2** | $3.18 \pm .096^{c}$ | 43.5 | $2.53 \pm .096^{e}$ | 55 | $2.38 \pm .05^{d}$ | 57.7 |
| **Ju-TGDb-3** | $3.13 \pm .096^{c}$ | 44.4 | $2.17 \pm .096^{f}$ | 61.5 | $2.03 \pm .096^{e}$ | 63.9 |
| **Ju-TDc-2** | $3.28 \pm .126^{c}$ | 41.7 | $2.83 \pm .096^{d}$ | 49.7 | $2.2 \pm .082^{de}$ | 60.9 |
| **P** | $5.63 \pm .41^{a}$ | | $5.63 \pm .41^{a}$ | | $5.63 \pm .41^{a}$ | |

**Where:** Each value is the mean ± SD of 4 replicate. Ju-TGDb-2, Ju-TGDb-3, Ju-TDc-2 are *Trichoderma* isolates and P: *P. infestans* Ju-PGD-5. Mean with the same letter are not significantly different by DMRT *($P < 0.05$)*

In 10% filtrates of *Trichoderma*-Ju-TGDb-1 and Ju-TGDb-2, the percentages of inhibition were very low on the growth of pathogen, while they inhibited more when the concentration adjusted to 20% and 40% (Figure 9).

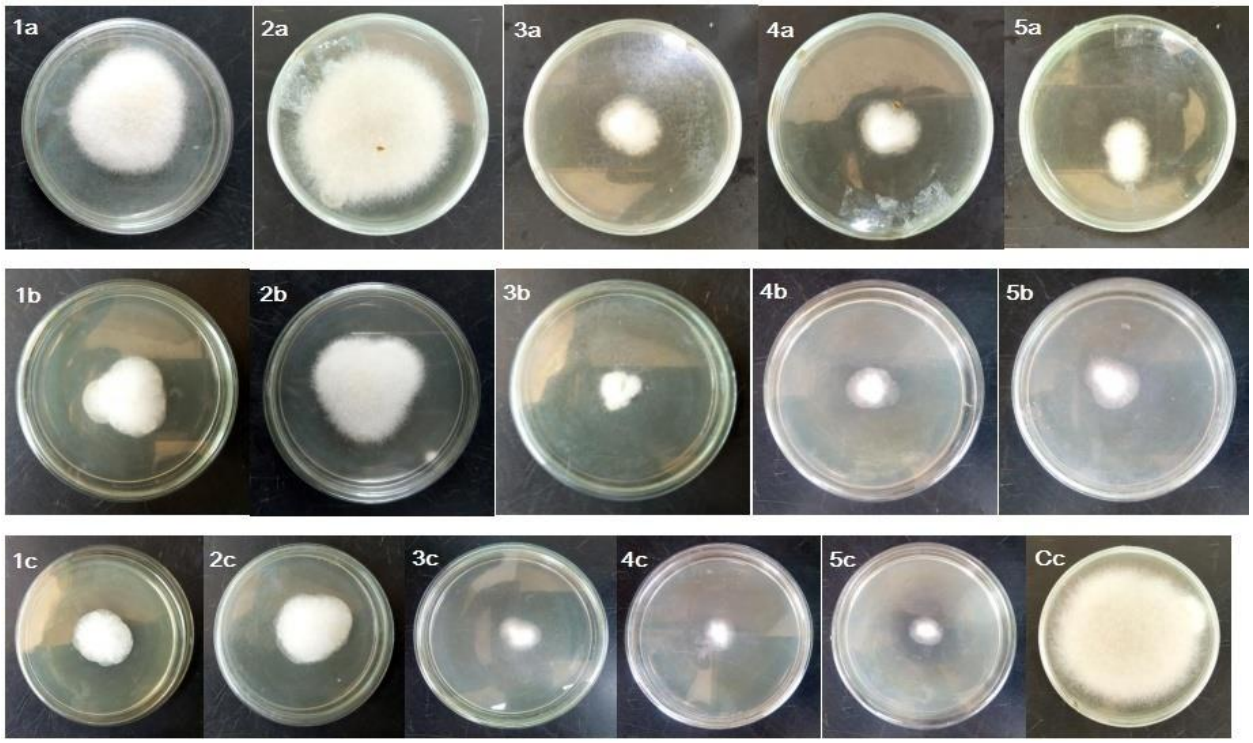


**Figure 9.** The effect of different concentrations of non-volatile compounds of *Trichoderma* isolates on the mycelial growth of *P. infestans*. **Where:** The 10% filtrates of Ju-TGDa-2 **(1a),** Ju-TGDb-1 **(2a),** Ju-TGDb-2 **(3a),** Ju-TGDb-3 **(4a),** Ju-TDWc-2 **(5a);** the 20% filtrates of Ju-TGDa-2 **(1b),** Ju-TGDb-1 **(2b),** Ju-TGDb-2 **(3b),** Ju-TGDb-3 **(4b),** Ju-TDc-2 **(5b)** and Ju-TGDa-2 **(1c),** Ju-TGDb-1 **(2c),** Ju-TGDb-2 **(3c),** Ju-TGDb-3 **(4c),** Ju-TDWc-2 **(5c)** and *P infestan*-Ju-PGD-5 **(Cc)** alone.

### 4.3.3. Volatiles compounds activities

The volatiles activities of most *Trichoderma* isolates were confirmed to successfully inhibit the extensions of pathogen's mycelia in only 3 days after the inoculation of BCAs (Table 6). After inoculation of pathogen, the effective isolates with high inhibitions were *Trichoderma*-Ju-TGDb-2 (42.5%), Ju-TDc-2 (37.2%) and Ju-TGDb-3 (19.1%), while Ju-TGDb-1 showed no inhibition of the mycelial growth of pathogen (Table 6). On day 6, the growth of pathogen reduced to 34.7%, 63.5%, 45.8% and 47.7% by *Trichoderma* Ju-TGDa-2, Ju-TGDb-2, Ju-TGDb-3 and Ju-TDc-2 respectively. Growth inhibitions of pathogen after 7 days were 35.8%, 64.1%, 46.6% and 48.6% in *Trichoderma* Ju-TGDa-2, Ju-TGDb-2, Ju-TGDb-3 and Ju-TDc-2, respectively (Table

6). The inhibition percentage varied between 42.5 to 2.5 in 3 days, 63.5 to 34.7% in 5 days and 64.1 to 35.8% in 6 days (Table 6).

**Table 6.** The effect of volatile compounds of *Trichoderma* isolates on the mycelial growth of *P infestans* 7 days

| Treatments | 5 days | | 6 days | | 7 days | |
|---|---|---|---|---|---|---|
| | **Mean ± S.D** | **%** | **Mean ± S.D** | **%** | **Mean ± S.D** | **%** |
| **Ju-TGDa-2** | $5 \pm .245^{b}$ | 2.5 | $5.78 \pm .096^{b}$ | 34.7 | $5.78 \pm .096^{b}$ | 35.8 |
| **Ju-TGDb-1** | $5.85 \pm .129^{a}$ | | $8.98 \pm .05^{a}$ | | $9 \pm 00^{a}$ | |
| **Ju-TGDb-2** | $2.95 \pm .526^{d}$ | 42.5 | $3.23 \pm .096^{d}$ | 63.5 | $3.23 \pm .096^{e}$ | 64.1 |
| **Ju-TGDb-3** | $4.15 \pm .191^{c}$ | 19.1 | $4.80 \pm .082^{c}$ | 45.8 | $4.90 \pm .082^{c}$ | 45.6 |
| **Ju-TDc-2** | $3.22 \pm .15^{d}$ | 37.2 | $4.63 \pm .369^{c}$ | 47.7 | $4.63 \pm .369^{d}$ | 48.6 |
| **P** | $5.13 \pm .189^{b}$ | | $8.85 \pm .129^{a}$ | | $9 \pm 00^{a}$ | |

**Where:** Each value is the mean ± SD of 4 replicate. Ju-TGDb-2, Ju-TGDb-3, Ju-TDc-2 are *Trichoderma* isolates and P: *P. infestans* Ju-PGD-5. Mean with the same letter are not significantly different by DMRT *($P < 0.05$).*

## 4.5. Pot experiment in greenhouse

The present of late blight were assessed on all tomato plants with difference treatments and the highest severity of late blight were recorded to be 19.2% in plants treated with only pathogen (Figure 10). The first symptoms of late blight on tomato leaves were irregularly shaped, water-soaked lesions mostly found on the younger in the top part of the plant canopy. The white cottony growths were visible on underside of some leaves. As the disease progresses, lesions enlarge causing leaves to brown and die. Late blight was also encountered in tomato fruits in all stages of development. Rotted fruit were greasy that eventually become leathery and chocolate brown in color; these spots enlarge to the point of encompassing the entire fruit. In plants treated with both BCAs and pathogen, the severity of late blight was higher in plants treated with *Trichoderma* Ju-TGDb-2 + P were 14.8% (Table 7).

The lower severity was seen in plants treated with *Trichoderma* Ju-TDc-2 + P, Ju-TGDa-2 + P and Ju-TGDb-3 + P with 8.8%, 12.1% and 12.8%, respectively (Table 7). The sign and symptoms completely absent in plants treated with *Trichoderma* Ju-TDc-2 treated plants. In some plants treated with only BCAs, the severity of disease appeared to be high as 13.1% in

plants treated with only *Trichoderma* Ju-TGDa-2 according to the study and low as 3.9 and 3.3% in plants treated with Ju-TGDb-3 + P and Ju-TGDb-1 + P (Table 7).

**Table 7.** The disease severity of tomato plants treated with *Trichoderma* isolates against the development of late blight in Greenhouse, 2019

| Treatments | No. of plants affected (affected leaves) | Total assessed units (All leaves) | Disease severity index (%) |
|---|---|---|---|
| **Ju-TGDa-2 + P** | $9.67^{bac}$ | 16 | 12.1 |
| **Ju-TGDa-2** | $7.33^{dcb}$ | 16.33 | 13.1 |
| **Ju-TGDb-1 + P** | $13.67^{ba}$ | 16 | 17.8 |
| **Ju-TGDb-1** | $2.67^{dc}$ | 16.33 | 3.3 |
| **Ju-TGDb-2 + P** | $12.33^{ba}$ | 16.33 | 14.8 |
| **Ju-TGDb-2** | $0^{d}$ | 14.67 | 0 |
| **Ju-TGDb-3 + P** | $10.67^{ba}$ | 16.67 | 12.8 |
| **Ju-TGDb-3** | $3^{dc}$ | 16 | 3.9 |
| **Ju-TDc-2 + P** | $7^{dcb}$ | 16 | 8.8 |
| **Ju-TDc-2** | $0^{d}$ | 15 | 0 |
| **P** | $15.33^{a}$ | 16 | 19.2 |
| **Mean** | 7.42 | 14.58 | 10.16 |
| **SD** | 6.245 | 4.918 | |

**Where:** Each value is the mean of 3 replicate. Ju-TGDa-2, Ju-TGDb-1, Ju-TGDb-2, Ju-TGDb-3, Ju-TDc-2 are *Trichoderma* isolates and P: *P. infestans*. Mean with the same letter are not significantly different at DMRT for Disease severity index *($p < 0.05$).*


**Figure 10.** The growth of tomato seedlings treated with different *Trichoderma* isolates. Where: **A:** Young plants, **B:** Plants selected, **C:** First sign of disease, **D:** Fully destructed leaves, **E:** Rotted fruits, **F:** Health plants, **G:** Health fruits & **H:** Total fruits yielded.

### 4.5.1. Measurement of Tomato growth

The highest shoot length was recorded to be 89.67 cm in plants treated with only *Trichoderma* Ju-TGDa-2 while the lowest was recorded to be 63.67 cm in those treated with only pathogen (Table 8). The highest score of root length were recorded to be 29.67 cm in plants treated with only *Trichoderma* Ju-TGDa-2 + P, Ju-TGDb-3 + P and Ju-TGDb-3, while shorter as 26 cm in those treated with *Trichoderma* Ju-TGDb-2. Therefore, the overall mean score of root and shoot lengths were 28.21 cm and 78.7 cm, respectively (Table 8).

**Table 8.** The shoot and root length of tomato plants treated with *Trichoderma* isolates in Greenhouse.

| Treatments | Shoot length (cm) | Root length (cm) |
|---|---|---|
| **Ju-TGDa-2 + P** | $75.67^{ba}$ | $29.67^{a}$ |
| **Ju-TGDa-2** | $89.67^{a}$ | $28.33^{a}$ |
| **Ju-TGDb-1 + P** | $80.67^{ba}$ | $28.33^{a}$ |
| **Ju-TGDb-1** | $85^{ba}$ | $27.33^{a}$ |
| **Ju-TGDb-2 + P** | $83^{ba}$ | $26^{a}$ |
| **Ju-TGDb-2** | $75.67^{ba}$ | $28^{a}$ |
| **Ju-TGDb-3 + P** | $74.33^{ba}$ | $29.67^{a}$ |
| **Ju-TGDb-3** | $73.67^{ba}$ | $29.67^{a}$ |
| **Ju-TDc-2 + P** | $83.67^{ba}$ | $27.67^{a}$ |
| **Ju-TDc-2** | $80.67^{ba}$ | $28.67^{a}$ |
| **P** | $63.67^{b}$ | $27^{a}$ |
| **Mean** | 78.7 | 28.21 |
| **SD** | 7.069 | 1.187 |

**Where:** Each value is the mean of 3 replicate. Ju-TGDa-2, Ju-TGDb-1, Ju-TGDb-2, Ju-TGDb-3, Ju-TDc-2 are *Trichoderma* isolates and P: *P. infestans*. Mean with the same letter are not significantly different at DMRT for shoot and root length *($p < 0.05$).*

### 4.5.2. Fresh and Dry Weight

The mean gram fresh weight of all tomato plants cultivar was 104.11g, while the dry weight was 28.44 g (Table 9). In plants treated with both BCAs and pathogen, the highest fresh and dry weight recorded was 127.67 g and 35.5 g, respectively in *Trichoderma* Ju-TDc-2. The lowest fresh and dry weights recorded were to be 50g and 12g in plants treated with pathogen only.

**Table 9.** The fresh weight and dry weight of tomato plants treated with *Trichoderma* isolates in Greenhouse.

| Treatments | Fresh weight (g) | Dry weight (g) |
|---|---|---|
| **Ju-TGDa-2 + P** | $92.67^{ba}$ | $20^{bc}$ |
| **Ju-TGDa-2** | $101.67^{ba}$ | $25.67^{bac}$ |
| **Ju-TGDb-1 + P** | $98.83^{ba}$ | $30.67^{bac}$ |
| **Ju-TGDb-1** | $137.5^{a}$ | $34.83^{ba}$ |
| **Ju-TGDb-2 + P** | $106.5^{a}$ | $25.33^{bac}$ |
| **Ju-TGDb-2** | $109^{a}$ | $30.67^{bac}$ |
| **Ju-TGDb-3 + P** | $95.1^{ba}$ | $20.5^{bc}$ |
| **Ju-TGDb-3** | $110.33^{a}$ | $34^{ba}$ |
| **Ju-TDc-2 + P** | $115.5^{a}$ | $35.5^{ba}$ |
| **Ju-TDc-2** | $127.67^{a}$ | $43.67^{a}$ |
| **P** | $50^{b}$ | $12^{c}$ |
| **Mean** | 104.11 | 28.44 |
| **SD** | 22.438 | 8.862 |

**Where:** Each value is the mean of 3 replicate. Ju-TGDa-2, Ju-TGDb-1, Ju-TGDb-2, Ju-TGDb-3, Ju-TDc-2 are *Trichoderma* isolates and P: *P. infestans*. Mean with the same letter are not significantly different at DMRT for fresh weight and dry weight *($p < 0.05$).*

### 4.5.3. Relative yield loss of tomato plants

The highest grams yielded were recorded to be 29.47 g in plants treated with only *Trichoderma* Ju-TGDb-3 (Table 10). Plants treated with *Trichoderma* Ju-TDWc-2 and pathogen yielded 17g more compared to Ju-TGDb-2 + P (12.83 g), Ju-TGDa-2 + P (10.30 g), Ju-TGDb-3 + P (9.17 g) and Ju-TGDb-1 + P (1.17 g), while the plants treated with only pathogen yielded none (Table 10). The relative percent yield loss were recorded to be 54.6%, 95.3%, 43.4%, 68.9%, 33.3% and 100% in plants treated with *Trichoderma* Ju-TGDa-2, Ju-TGDb-1, Ju-TGDb-2, Ju-TGDb-3, Ju-TDc-2 and P (Figure 11).

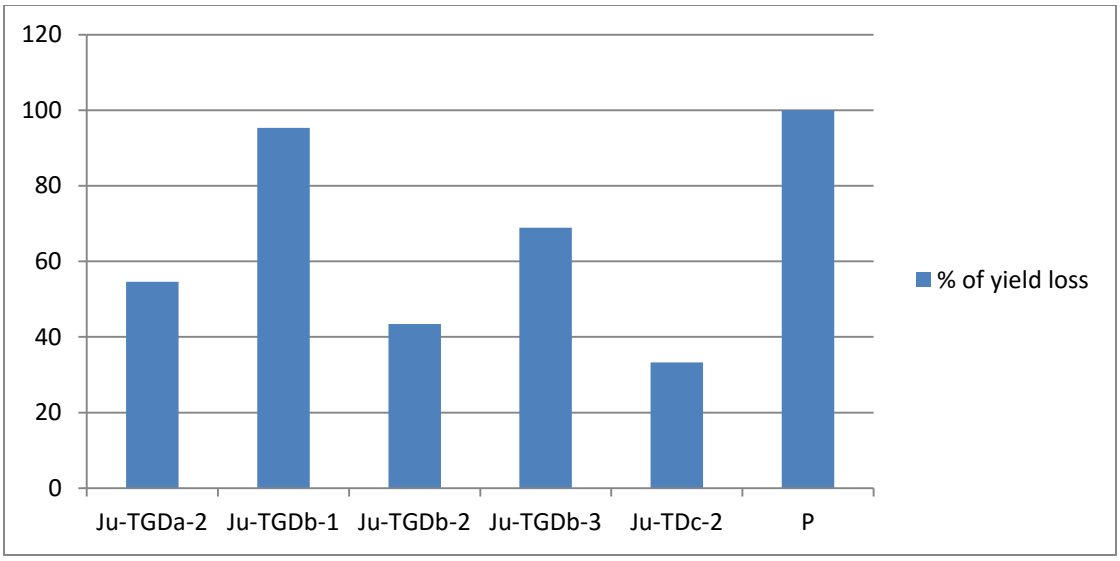


**Figure 11.** The yield of tomato plants treated with *Trichoderma* isolates in Greenhouse. Where: Each value is the mean of 3 replicate. Ju-TGDa-2, Ju-TGDb-1, Ju-TGDb-2, Ju-TGDb-3, Ju-TDc-2 are *Trichoderma* isolates and P: *P. infestans*.

## 5. Discussion

According to the study, the *Phytophthora infestans* was isolated from leaves, stems and fruits of tomato plants successfully. It exists in tomato plants by producing of white, profusely branching, aseptate mycelium, sporangia, and oospores. The pathogen has semi-papillate, caduceus sporangia with amphigynous antheridia and compound sympodial sporangiophore similar to the finding by Loliam *et al.* (2012) and Sendall & Drenth, (2001). According to the host from which it was isolated, the oomycetes suspected to be *Phytophthora* isolated from diseased tissue can be assigned to be *P. infestans.*

*Trichoderma* spp. that are common saprophytic fungi found in almost any soil and rhizosphere micro flora, have been investigated as potential biocontrol agents because of their ability to inhibit plant pathogenic fungi, particularly many common soil borne pathogens. In this study, the potential *Trichoderma* isolates were successfully isolated from tomato plants rhizospheric soil. They are abundant around plants roots primarily not to protect plants but looking for shelter and adherence to the fast developing root of plants. Plants roots are their important source of exudates releases which are the main source of nutrition. From plants rhizosperic soil, the is were confirmed as *Trichoderma* spp. because of their fast growth over pathogen, small conidia, color which varied from white and light green to dark green, and the presence of hyaline septate hyphae. The identification was carried out according to the morphological and microscopic characteristics followed by the online identification key provided by Samuels (http://nt.arsgrin.gov/taxadescriptions/keys/FrameKey.cfm?gen=Trichoderma). In this study, the chlamydospores though to be detected for characterization were not encountered from all the six isolates of *Trichoderma*. According to Haggag & Abo-Sedera (2005), all *Trichoderma* spp. tested form either conidiospore or chlamydospore during incubation in peanut haulms compost in comparison with peat, not on PDA. The morphological characteristics were generally found to be highly variable (Kucuk & Kivanc 2004). This is in accordance with Druzhinina & Kubicek (2003) that morphological analyses is highly prone to error and consequently roughly 50% of the *Trichoderma* spp. deposited in culture collection under names obtained by morphological analyses alone are wrong. The authors further stated that using gene sequence analysis is becoming more and more popular and can complete most of the phylogenetic free analysis.

In dual culture assay, the colonies of *Trichoderma*-Ju-TGDa-2, Ju-TGDb-3, Ju-TDc-2 and Ju-TGDb-2 were confirmed to be better BCAs. They regarding as good BCAs according to Kannangara *et al.* (2017) who indicates that the BCAs with higher inhibition (≥40%) are good antagonists. These isolates grow faster with time giving them an important advantage in the competition for space and nutrients with plant pathogenic fungi. Similarly, the *Trichoderma harzianum* and *Trichoderma viride* were the best antagonists for growth inhibition against several plant pathogens with 60%-80% inhibition (Rahman *et al.,* 2009; Siameto *et al.,* 2010). Herath (2015) has reported that the radial growth of tested pathogen was reduced by *Trichoderma erinaceum* with greatest reduction occurring in *Rhizotonia solani* by 72.66%. *Trichoderma harzianum, Trichoderma virens* and *Trichoderma viride* are known to have strong antifungal activities against a range of plant pathogens (Lunge & Patil, 2012; Imran *et al.,* 2012). Shaigan *et al.* (2008) have found that among five species of this genus, *Trichoderma viride* had greater inhibition than *Trichoderma harzianum, Trichoderma longibrachiatum Trichoderma paraseramosum,* and *Trichoderma hamatum.* In addition, Amin *et al.* (2010) have reported that *Trichoderma viride* highly inhibited the mycelia growth of *Rhizotonia solani, Sclerotium rolfsii* and *Sclerotinia sclerotiorum* in comparison with *Trichoderma harzianum.*

The non-volatile compounds activities of 10%, 20% and 40% of *Trichoderma* isolates successfully inhibit the growth of pathogen's mycelia. Their inhibition ability of pathogen increased with the dilution factors. Seshagiri & Eswaran (2002) have reported that mycelial growth of pathogen decreases with the increase in the concentration of the culture filtrate produced by fungal antagonists from 5% to 40% and no growth at 50%. The maximum inhibition of the mycelia growth of *Macrophomina phaseolina* was observed with the culture filtrate of *Trichoderma harzianum* used as 40% concentration (Devi, 2011). The culture filtrates of *Trichoderma viride* inhibited mycelial growth of *Sclerotinia sclerotiorum* due to production of an antibiotic like substance (Shafi *et al.,* 2017). It is important to mention that *Trichoderma* spp. are known to produce a number of antibiotics such as Trichodernin, Trichodermol, Harzianum A and Harzianolide (Dennis and Webster, 1971; Kucuk & Kivanc, 2004) as well as some cell walls degrading enzymes, thereby destroying cell wall integrity (Elad, 2000)). These may also play a major role in mycoparasitism because of changes in cell wall integrity.

The volatile compound produced by *Trichoderma* isolates Ju-TGDa-2, Ju-TGDb-2 and Ju-TGDb-3 is toxic and effectively inhibited the growth of *P. infestans* mycelia above 40% concentration. This activity is one way that made *Trichoderma* spp. better BCAs against phytopathogen. These isolates could grow well and distantly stop the extension of mycelial growth of pathogen by production a plenty of active volatile compounds. They can produce volatile compounds that inhibit the growth of plant pathogenic fungi via air diffusion (Minerdi *et al.,* 2009; Kishmoto *et al.,* 2007; Ryu *et al.,* 2003). Vinale *et al.* (2009) have reported that volatile secondary metabolites play a key role not only in mycoparasitism by *Trichoderma harzianum* and *Trichoderma atroviride*, but also in their interactions with tomato and canola seedlings. Stoppacher *et al.* (2010) have reported that the activities of volatile compounds produced by *Trichoderma* species include alcohols, ketones, alkanes, furanes, pyrones, and terpenes, which have varying degrees of antagonistic activity against pathogenic fungi and are known to be produced by *Trichoderma gamsi.* Compounds such as *n*-alkanes, cyclohexane, cyclopentane, fatty acids, alcohols, esters, sulfur containing compounds, simple pyrane and benzene derivatives have been identified on cultures of *Trichoderma Harzianum* and *Trichoderma atroviride* (Meena *et al.,* 2017).

In greenhouse, the late blight infection is mostly encountered in upper part of the tomato plants because of the presence of essential nutrients required for their growth and survival. It infection is high in the green parts of the tomato plants esp. leaves, stem and fruits due to large concentration of solinin . In spite of less application of *Trichoderma* as biocontrol agent of foliar diseases there is a fact that *Trichoderma* species have a beneficial effects in controlling phytopathogen in the greenhouse/field (Blakeman & Fokemma, 1982). This study showed that the isolates Ju-TDc-2, Ju-TGDa-2 and Ju-TGDb-3 of *Trichoderma* effectively reduce the severity of late blight. They effectively promoted growth, fresh and dry weights as well as the yields of tomato plants. Despite an environment highly conducive to *P. infestans* infection, lesion development in tomato plants treated with Ju-TDc-2, Ju-TGDa-2 and Ju-TGDb-3 was delayed or less than that observed for the control with only pathogen. This could be attributed to the ability of the antagonistic isolates to survive on the plants for a long period in the test conditions. It is showed that three antagonists were able to colonize the planting material when applied as a dip thereby made the plants resistant to pathogen. This beneficial effects have been attributed to many factors such as promotion of plant growth by improvement on water and nutrient uptake,

promotion of plant resistance and effects mediated by environmental factors (Li *et al.,* 2018; Jogaiah *et al.,* 2018; Animashaun *et al.,* 2017). These mechanisms have been favored by the ability of *Trichoderma* for colonizing plant roots (Charoenrak & Chamswarng, 2016) which can even reduce foliar diseases (Harman *et al.,* 2004). Some *Trichoderma* species are capable to improve soil conditions for root development by reducing plant stress (Shoresh *et al.,* 2005; Youssef *et al.,* 2016) and by activation of physiological and biochemical mechanisms to induced resistance to pathogens by plants (Hanson & Howell, 2004; Harman *et al.,* 2004; Hoitink *et al.,* 2006). The study by Hoitink *et al.,* (2006) revealed that inoculants of *Trichoderma* spp. reduce the disease by inducing systemic resistance in plants against aggressive foliar *Phytophthora* spp. and they are known to induce systemic resistance (ISR) in plants against fungal diseases (Sawant, 2014). Apart from parasitism, *Trichoderma harzianum* T39 has shown to be an elicitor of systemic resistance in plants as soil applications of live spores in the root zone or even the application of dead cells could provide reduction in foliar diseases (Elad, 2000). The relative percent yield loss was recorded to be higher in plants treated with only pathogen than those treated with both bio agent and pathogen followed by plants treated with *Trichoderma* Ju-TGDb-1, while the yield loss in plants treated with *Trichoderma* Ju-TGDb-2 and Ju-TDc-2 is low.

According to Anees *et al.* (2010), the same *Trichoderma* species can exhibit different abilities to control the same pathogen in different occasions. The presence of different in inhibition of the same species at different situations could be due to the distinctive biological control mechanisms established by the isolates (Anees *et al.,* 2010). Another possible explanation for this situation is that the responsible gene expression efficiency of biological control abilities may be different in different isolates. The isolates that can express these genes more rapidly and efficiently are usually better biological control agents than the other isolates (Harman, 2006). In contrast to isolates *Trichoderma* Ju-TGDa-2, Ju-TGDb-3 and Ju-TDc-2 which were more effective against pathogen, the study also confirmed that *Trichoderma* isolates Ju-TGDb-1 and Ju-TGDb-2 were less or non-effective in enhancing the growth of plants, which led to loss of yields. According to Bal & Altintas (2008), application of *Trichoderma harzianum* did not increase the yield of tomato plants. This is because of the environmental parameters such as abiotic (soil type, soil temperature, soil pH, and water potential) and biotic (plant species and variety, microbial activity of the soil) factors as well as other factors such as method and timing of applications may have influenced the biological control efficacy of *Trichoderma* isolates

(Hajieghrari *et al.*, 2008). As it has been mentioned, the responses varied according to the species of *Trichoderma* and even the beneficial effect of *Trichoderma* is also modulated by the tomato plant genotype (Tucci *et al.*, 2011).

# 6. Conclusion and recommendations

## 6.1. Conclusion

This investigation confirmed the use of *Trichoderma* spp. in controlling the tomato late blight pathogen *(P. infestans)*, which is one of the plant pathogens that cause serious destruction of tomato plants. The work indicated that *Trichoderma* antagonists could be isolated from tomato growing fields, while *P. infestans* are isolated from the tomato plants leaves, stems and fruits. The dual culture techniques revealed that the *Trichoderma* Ju-TGDa-2, Ju-TGDb-1, Ju-TGDb-2, Ju-TGDb-3 and Ju-TDc-2 are good BCAs because they grow faster. They effectively inhibit the extension of *P. infestans* mycelia by producing mycelia which rapidly occupy the most parts of the plates to restrict the pathogen growth. The non-volatile compounds of *Trichoderma* Ju-TGDa-2, Ju-TGDb-2, Ju-TGDb-3 and Ju-TDc-2 produce antifungal which suppress the growth of pathogen. Volatile compounds of *Trichoderma* Ju-TGDa-2, Ju-TGDb-2, Ju-TGDb-3 and Ju-TDc-2 are highly toxic to other microbes. This demonstrated that the power of these antagonists act by the secretion of diffusible compound which are able to distantly stop the development of the pathogenic agent. *Trichoderma* isolates Ju-TDc-2, Ju-TGDa-2 and Ju-TGDb-3 reduce the severity of late blight as well as enhancing the growth of tomato plants. The *Trichoderma* Ju-TGDb-2 and Ju-TDc-2 are confirmed to be the most effective because they reduce the relative percent yield loss. They improve the growth of plant by making them resistance to reduce late blight which is one of the foliar diseases. They are capable to improve soil conditions for root development which in turn reducing plant stress.

## 6.2. Recommendations

The follow key points are recommended in order to improve the growth and yields of tomato plants:

- It is important that the potential *Trichoderma* isolates should be further evaluated for their safety to human.
- The protocol and their applications should be formulated and evaluated in fields.
- More work is needed to genetically improve these potential *Trichoderma* isolates against the development of late blight.

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## Appendix 1: Raw Data

**Appendix 1.** Colony morphologies and microscopic observations of *Trichoderma* isolates isolated from soil of tomato plants' rhzhospheres

| Codes | Colony color | | | | | Microscopic observation | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **2 days** | **3 days** | **4 days** | **5 days** | **Colony reverse color** | **Hyphae** | **Phialide** | **Conidia** | **Chlamydospore** |
| **Ju-TGDa-2** | White 1 ring | White 1 ring | White with 3 rings | White 3 rings | Colorless | Septate hyaline | Evenly branched | Light round | Not recorded |
| **Ju-TGDa-14** | White | White | White margins | White | Uncolored | Septate hyaline | Unevenly branched | Light oval-round | Round, oval black |
| **Ju-TGDb-1** | White cottony | White | Green | Green | Colorless | Septate hyaline | Long gaped evenly branch | Light round | Not recorded |
| **Ju-TGDb-2** | White cottony at center | White green | 3 green circular rings | 3 green circular rings, hyaphae raised | Colorless | Septate hyaline | Long gaped | Light oval-round | Round dark black |
| **Ju-TGDb-3** | Crystalline white at center | White to light green | 3 green circular rings | 3 green circular rings with white margin, hyaphae raised | Dull yellowish | Septate hyaline | Short evenly branched | Light oval-round | Not recorded |
| **Ju-TGDc-3** | White cottony at center | White | White | White | Uncolored | Septate hyaline | Long gaped | Light gray-dark ovoid | Not recorded |
| **Ju-TDd-4** | White at the center | White | White | White cottony | Uncolored | Septate hyaline | Evenly branched | Round light | Rounded black |
| **Ju-TDa-9** | White | White | 3 green rings | 3 green rings | Colorless | Septate hyaline | Branched | Round light | Rounded black |
| **Ju-TDc-2** | White cottony at center | White | White | White with yellow pigments at the surface | Yellow | Septate hyaline | Long gaped evenly branch | Light round | Not recorded |
| **Ju-TDa-11** | White | White | Deep green | Deep green | Colorless | Septate hyaline | Evenly branched | Round light | Not recorded |
| **JUT-9** | White cottony at center | White | White | Yellow with White pigments at the surface | Yellow | Septate hyaline | Long gaped evenly branch | Light round | Not recorded |

**Source:** primary (2019)

**Appendix 2.** Morphological characteristic of *P. infestans*

| Isolates | Colony color | Spore structure | | |
|---|---|---|---|---|
| | | Hyphae | Sporangia | Oogonia |
| **Ju-PGDs1** | White | Branched, hyaline aseptate hyapae | Lemoniform | Spherical smooth walled |
| **Ju-PGDs2** | White | Branched, hyaline aseptate hyapae | Lemoniform | Spherical smooth walled |
| **Ju-PGDs3** | White | Branched, hyaline aseptate hyapae | Lemoniform | Spherical smooth walled |
| **Ju-PGDs4** | White | Unbranched hyaline aseptate hypae | Lemoniform | Spherical smooth walled |
| **Ju-PGDs5** | White | Branched, hyaline aseptate hyapae | Lemoniform | Spherical smooth walled |
| **Ju-PGDs6** | White | Branched, hyaline aseptate hyapae | Lemoniform | Spherical smooth walled |
| **Ju-PJM1** | White | Branched, hyaline aseptate hyapae | Lemoniform | Spherical smooth walled |
| **Ju-PJM2** | White | Unbranched hyaline aseptate hypae | Lemoniform | Spherical smooth walled |
| **Ju-PJM3** | White | Branched, hyaline aseptate hyapae | Lemoniform | Spherical smooth walled |
| **Ju-PJM4** | White | Unbranched hyaline aseptate hypae | Ovoid | Spherical smooth walled |
| **Ju-PJM5** | White | Branched, hyaline aseptate hyapae | Lemoniform | Spherical smooth walled |
| **Ju-PGD1** | White | Branched, hyaline aseptate hyapae | Lemoniform | Spherical smooth walled |
| **Ju-PGD2** | White | Branched, hyaline aseptate hyapae | Lemoniform | Spherical smooth walled |
| **Ju-PGD3** | White | Branched, hyaline aseptate hyapae | Ovoid/lemoniform | Spherical smooth walled |
| **Ju-PGD4** | White | Branched, hyaline aseptate hyapae | Lemoniform | Spherical smooth walled |
| **Ju-PGD5** | White | Branched, hyaline aseptate hyapae | Lemoniform | Spherical smooth walled |
| **Oomycete-Ju-GD6** | White | Branched, hyaline septate hyapae | Lemoniform but observed inside the hypae | Not seen |
| **Oomycete-Ju-GD31** | White | Branched, hyaline septate hyapae | Lemoniform but observed inside the hypae | Not seen |

**Source:** primary (2019)

# Appendix 2: Medias

## Carrot agar

| Ingredients | gms / Litre |
|---|---|
| Carrots | 200.0 g |
| Distilled water | 1000 ml |
| Agar | 20.0 g |

## Czapek  Dox Agar

**Composition****

| Ingredients | gms / Litre |
|---|---|
| Sucrose | 30.000 |
| Sodium nitrate | 2.000 |
| Dipotassium phosphate | 1.000 |
| Magnesium sulphate | 0.500 |
| Potassium chloride | 0.500 |
| Ferrous sulphate | 0.010 |
| Agar | 15.000 |
| Final pH (at 25°C) | 7.3±0.2 |

**Formula adjusted, standardized to suit performance parameters

## Potato Dextrose Agar (PDA)

| Ingredients | gms / Litre |
|---|---|
| Potato infusion | 200 gm |
| Dextrose | 20 gm |
| Agar | 20 gm |
| Distilled water | 1 liter |

# Declaration

I undersigned Registration I.D. Number RM/0961/10 do here by declare that this thesis entitled **"Biocontrol of *Trichoderma* isolates against *P. infestan* and enhancement the growth of tomato plants in Jimma, Southwestern, Ethiopia",** is my original work and that it has not been submitted partially or in full by any other person for an award of a master degree in any other university/institution.

| **Name of Investigator/participant** | **Signature** | **Date** |
|---|---|---|
| _____ | _____ | _____ |

This thesis has been submitted for examination with my approval as university supervisors

| **Name of Principal advisor** | **Signature** | **Date** |
|---|---|---|
| _____ | _____ | _____ |
| **Name of Co-advisor** | **Signature** | **Date** |
| _____ | _____ | _____ |