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Addis Ababa University

School of Graduate Studies

College of Natural Sciences

Department of Microbial, Cellular and Molecular Biology

Characterization and Pathogenicity of Blast Pathogen (*Pyricularia grisea*) of Wild Finger Millet (*Eleusine africana)* and its Control Using Biological Antagonists and Fungicides.

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A Thesis submitted to the school of graduate studies of Addis Ababa University in partial fulfilment of the requirements for the degree of Master of Science in Applied Microbiology

Feb, 2017

Addis Ababa, Ethiopia

APPROVAL SHEET

Addis Ababa University

School of Graduate Studies

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ACKNOWLDGEMENTS:

I praise the God for helping me and showering with his blessings from beginning of my life.

I wish to express my deep felt gratitude and appreciation to my advisors Dr. Kassahun Tesfaye and Dr. Tesfaye Alemu for their encouragement, keen interest in this work and support with valuable suggestions and guidance which has made me to complete this Thesis.

I am grateful to Dr. Kassahun Tesfaye for providing necessary facilities for the research.

My special thanks and respect go to Dr. Dagnachew Lule and Alemayehu Getahun (Ph.D. candidate) for giving me essential information that is required for this study.

Last but not list, I am indebted to my best friend Brehanu Taddese (MSc.) for his constant help and for being so proud of my efforts.

TABLES OF CONTENT

Page

TABLES OF CONTENT i

List of Tables iv

List of Figures vi

List of Appendices vii

List of Abbreviations viii

ABSTRACT ix

1. INTRODUCTION 1

2. OBJECTIVES OF THE STUDY 4

2.1. General objective *4*

2.2. Specific objectives: *4*

3. LITERATURE REVIEW 5

3.1. Distribution *5*

3.2. Economic importance *5*

3.3. Pathogen diversity *5*

3.4. Symptoms *6*

3.5. Morphology of the fungus *7*

3.6. Biology of the pathogen *7*

3.7. Factor affecting the growth of the pathogen *9*

3.7.1. Media 9

3.7.2. Temperature 9

3.7.3. PH 9

3.8. Biochemical studies *9*

3.8.1. Carbon utilization 9

3.8.2. Nitrogen utilization 10

3.9. Sporulation *10*

3.10. Disease development *10*

3.11. Pathogenicity test *11*

3.12. Mechanism of action of P. grisea species as Pathogen *11*

3.12.1. Phytotoxin Production 11

3.13. Disease management *11*

3.13.1. Cultural practice 12

3.13.2. Resistant varieties 12

3.13.3. Biological control 12

3.13.3.1. Mechanism of action of *Trichoderma* and *Pseudomonas* species as bioagents 12
3.13.4. Compatibility Studies 13

4. MATERIALS AND METHODS 14

4.1. Experimental site 14

4.2. Sampling sites for diseased wild finger millet samples............................14

4.3. Diseased plant sample collection............................14

4.4. Isolation of the test pathogens 16

4.5. Maintenance of isolates............................16

4.6. Variations of Pyricularia grisea isolates............................16
4.6.1. Morphological Characterization............................16
4.6.2. Cultural characterization............................17
4.6.3 .Physiological Studies. 18
4.6.3.1. Effect of different temperature levels............................18
4.6.3.2. Effect of hydrogen ion concentration (pH)............................18
4.6.4. Biochemical Characterization............................19
4.6.4.1. Carbon utilization and Nitrogen utilization 19

4.7. Preparation of inoculums of isolates............................19

4.8. In Vivo (Greenhouse) studies............................20
4.8.1. Pathogenicity Studies............................20
4.8.1.1 Finger millet blast disease assessment and scoring 21
4.8.1.2. Assessment of yield loss............................22

4.9. In vitro evaluation of Trichoderma and Pseudomonas isolates against the isolated pathogens............................23

4.10. In vitro evaluation of fungicides............................24

4.11. Compatibility Studies of Trichoderma and Fungicides 24

4.12. Statistical Analysis............................25

5. RESULTS 26

5.1. Collection and isolation of different isolates of the pathogen 26

5.2. Cultural, morphological, physiological and biochemical characteristics of the test pathogen............................26
5.2.1. Conidial characteristics of the test pathogen isolates 26
5.2.1.1. Septation, Shape, size and color of conidia............................26
5.2.2. Cultural characteristics and Sporulation pattern isolates on different solid media 28
5.2.2.1. Host seed extract + 2% sucrose agar 28
5.2.2.2. Oatmeal agar 29
5.2.2.3. Potato Dextrose Agar 29
5.2.2.4. Richards's Agar Medium............................29
5.2.1.2. Effect of different culture media on mycelia growth of Pathogen............................31
5.2.3. Physiological variability 32
5.2.3.1. Temperature............................32
5.2.3.2. pH (Hydrogen) ion concentration:............................32
5.2.4. Biochemical studies............................34
5.2.4.1. Effect of carbon sources on the mycelial growth of *P. grisea* isolates............................34
5.2.4.2. Nitrogen utilization............................34

5.3. Pathogenicitytest 35

5.4. Yield loss caused by P. grisea isolates *36*

5.5. In vitro evaluation of antagonistic activity of Trichoderma species and P. fluorescence against the test pathogen. *37*

5.6. In vitro evaluation of fungicides against the test pathogen *38*

5.7. Selection of selective fungicides and fungicides resistant antagonists for compatibility studies. *40*

6. DISCUSSION **42**

7. CONCLUSIONS AND RECOMMENDATIONS **50**

7.1. CONCLUSIONS *50*

7.2. RECOMMENDATIONS *51*

REFERENCES **52**

APPENDICES **62**

List of Tables

Table 1. Wild Finger Millet blast disease survey sites in West and North-Western Ethiopia.......15

Table 2. Sporulation rate of *P. grisea* isolates on different substrates..18

Table 3**.** Finger millet varieties used for pathogenicity test on seedlings....................................21

Table 4.Conidial size of nine *P. grisea* isolates after three weeks of incubation at 27±1° C........28

Table 5. Effect of different culture media on colony characters development of *Pyricularia grisea* isolates incubated at, 27±1°C..30

Table 6. Evaluation of different culture media for the mycelial growth of *P. grisea* isolates.......31

Table 7. Effect of different temperature on mycelial growth of *P. grisea* isolates........................32

Table 8. Effect of different pH values on dry mycelial weight of *P. grisea* isolates.....................33

Table 9.Effect of different carbon sources on mycelial growth of *P. grisea* isolates....................34

Table 10.Effect of different nitrogen sources on mycelial growth of *P. grisea* isolates...............35

Table 11.Percentage disease incidence and severity on seven varieties inoculated with Six *P. grisea* isolates under green house condition..36

Table 12**.** Yield losses caused by *P. grisea* isolates on the seven finger millet varieties under green house condition..37

Table 13.In vitro evaluation and testing of *T. harzianum*, *T. viride* and *P. fluorescence* against mycelial growth of *P. grisea* isolates..38

Table 14**.**The mean percent of mycelial growth inhibition of fungicides against *P. grisea* isolates on PDA medium after 7 days of incubation at 27 ±1oC...39

Table.15. Evaluations of different concentration of fungicides on mycelial growth of *P.grisea*...39

Table 16. Screening of *Trichoderma* species for tolerance to Curzate at different concentration afterseven days of incubation at 25°..41

Table 17**.** Screening of *Trichoderma* species for tolerance to Sancozeb at different Concentration seven days after incubation at 25°C..41

List of Figures

Figure 1.Life cycle of *Pyricularia grisea*, causal organism of gray leaf spot...............................8

Figure 2. Collection of blast samples from farmer's field………………………………………14

Figure 3. Pathogenicity test in the greenhouse...21

Figure4. Microphotograph showing conidia of *P. grisea* (A) with one septation, (B) with two septation and (C) with three septation (40x)..27

Figure5. Microphotograph showing conidial mass of *P. grisea* with different shapes and size...27

Figure 6. Microphotograph showing conidia and conidiophores of *pyricularia grisea*(20, 40 and 100x)..28

Figure 7. Percentage mycelial growth inhibition of *P. grisea* isolates by Sancozeb ® 80%W...40

List of Appendices

Appendix 1. Composition and preparation of different growth media…………………………..62

Appendix 2. Growth characters of *P. grisea* isolates at different temperature levels..................64

Appendix 3. Effect of different culture media on mycelial growth of *P.grisea* isolates………..66

Appendix 4. Concentric ring pattern of seed and leaf blast *P.grisea* isolates………………..…67

Appendix 5. Effect of different Carbon sources on mycelial growth of *P. grisea* isolates……...67

Appendix 6. Finger millet seedlings showing blast symptoms following inoculations………...68

Appendix 7. Percent mycelial growth inhibition of *P. grisea* isolates by Curzate fungicides at different concentration...70

Appendix 8. Percentage Compatibility of *Trichoderma* species with Sancozeb and Curzate at different concentration after seven days of incubation at 25°C...71

Appendix 9. Disease intensity index…………………………………………………………71

List of Abbreviations

µm= micrometer

AAUT1= Addis Ababa University *Trichoderma* isolate 1

AAUT2= Addis Ababa University *Trichoderma* isolate 2

E. Africana= Eleusine Africana

E. coracana= Eleusine coracana

IPM= Integrated Pest Management

OMA= Oat Meal Agar

P.grisea /pg = Pyricularia grisea

Pg1-26= Pyricularia grisea isolates

PDA= Potato Dextrose Agar

RA= Richard's Agar

SDW= Sterile Distilled Water

T. harzianum= Trichoderma harzianum

T. viride=Trichoderma viride

% YL = Percent Yield Loss

ABSTRACT

Blast (Pyricularia grisea) is an important disease of wild finger millet (Eleusine africana). It causes disease on a wide variety of alternative hosts including agriculturally important plants such as finger millet (E. coracana) in Ethiopia. A total of twenty six isolates of P. grisea were isolated from infected wild finger millet plants from 5 different zones of Ethiopia. From the initial twenty six isolates, only nine isolates were selected for the detailed morphological, cultural, physiological and biochemical characterization based on their geographical location and isolate type, whether it is isolated from stem, leaf, neck or seed parts. Among the different media used host seed extract + 2% sucrose agar and oatmeal agar were found to be the best for mycelial growth and sporulation of the isolates, dextrose was the most preferred among the carbon sources and $NaNo_3$ *proved to be the best source of nitrogen for all the isolates. The shape, color and compactness of the fungal colonies varied with the media used. At pH 6.5 all the isolates exhibited maximum mean dry mycelial weight, and found optimum pH. The optimum temperature range for the maximum growth of the isolates of P. grisea was found to be 20°C to 30°C. All the six P. grisea studied under green house for their pathogenicity, were able to infect all the tested varieties. Among an In vitro evaluated fungicide against the test pathogen, both Curzate and Sancozeb fungicides showed varied levels of antifungal activity and the highest concentration (800 and 1000ppm) of fungicides were found of inhibiting the growth of the isolates of P. grisea. With regard to the efficacy of the fungicides, Sancozeb was found to be the most effective to inhibit the isolates. Among in vitro tested biocontrol agents viz. Trichoderma viride, Trichoderma harzianum and Pseudomonas fluorescence, the growth of Trichoderma species was fast, the clear inhibition zones were not formed in all incubated Petri plates, therefore it was more effective than the bacterial antagonist. Compatibility of the biocontrol agents with different fungicides (Curzate and Sancozeb) that is., Trichoderma harzianum and Trichoderma viride in an in vitro condition resulted in that, both Curzate and Sancozeb, in lower concentrations (100, 200, and 400ppm) were found well tolerated with both Trichoderma species and hence they were shown effective in managing plant pathogens. Combining antagonists with synthetic chemicals eliminates the chance of resistance development and reduces the fungicide application.*

Keywords: *Finger millet, Pyricularia grisea, biocontrol, fungicide, compatibility, pathogenicity,*

1. INTRODUCTION

Millets are a group of highly variable grass species belonging to different genera that had been originated in tropical and subtropical parts of Africa and Asia. There are two subspecies of African finger millet, the wild form (*Eleusine coracana subsp. africana)* and a cultivated form *(Eleusine coracana subsp.coracana).* Finger millet is thought to have originated from Uganda or neighbouring Ethiopian highlands where wide diversity of the genus *Eleusine* exists (Hilu *et al*., 1979). In the semi arid tropics of East Africa, finger millet (*Eleusine coracana*) is a staple food for millions of people. It ranks third in importance among millets after pearl millet (*Pennisetum glaucum*) and foxtail millet (*Setaria italica*) in the semi arid tropics and subtropics of the world (Reddy *et al*., 2009). It was recently estimated that finger millet accounts for 3.8 m ha which is 10% of millet products globally (Mgonja *et al*., 2007).

The cereal plays an important role in the diets and economy of subsistence farmers and is especially important for the weak and immuno-compromised (Takan *et al*., 2012). The grains are rich source of minerals (calcium, iron, zinc, and manganese) and amino acids (tryptophan, cystine, and methionine), which are crucial to human health and growth, and these are deficient in most cereals. These nutritive values make the crop particularly important in the diets of children, pregnant and lactating mothers (National Research Council 1996). Despite its importance, its productivity has been limited by blast disease caused by *Pyricularia grisea* in the eastern African countries (Lenne *et al*., 2007)

The blast, *Pyricularia grisea* is one of the biological constraints that affect the yield, utilization and trade of finger millet within East Africa (Lenne *et al*., 2007). The disease affects of finger millet, all stage of growth. Neck and panicle blast are the most destructive form of the disease (Takan *et al*. 2004; Takan *et al*. 2012). The most susceptible stage for leaf blast is seedling stage, whereas for neck and finger blast is pre-flowering stage. Growing cultivars with durable resistance is the best means of combating the blast disease of finger millet. Resistance is often assessed at the seedling stage, which did not correlate well with neck and finger infection. Hence, neck and finger blast are more destructive than leaf blast were considered important parameters for blast resistance (Nagaraja *et al*. 2007). Most of the landraces and a number of genotypes cause failure of the grain and major yield losses (Timper *et al*., 2002 and Thakur *et al*., 2011).

In Ethiopia, studies have been conducted to characterize this pathogen on the cultivar finger millet (Boneya (KEN#411), Bako Local check and Tadesse (KEN#1098)) by Getachew *et al*. (2013). However no studies have been conducted to characterize this pathogen on the wild finger millet. Although the growth requirements for fungi may vary from isolate to isolate, cultures of the same species and genera tend to grow best on similar media (Aneja, 2005). The growth characteristics of *Pyricularia grisea* can be easily studied on artificial medium. Oatmeal agar was known for better growth and sporulation of *P. grisea* at 30°C and pH 6.5 than other media (Getachew *et al*., 2013). Optimum growth of the fungus has been determined at, 28°C and pH 6.5 on potato dextrose agar media (Srivastava *et al*., 2009). The carbon compounds, maltose, sucrose, dextrose and fructose were the best carbon sources for *P. grisea* isolates (Getachew *et al*., 2013). Sodium nitrate used in place of ammonium salts in a synthetic medium, significantly improved the growth (Okeke *et al.,* 1992).

The pathogenicity test were carried out to authenticate that weather the pathogen isolates are causative agents of blast disease of finger millet, its virulence and Varietal susceptibility to the pathogen (Bhojya, 2013). The degree of pathogenicity varies depending on the fungus, the isolate, and host they invade. It has been suggested that the pathogenicity of the blast fungus *P. grisea* is largely restricted to its host species of origin. However Weed and wild grass hosts growing near cultivated plants were able to support the pathogen, under field conditions and serve as potential sources of inoculums for the disease (Takan *et al*., 2004) and are the most important sources of inoculums which are present throughout the year (Khadka *et al*., 2012). The fungus has a wide host range, but the most common alternate hosts are mostly grass weeds such as *Eleusine indica, Eleusine africana*, *Digitaria* spp., *Setaria* spp. and *Doctylocterium* spp. These serve as primary sources of inoculums and other grains that sustain finger millet blast are volunteer upland rice plants (Sreenivasprasad *et al*., 2005).

Characterization in morphological, cultural, physiological and biochemical features of the pathogen are of immense use in understanding the nature of the pathogen and understanding the physiological and biochemical requirements for the growth and development of the pathogen, which could serve as an input in disease management to minimize the effect of blast disease on finger millet (Bhojya, 2013).The implication of isolation of *P. grisea* isolates from weeds as an inoculum source helps to manage weeds because some isolates of blast pathogen from weeds also infect finger millet, hence the need for proper crop rotation and removal of alternate host species (Getachew *et al*., 2013).

Plant diseases need to be controlled to maintain the quality and abundance of the target crop through elimination of the pathogen from host by use of pesticides, combating virulence mechanisms of the pathogen, biocontrol, fungicidal control and use of resistant varieties (Agrios, 2005). Biological control through the use of antagonistic micro-organisms is a potential, non chemical means of controlling plant disease by reducing inoculum levels of pathogens. Such management would help in preventing the environmental pollution, development of resistant strains and decrease heavy dependence of modern agriculture on costly chemical fungicides (Harman *et al*., 2004; Bhojya, 2013). Fungi in the genus *Trichoderma* have been known since at least the 1920s for their ability to act as biocontrol agents against plant pathogens (Harman *et al*., 2004). *Trichoderma harzianum* and *Trichoderma viride* are the most studied of all the *Trichoderma* species for biological control and the most effective in reducing diseases caused by soil borne plant pathogens (Tesfaye Alemu and Kapoor, 2010). And bacteria of the genus *Pseudomonas* comprise a large group of the active biocontrol strains as a result of their general ability to produce a diverse array of potent antifungal metabolites (Srivastava and Shalin, 2008).

Fungicides can control and eradicate established infections, but they are also vulnerable to fungi developing resistance, as they generally only target one step in a biosynthetic pathway to kill the fungus. By alternating between the different classes of fungicides, the fungal population has less opportunity to build up resistance to one chemical (Nene and Thapliyal, 1993). The combination of bioagents with fungicides would provide similar disease suppression as achieved with higher fungicide use (Monte, 2001). In addition, this strategy may display even better control of resistant strains of fungal pathogens and may help the commercial growers to reduce the amount of fungicide use, thus lowering the amount of chemical residue in the marketed products (Bikila, 2015). Therefore, the present study was conducted to determine the intensity of the blast disease, to characterize its isolates and its pathogenicity on *E. africana*, target points for disease control and evaluate its biocontrol and fungicidal control.

2. OBJECTIVES OF THE STUDY

2.1. General objective

The general objective of this study is to isolate and characterize the wild finger millet blast fungus *(Pyricularia grisea*) and understand the effect of the pathogen on *Eleusine africana*, and also to verify the importance of in the disease cycle and using it as target points for disease control.

2.2. Specific objectives:

- ❖ To isolate and characterize the pathogen isolates from infected finger millet plants
- ❖ To study cultural, morphological, physiological, biochemical and pathogenic diversity of *P. grisea* isolates,
- ❖ To make *in vivo* evaluation of pathogenicity of *P. grisea* isolates
- ❖ To estimate the losses caused by *Pyricularia grisea*
- ❖ To carry out *in vitro* evaluation of fungicides and biocontrol agents against *P. grisea* isolates,
- ❖ To examine compatibility of selective fungicides and biological agents

3. LITERATURE REVIEW

3.1. Distribution

Wild finger millet was possibly grown as a weed with and adjacent to the cultivar finger millet, which is important crop in areas of Gojjam, Gonder, Wollega, Iluababora, Gamo-Gofa, Eastern Hararghe and Tigray (Molla Fentie, 2012) and in area of Arsi Negele, Shashemene and Siraro Woredas (Chimdo Anchala *et al.*, 2006). After the recent release of high yielding varieties for these areas, currently, millet has become popular due to its advantages of agronomic practice (drought tolerant, storability of seeds and traditional food making quality) (Taye Tadesse *et al.*, 2002).

3.2. Economic importance

Blast caused by heterothallic ascomycete *Magnaporthe grisea* (Hebert) Barr. (Anamorph: *Pyricularia grisea*) is the most important constraint to finger millet production in most finger millet growing environments (Singh and Kumar, 2010). And it was the major finger millet disease and highest priority production constraint in east Africa where most landraces are susceptible to the pathogen (Lukose *et al.*, 2007). It is an important disease particularly in pearl millet forage cultivars in the southern United States and more recently it has emerged as a serious disease of dual purpose (grain and fodder) pearl millet hybrids in India (Lukose *et al.*, 2007; Anonymous, 2009). The disease causes chronic yield losses of grain and forage (Thakur, 2011).The yield losses estimated to be10.1- 41.4% in Ethiopia (Getachew *et al.*, 2013), 10-50% in Kenya and 10-80% in Uganda (Sreenivasaprasad *et al.,* 2004). In India, the average loss due to blast has been reported to be around 28-36% (Nagaraja *et al.,* 2007). The production losses caused by blast can vary from very low to almost 100% (Kohli *et al.*, 2011).

3.3. Pathogen diversity

Magnaporthe grisea species complex has a very wide host range among the grasses and has even been reported from a few other monocot families (Takan *et al.*, 2012). At the strain or pathotype level, however, this group is characterized by a high degree of host specificity and molecular genetics differentiation on different hosts (Zellerhoff *et al.*, 2006; Faivre-Rampant *et al.*, 2008). Even species within a single genus or cultivars of a single crop often have host-

specific pathotypes of this pathogen that are avirulent on plants of close congeners (Yoshida, 2009). The limited variation in *P. grisea* could be due to its predominant asexual reproduction as Uddin (2000) reported sexual reproduction to be rare. This would imply that identification of resistance genes for virulent pathogen genes would fairly control blast in East Africa, as there would not be pathogen race diversity in a region to easily break deployed resistances. This gives hope of the usefulness of vertical resistance. All farmers' varieties in all locations show varying degrees of susceptibility to finger millet blast disease with neck and head blast being more frequent than foliar blast.

Sonah *et al.* (2009) studied the cultural and morphological variability of *M. grisea* isolates collected from rice and non-rice hosts revealed that isolates that showed fast vegetative growth as gray-green or gray- white produced large number of spores than those with lower vegetative growth (submerged or subdued growth patterns). Isolates derived from non-rice hosts also showed abnormal spore morphology which was longer, cylindrical and obpyriform. Information on regional and global population diversity at the lineage level is useful to understand the epidemiological properties of the blast disease in neighbouring areas (Sonah *et al*., 2009). Takan *et al.* (2012) studied the compatibility of isolates representing diverse sampling location and host range revealed that all isolates were compatible to the tested finger millet varieties and only showed differences in aggressiveness and over all differences between isolates and varieties were highly significant for lesion number and leaf area affected (Motlagh, 2015). Using amplified fragment length polymorphism (AFLP) analysis, compared isolates causing leaf, neck and head blast and found them genetically similar, suggesting that the same strains probably cause the different symptoms under suitable conditions (Takan *et al*., 2004).

3.4. Symptoms

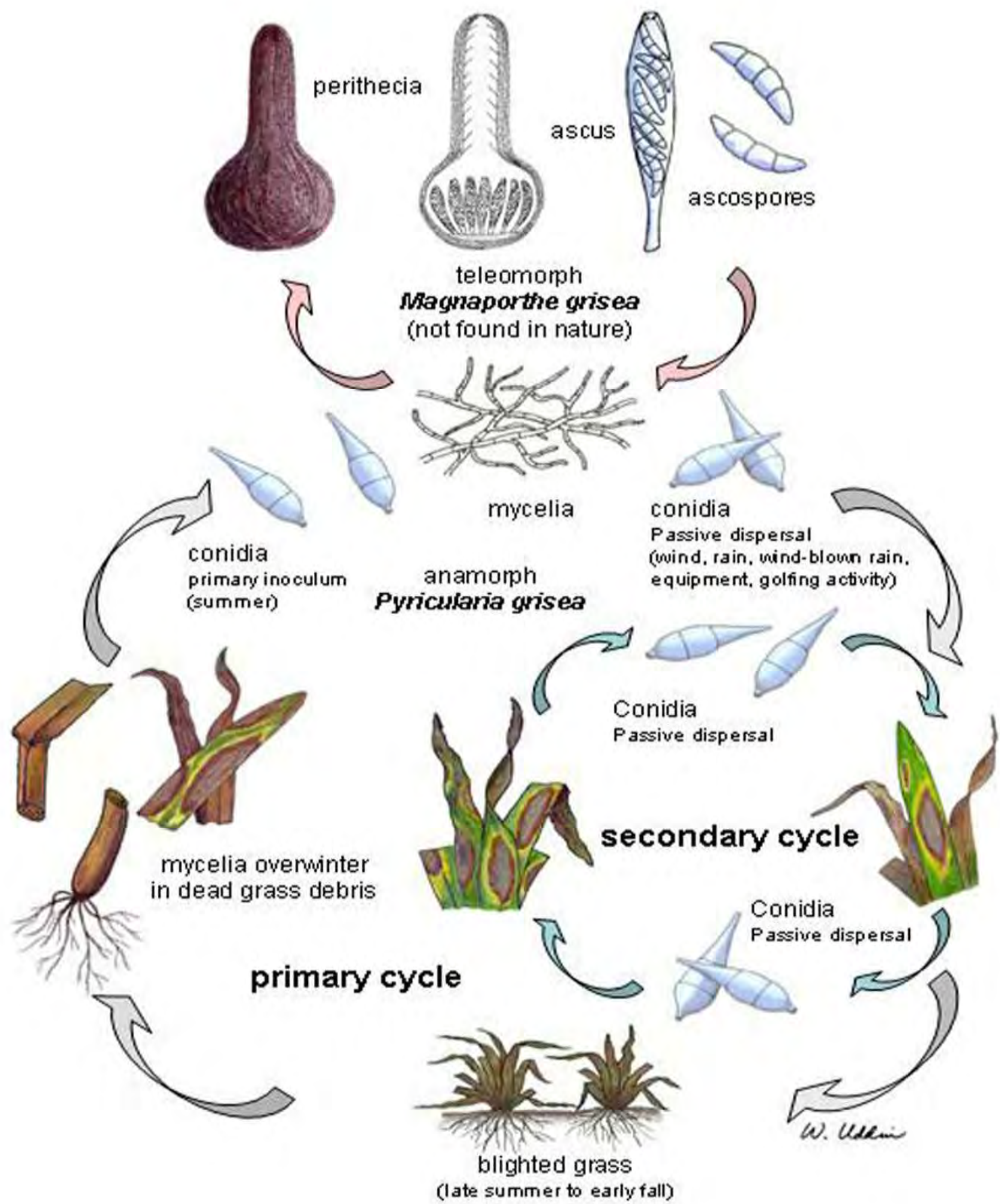
Symptom development usually occurs within several hours to several days after inoculation, depending on host species and pathogen isolate (Tredway *et al*., 2003). The symptoms appear at all the stages of plant growth and the disease affects the crop at all growth stages from seedling stage, causing lesions and premature drying of young leaves, to affecting the panicle causing neck and/or finger blast (Getachew *et al*., 2014).When the young healthy seedlings catch the disease, patches of seedlings give burnt appearance due to severe leaf blight. weather conditions.

3.5. Morphology of the fungus

The genus *Pyricularia* (Cooke) Sacc. species, *P. grisea* (Cooke) Sacc. which originally was described from crabgrass (*Digitaria sanguinalis* L). The name ''*Pyricularia*'' refers to the pyriform shape of the conidia (Rossman *et al*., 1990). Morphologically *Pyricularia grisea*, is a very close relative of rice blast (*Pyricularia oryzae*), and is the most serious and widely spread disease (Mgonja *et al.*, 2007). The fungus is characterized by simple or sparely-branched conidiophores, 2-4 celled, mostly three-celled with a small appendage on the basal cell And by observing conidia are hyaline, pyriform, septate (1-3 septa) asexual conidia on diseased leaf tissue (Getachew *et al*,. 2013). They are slightly swollen at the base, tapering towards the apex. Specimens from different hosts are morphologically indistinguishable. *Pyricularia grisea* produces gray conidiophores which yield terminal pyriform asexual spores termed conidia (Thakur, 2011). Conidia variable in size and shape, terminal, pyriform to obelavate, base rounded, apex narrowed, not or slightly constricted at septa, almost hyaline to pale olive, 14 – 40 x 6 -13 µm in size, usually 19-23 x 7-9 µm, with small basal appendage (Nishikado, 1927). Mycelium in cultures aerial or submerged, hyaline or olivaceous, 1.5 – 6.0 µm in width, septate branched (Meena, 2005). Conidia germinate from the apical or basal cell and less frequently from the middle cell. Conidia measure approximately 17-31x 6-9 µm and germinate by producing appressorium (Wilson, 2000).

3.6. Biology of the pathogen

Heterothallic sexual (teleomorph) stage of the fungus, *Magnaporthe grisea*, and anamorph: *Pyricularia grisea* belongs to Phylum Ascomycota (Order *Diaporthales*) (Agrios, 2005). Sexual reproduction occurs when two mating types of the fungus (MAT1-1andMAT1-2,) and when fertile isolates carrying opposite mating types are paired together on an appropriate growth medium, they will form sexual fruiting bodies called perithecia within 21 days. Sporulation occurs when the appearance of necrotic disease lesions is accompanied by the development of aerial conidiophores. Conidia are arrayed at the tips of these aerial hyphae (Getachew *et al*., 2013). Mitotic divisions of a single progenitor nucleus occur in the conidiophore, leading to the production of the first three-cell conidium. Thereafter, the hyphal tip moves to the side of the conidium and produces a second spore until three to five conidia are produced in a whorl at the conidiophores tip (Talbot, 2003). Dispersal of inoculums is by the wind, windblown rain, water-splash from sprinkler irrigation, movement by ground maintenance equipment (Uddin *et al*., 2003)



Source: (Uddin *et al*., 2003)

Figure 1. Life cycle of *Pyricularia grisea*, causal organism of gray leaf spot

3.7. Factor affecting the growth of the pathogen

Three basic elements are required for the development of an infectious disease: a susceptible host, a virulent pathogen and favourable weather conditions for infection, host colonization and propagule production (Agrios, 2005).The major factors affecting growth are medium, temperature, light, aeration, pH and Water activity.

3.7.1. Media

Types of media affect colony morphology and color, whether particular structures are formed (Aneja, 2005).The growth characteristics of *P. grisea,* the blast pathogen of graminaceous hosts (millets) can be easily studied in artificial medium. Optimum growth of the fungus has been determined at, 28°C and pH 6.5 on potato dextrose agar media (Srivastava *et al*., 2009).

3.7.2. Temperature

Temperature and relative humidity play an important role governing growth, reproduction and survival of the fungus, (Munoz, 2008). The optimum temperature for growth and conidial production of *P. grisea* were ranges from 20^{0}C to 35^{0}C (Getachew *et al.,* 2013).

3.7.3. PH

Most common fungi grow well over the range of pH 3 to 7, although some can grow at pH 2 (Aneja, 2005). Every organism has its minimum, maximum and optimum pH for growth. Most of the strains of *P. grisea* grew on media with a hydrogen ion concentration between 5 - 10 (Getachew *et al*., 2013).

3.8. Biochemical studies

3.8.1. Carbon utilization

Utilization of carbon compounds, maltose, sucrose, glucose, insulin and mannitol as well as organic acids such as succinic acid were the best carbon sources whereas; lactose and galactose were not suitable for *P. grisea* (Otani, 1953). Getachew *et al.* (2013) reported that dextrose, maltose, sucrose and fructose, were identified as the most suitable carbon sources for isolates of *P. grisea*.

3.8.2. Nitrogen utilization

Nitrogen is an important element for protein synthesis. (Okeke *et al.* (1992) and Getachew *et al*. (2013)) demonstrated that on synthetic media, nitrate nitrogen was better for the growth of *P. grisea* than ammoniac nitrogen. They also reported that sodium nitrate used in place of ammonium salts in a synthetic medium, significantly improved the growth.

3.9. Sporulation

Spores often are produced under conditions that are not suitable for vegetative growth (Dhingra and Sinclair, 1993). Potato dextrose agar, oatmeal agar, malt extract agar, host extract agar and Richard's agar medium are useful for sporulation of *P. grisea* isolates (Meena, 2005).

3.10. Disease development

Magnaporthe grisea survive over winters as dormant mycelium in dead leaves. Harmon and Latin (Harmon and Latin (2001)) found that survival of *M. grisea* was greatly reduced during the winter, but they successfully induced sporulation of the fungus from infected plant debris in the spring. It survives the period of inactive plant growth as mycelium or conidia in or on senesced host leaves (Greer and Webster (2001)). Conidia produced from the leaf debris apparently serve as the primary inoculums for leaf infections early in the growing season, although details of this early infection process need to be determined. High temperature, high relative humidity and leaf wetness are critical environmental factors that influence disease development (Ruiz, 2003). Leaf wetness duration is also important in the development of gray leaf spot (Uddin and Viji (2002)). Disease incidence and severity increased with increased leaf wetness duration at all temperatures. Shorter leaf wetness duration was required for disease development under warmer temperatures (Uddin and Viji (2002)). In addition to leaf wetness duration, relative humidity is also an important environmental component influencing gray leaf spot development. Gray leaf spot normally develops from early August to mid-October. Environmental conditions prevailing during this late summer period and availability of inoculums are major determinants in the development of gray leaf spot epidemics. Study showed that the effect of temperature on gray leaf spot incidence and severity is cubic, indicating that disease incidence and severity increase with increases in temperature from 20 to 28 ^{0}C (68 to 82 F) and decrease with increase in temperature above 28 ^{0}C (82 F) (Uddin *et al.*, 2003).

3.11. Pathogenicity test

In order to substantiate an organism, group of organisms or combination of organisms and environmental factors are the causal agents for a disease, it must fulfil Koch's postulates (Aneja, 2005). Pathogenicity test was conducted to confirm that *P. grisea* isolates were the causal agents of finger millet blast disease. The pathogenicity test begins with preparation of the sporulated spore or raw mycelium from the test organism. It has been adjusted to different concentration/loads of spores during this test: $1x10^5$ spores/ml of *P. grisea* isolates were used (Takan *et al.*, 2004). The spore or mycelium of *P. grisea* isolates could be loaded with or without wounding the plant (Denman *et al*., 2005).

3.12. Mechanism of action of *P. grisea* species as Pathogen

3.12.1. Phytotoxin Production

The pathogen *Pyricularia grisea* is known to produce a wide array of phytotoxic compounds, including polyketides such as pyriculol, epipyriculol, dehydroxypyriculol, and pyricuol (Kim *et al*.,1998), pyricularin, tenuazonic acid, and cytochalasins (Talbot, 2003). The cytochalasin pyrichalasin H is a host-specific phytotoxin produced only by pathotypes on *Digitaria*; concurrent application of this phytotoxin can render otherwise nonvirulent strains from other hosts pathogenic on *Digitaria* (Tsurushima *et al*. 2005). Application of some other phytotoxins produced by the fungus, including pyriculol and tenuazonic acid, can cause typical brown leaf necrosis (Tshurushima *et al*., 2009).

3.13. Disease management

All plant diseases result from a three-way interaction between the host, pathogen and the environment (Agrios (2005); Ahmad (2011) and Getachew *et al*. (2013)).Therefore, disease can be controlled by manipulating one or more of these factors so that conditions are unsuitable for replication, survival or infection by the test pathogen. Recently there are a lot of interest and management approaches to control plant diseases using biological antagonists, fungicides, resistant varieties which lead to an application of an integrated pest management (TeBeest *et al*. (2007) and Maciel, (2011)). An integrated approach that entails various cultural management practices and a sound fungicide program provide effective control of the blast (Uddin *et al*., 2003).

3.13.1. Cultural practice

Among cultural practices that are recommended against the blast; early sowing of seeds after the onset of the rainy season is more advisable than late sown crops (Sreenivasprasad *et al*., 2004). Early sowing allows escape from the build-up of inoculums originating from neighbouring farms. And row planting has often been advocated as a solution (Getachew *et al*., 2013).

3.13.2. Resistant varieties

Planting resistant varieties against the finger millet blast is the most practical and economical way of controlling millet blast. Several races of the *Pyricularia* species exist, resistance to the Indian isolate of *P. grisea* was found to be governed by a single dominant gene, and so single-gene resistance may not be effective against all strains of each pathogen (Harish *et al*., 2007). Multigenic resistance, controlled by several genes could be applied to slow down the development of individual infection loci on a plant, there by slowing down the spread and development of the disease (Agrios, 2005).

3.13.3. Biological control

Some pathogens occupy the niches and exclude pathogens from becoming established, thereby protecting plants from infection (Vinale *et al.,* 2008). Antagonists that produce antibiotics kill pathogens and eradicate or control them from substrates. Fungi in the genus *Trichoderma* have been known since at least the 1920s for their ability to act as biocontrol agents against plant pathogens (Bikila*,* 2015). They are very useful filamentous fungi that have been widely used as antagonistic fungal agents against several pests as well as plant growth enhancers (Lorito *et al*., 2010). *Pseudomonas fluorescence* was also known for antifungal activity against different plant pathogenic fungi (Srivastava, 2009).

3.13.3.1. Mechanism of action of *Trichoderma* and *Pseudomonas* species as bioagents

Mechanism of action of *Trichoderma* spp. as a biocontrol agent, includes, antibiotic production, mycoparasitism, competition for space and nutrients, and induction of systemic resistance (Rocco and Perez, 2001). The lytic enzymes degrade the cell walls of the pathogenic fungi, enabling *Trichoderma* to utilize both their cell walls and cellular contents for nutrition (Radjacommare *et al*., 2010). A given *Trichoderma* host interaction may involve any of these mechanisms individually or encompass more than one of them acting

simultaneously and in fact it seems advantageous for a biocontrol agent to suppress a plant pathogen using multiple mechanisms (Getachew *et al*, 2014).

Pseudomonas fluorescence is effective organisms for the biological control of soil borne plant pathogens (Diby *et al*., 2001). The mechanisms involved in disease suppression of plants by *Pseudomonas* spp. are diverse and include colonization, competition for iron, production of lytic enzymes, antibiotics and stimulation of plant defense (Haas *et al*., 2002). But one of the most important mechanisms responsible for the suppression of plant pathogens for *Pseudomonas* spp. is siderophore mediated competitions for iron (Henry *et al*., 1991).

3.13.4. Compatibility Studies

To develop an effective disease management program, the compatibility of potential bioagents with fungicides is essential. Combined applications of bioagents followed by small quantities of fungicides may help the antagonists and the relative cost of the formulations (Thoudan and Dutta, 2014). It has been reported that many *Trichoderma* species has an innate and/or induced resistance to many fungicides but the level of resistance varies with the fungicide. Integrating chemical resistant *Trichoderma* species has an importance in the framework of integrated disease management (Bikila, 2015). Disease prevention can be increased by using such tolerant species that keeps pathogens under sufficient pressure so that they cannot thrive.

4. MATERIALS AND METHODS

4.1. Experimental site

All experiments were carried out at the Mycological Research Laboratory and greenhouse of the Department of Microbial, Cellular and Molecular Biology, College of Natural Sciences at Addis Ababa University.

4.2. Sampling sites for diseased wild finger millet samples

Samples were collected from five different sampling sites in East Wollega, Assosa, West Wollega, Beles Chagni and West Gojam. Samples were collected during the main cropping season from October-November 2015. The areas were characterized by mixed crop-livestock system with finger millet as major crop along with other cereals.

4.3. Diseased plant sample collection

Samples were collected from leaf, neck, stem and head of infected plants in the farmer's field with an altitude ranges between 574 and 2239 meters above sea level. Samples were collected in 8 to 12 km intervals from farmer's field followed major roads to towns and localities in 11 districts with closer sampling sites separated by 10-12 km from each other. Blast infected plants in the field were cut from the mother plant (wild finger millet) and placed in an envelope, which was labelled with all necessary information including the name of the region, zone, district, localities, cultivars, GPS data and date of collection. Samples were kept in a refrigerator at 4°C for further study.

A. Assosa B. West Wellega C. East Wellega

Figure 2. Collection of blast samples from farmer's field.

Table 1. Wild Finger Millet blast disease survey sites in West and North-Western Ethiopia.

S No	Designation	Plant part	Site	Cultivar	Altitude (masl)	GPS data	
			Zone, District, Locality			**Longitude (E)**	**Latitude (N)**
1	*Pg*.003	Neck & Leaf	East Wellega, Wayu Tuka, Gute Badiya	Local	1902	036^{0} 38’11.8”	09^{0}00’58.6”
2	*Pg*.007	Neck, Leaf& Stem	East Wellega, leka dullecha, Ale Quessa	Local	2227	036^{0} 29’23.4”	08^{0}58’22.9”
3	*Pg*. 009	Head	East Wellega, Horda, Qawwissa	Local	2189	036^{0} 28’16.8”	08^{0}54’54.1”
4	*Pg*. 013	Leaf	East Wellega, Diga, Bikila	Local	1547	036^{0} 20’41.2”	09^{0}04’04.6”
5	*Pg*.015	Leaf	East Wellega, Diga, Arjo Gudetu	Local	1359	036^{0} 16’11.8”	09^{0}02’43.6”
6	*Pg*.025	Neck & Leaf	West Wellega, Nedjo, Waligalte Agar	Local	1882	035^{0} 29’13.0”	09^{0}32’14.1”
7	*Pg*.027	Neck, Leaf& Stem	West Wellega, Nedjo, Kote Qansi	Local	1885	035^{0} 26’55.1”	09^{0}32’17.6”
8	*Pg*.032	Stem	West Wellega, Kiltu kara, Wato dallee	Local	1605	035^{0} 14’25.7”	09^{0}40’44.6”
5	*Pg*.034	Leaf	West Wellega, Mana sibu, Qarsa Walga	Local	1752	035^{0} 04’38.7”	09^{0}49’35.9”
6	*Pg*.046	Neck, Leaf & Stem	Assosa, Saddal, Papararo	Local	574	035^{0} 11’17.6”	10^{0}52’21.4”
7	*Pg*.056	Leaf	B/n Beles & Chagni, Mandura, Hidade	Local	1118	036^{0} 21’26.4”	11^{0}08’04.0”
7	*Pg*.058	Leaf & Stem	B/n Beles & Chagni, Mandura, Jigda	Local	1468	036^{0} 27’56.1”	11^{0}04’19.2”
8	*Pg*.080	Head	West Gojjam, Adet, AARC	Local	2204	037^{0} 28’43.2”	11^{0}17’14.8”
9	*Pg*.085	Neck, Head, Leaf & Stem	West Gojjam, Wangedam, Bure	Local	2239	037^{0} 25’26.1”	11^{0} 21’45.5”

4.4. Isolation of the test pathogens

The fungal isolates were isolated, using standard tissue isolation procedure as described in Tuite (1969). Diseased plant parts were cut from the margin of infected portion using a sterilized knife and washed in tap water for two minutes in separate plates in order to minimize surface contaminants. They were then dipped in 70% ethanol for one minute to sterilize the surface and rinsed three times with sterile distilled water to remove the remaining contaminants (Tuite, 1969).The surface sterilized tissues were split open longitudinally using sterilized knife and directly transferred onto potato dextrose agar (PDA), amended with Chloramphenicol (0.035mg to 0.1mg) to inhibit bacterial contamination. The Petri plates were incubated in the dark at 27° C ± 1 ° C for 10 days. And purified by subculturing on PDA and incubated at 27°C ± + 1°C for ten days until the emergence of mycelia and sporulation for further study.

4.5. Maintenance of isolates

The fungal cultures of *P. grisea* isolates were sub cultured on potato dextrose agar slants and kept at 27±1°C for 15 days. Such isolates were stored in a refrigerator at 4°C and were renewed at monthly interval for further studies (Bhojya, 2013).

4.6. Variations of *Pyricularia grisea* isolates

4.6.1. Morphological Characterization

Isolates were identified based on their morphological growth pattern and spore shape on PDA midium. Morphological variation among the isolates were examined and characterized according to their conidial structure (size, shape, color and septation of conidia). Morphological studies were carried by preparing slide culture, PDA, water agar, an empty glass Petri-dish, two glass slide and one cover slide (for each Petri-dish) were sterilized independently. Then the water agar were poured into the Petri-dishes (to keep moisture), the two slides were placed on the solidified agar cross to each other and cut of potato dextrose agar plate into small squares of 1cm square each for each plates, were plated at the middle of the slide, the four sides of the PDA block were inoculated with the fungal colony under investigation with sterile needles and a cover slip was placed on top of the inoculated water agar block using sterile forceps and finally the sets were incubated at 27 ± ^{0}C to allow the intact morphology of the fungus to be seen under the microscope. After three weeks of sporulation, the length and width of 10 spores were measured under high power objective for each isolate using a micrometer. The average size and shape of conidia (length and width)

was determined using ocular and stage micrometer. Number of septa and color were also recorded (Mebratu *et al*., 2015). Microphotographs were taken to show the typical spore morphology of the pathogen (Figure.6). And the isolates were identified as field isolates of *P. grisea* of wild finger millet blast based on color, size and shape of the spore. Twenty six Purified isolates were stored on PDA slants at 4^oC for further study. From the total of twenty six isolates, fungal pathogen only nine were futher characterized based on their geographical location and isolates type, whether it is isolated from stem, leaf, neck or seed parts.

4.6.2. Cultural characterization

Radial mycelial growth and sporulation of isolates were studied by culturing nine fungal isolates obtained from *E. africana* on four different media; potato dextrose agar (PDA) (Potato 200 g + Dextrose 20 g + Agar – Agar 20.g + Distilled water (to make up) 1000 ml), Richards's agar (Sucrose ($C_{12}H_{22}O_{11}$) 50.00 g + Potassium dihydrogen phosphate (KH_2PO_4) 5.00 g + Potassium nitrate (KNO_3) 10.00 g + Magnesium sulphate ($MgSO_4$.$7H_2O$) 2.50 g + Ferric chloride ($Fecl_3$, $6H_2O$) 0.02g + Agar – agar 20.0g+Distilled water (to make up) 1000ml), oatmeal agar (OMA) (Oat flakes 30.0 + Agar – agar 20.0 g + Distilled water (to make up) 1000 ml) (Ainsworth (1981) and Tuite (1969)**)** and Host seed extract + 2% sucrose agar (Sucrose 20 g + Finger millet seed100g + Agar – agar 20 g + Distilled water (to make up)1000 ml) **(**Srivastava *et al*., 2009).

Twenty to twenty five ml of each of the medium were poured into each of sterilized Petri plates. Pure cultures of the isolates were prepared and fungal blocks of 4 mm disc of the mycelial mat were cut from the periphery of 10 days old culture of a pure culture of the isolates and were placed at the center of Petri-dishes in all four types of media. Each treatment was replicated three times. Cross lines with semi-permanent board were marked or drawn on the under surface of the lower plate along with the center of the fungal block. Each plate was wrapped with Parafilm tape to protect from contamination and they were incubated at 27 ± 1°C. The radial mean growth of the colonies was recorded at ten days after incubation. The diameters of the fungal colonies were measured with a measuring scale.

Sporulation was detected by microscopic observation from five days after incubation at the interval of two days up to 30 days after inoculation using lactophenol cotton blue teased mount techniques; were a loopful of culture was transferred to a clean slide and mixed well with lactophenol and a cover slip was placed on it. And finally sporulation rate of each isolate

on different media was assessed by microscopic counts. Other cultural characters such as, rate of growth, type of margin, colony color and were also recorded.

4.6.3 .Physiological Studies.

4.6.3.1. Effect of different temperature levels

The effects of temperature on the growth of different isolates were studied at different temperature level. A 4mm mycelial disc cut from margin of actively growing culture were inoculated to malt extract agar (malt extract 30g + Dipotassium hydrogen phosphate 2.0g + Ammonium chloride1.0g + Citric acid 1 N 15.0ml + Agar 12g + Distilled water 1000.ml) and incubated in darkness at six different temperatures (15, 20, 25, 30, 35 and 40°C) for 10 days. Each treatment was replicated three times. Colony diameters of each isolate on plates were measured and rate of sporulation were recorded according to (Meena, 2005).

Table 2. Sporulation rate of *P. grisea* isolates on different substrates.

Sporulation rate	No. of spores/ microscopic field
Abundant (+++)	>60
Moderate (++)	25-60
Fair (+)	10-25
Poor	<10
Nil	0 (no sporulation)

4.6.3.2. Effect of hydrogen ion concentration (pH)

The inoculums, 4mm diameter mycelial disc cut of the isolates were taken from the margin of 10 days old culture of the isolates grown on PDA and placed into 250 ml flasks containing 90 ml potato dextrose broth (PDB). The pH of PDB was varied in 0.5 units from 3 to 8.0.The pH values of the medium were adjusted to the desired pH range using 1N of HCl and 1N NaOH. Inoculated media were incubated at 27 ± 1°C, with each treatment replicated three times. After 14 days, the mycelial mats were separated and harvested from the filtrate using Whatman No 42 filter paper and washed three times with distilled water so as to wash out the

adhering salts. Mycelial mats were oven dried at 65°C for 48 hours and the dry weight of each isolate determined on electronic balance (Wagtech Was 220/ C/2). The mycelial dry weight per culture was determined by subtracting the weight of filter paper from the weight of filter paper + mycelial mat (Meena, 2005).

4.6.4. Biochemical Characterization

4.6.4.1. Carbon utilization and Nitrogen utilization

The isolates were tested for carbohydrate and nitrogen utilization. The carbon sources were replaced with 50.0g of each of carbon sources (dextrose, fructose, maltose and sucrose) and nitrogen sources were replaced with 10.0 g of each of nitrogen sources (KNO_3 $NaNO_3$, L-arginine monohydrochloride and glycine) in Richards's basal medium [Sucrose ($C_{12}H_{22}O_{11}$) 50.00 g, Potassium dehydrogenate phosphate (KH_2PO_4) 5.00 g, Potassium nitrate (KNO3) 10.00 g, Magnesium sulphate ($MgSO_4$. $7H_2O$) 2.50 g, Ferric chloride (FeCl3. $6H_2O$) 0.02g, agar 20.0g and distilled water (to make up) 1000ml]. All the carbon and nitrogen sources were dissolved properly and sterilized at 121°C for 15 minutes. By using a sterile cork borer, mycelial discs measuring 4 mm taken from the periphery of 10 days old pure culture on PDA were cut and used for inoculation to the Petri plates and the plates were incubated at 27 ± 1°C for 10 days. Colony diameters of each isolate on plates were measured.

4.7. Preparation of inocula of isolates

Among the nine isolates used for cultural, physiological and biochemical characterization representing their geographical location and isolates type, whether it is isolated from stem, leaf, neck or seed parts; only six isolates were selected for Pathogenicity study under greenhouse condition based on their sporulation rate, comparative growth on culture media, fungal pathogen. The inoculums of each isolate for pathogenicity test were prepared from 15 days old culture grown on oatmeal agar (OMA) at, 27±1°C. Conidia were harvested by scraping the surfaces of the colonies with a spatula by washing the media with sterilize distilled water and filtered them through nylon mesh cloth. Concentration of spore suspensions of different isolates were then adjusted by Haemocytometer to be (10^5) spores/ml for the isolates with sterile distilled water (Takan *et al.*, 2004). Spore suspension (200 ml) was prepared for each tested isolates for inoculation.

4.8. *In Vivo* (Greenhouse) studies

4.8.1. Pathogenicity Studies

For the pathogenicity test, in the greenhouse, disinfected viable seeds of three of *Eleusine coracana* varieties (Susceptible; variety Wama, tolerant; variety ACC.23547 and resistant; variety ACC.BKFM 0031as a standard and four varieties of *Eleusine africana* which were supplied by Dr. Dagnachew Lule from Bako Agricultural Research Center (BARC) (Table 3) were surface-disinfected (1.5% sodium hypochlorite solution, 2 min; sterile distilled water (SDW), 2 min) and planted in 30cm plastic pots filled with 4kg of autoclaved soil with three replications and control group for each seven varieties . In every three days each pot was watered with 200 ml of tap water early in the morning. The crop was thinned down to 3 stands per pot after 3 weeks of growth. When the seedlings were eight weeks old the leaves were thoroughly cleaned with sterile distilled water and the leaves were predisposed to nearly 95% humidity for 24 hours (Sreenivasaprasad *et al*., 2005).

Inoculation of each pot was done with the test pathogen isolate, Spore suspensions of $1x10^5$spore/ml sprayed on leaves by using hand sprayer (Han *et al*., 2003). Plants were inoculated twice; first at 64 days after planting on May 15, 2016, to initiate leaf and stem blast, followed at 94 days after planting; on July 15, 2016, mainly to intensify head blast infection. Three non-symptomatic seedlings per each treatment /isolate were prepared for inoculation. Additional three seedlings were included as controls. Each pot [7 varieties (3 seedlings of each) x 1isolate] for six isolates of was replicated three times. Three pots were considered as one replicate. Virulence was determined based on seedling mortality and disease development, which subsequently led to disease incidence and severity of the six the isolates on a leaf. The mean minimum and maximum temperature in the greenhouse during the study period were 15°C and 30°C respectively. After 30 days of inoculation, plant disease assessment was made in every ten days interval and recorded for the percentage of disease incidence and severity of leaf infection (Dagnachew, *et al*., 2014).

Table 3. Finger millet varieties used for pathogenicity test on seedlings.

Varieties		Date of Sowing	Inoculation	Data collection
E. coracana	Wama	11-3-2016	15-5-2016 and 15-7-2016	15-6-2016, 02-10-2016
	ACC.23547	11-3-2016	15-5-2016 and 15-7-2016	15-6-2016, 02-10-2016
	ACC.BKFM0031	11-3-2016	15-5-2016 and 15-7-2016	15-6-2016, 02-10-2016
E. africana	AAU-EIU-8	11-3-2016	15-5-2016 and 15-7-2016	15-6-2016, 02-10-2016
	ACC.AAU. EIU-15	11-3-2016	15-5-2016 and 15-7-2016	15-6-2016, 02-10-2016
	AAU-EIU-46	11-3-2016	15-5-2016 and 15-7-2016	15-6-2016, 02-10-2016
	AAU.EIU-65	11-3-2016	15-5-2016 and 15-7-2016	15-6-2016, 02-10-2016

a. Surface sterilization of the seed b.Conidia spray on the foliage c. Incubation of seedling

Figure 3. Pathogenicity test in the greenhouse.

4.8.1.1 Finger millet blast disease assessment and scoring

The plants were rated for disease incidence (DI) as the presence or absence of disease (percentage of infected leaves on the plant). DI was calculated using the number of infected plants and expressed as a percentage of the total number of plants assessed (Jamal *et al.*, 2011).

$$\textbf{Disease Incidence (\%)} = \frac{\textbf{Number of infected plant units X 100}}{\textbf{Total number of units assessed}}$$

Scoring scale of blast disease green house condition was rated according to the International Rice Research Institute (IRRI) scale of 1-9 (0 = No lesions; 1 = Small brown speaks of pin point size or large brown speck without speculating centre; 2 = Small round dish to slightly

elongated necrotic grey spots about 1-2 mm in diameter with distinct brown margin lesions are mostly found on lower leaves; 3 = Lesion type is same as in scale 2, but significant number of lesion are one on upper leaves; 4 = Typical susceptible blast lesion, 3 mm or longer infecting lesions on 2% of leaf area; 5 = Typical blast lesion infecting 2-10% of the leaf area; 6 = Typical blast lesion infecting 11-25% of the leaf area; 7 = Typical blast lesion infecting 26-50% of the leaf area; 8 = Typical blast lesion infecting 51-75% of the leaf area; 9 = More than 75% leaf area affected) (IRRI, 1996; 2009). The plants were rated for disease severity (DS) as the severity percentage of disease damage. Blast disease severity and incidence were assessed at, 30 days after inoculating the plant under natural infection by using the following formula (Waller *et al*., 2002).

Disease Severity% = nxv/9N x100; Where:

(n)= Number of plants in each category, (v) = Numerical values of symptoms category.

(N)= Total number of plants, (9) = Maximum numerical value of symptom category.

Incidence (I) and severity data (S) were used to calculate disease intensity index (DII), DII= (I× S)/9

Re- isolation of the test pathogen isolates was done in the laboratory from leaves and stems of the diseased plant parts that were taken from greenhouse. Diseased plant tissues were cut from the margin of infected portion using a sterilized knife and surface sterilized (Tuite, 1969) with, 70% ethanol for 2 min; and washed three times in sterile distilled water for 2 min. The surface sterilized tissues were directly transferred onto potato dextrose agar (PDA), amended with Chloramphenicol (0.035mg to 0.1mg). The Petri plates were incubated in the dark at 27° C for 10 days until the emergence of mycelia and sporulation. Isolates were purified by sub-culturing on PDA and maintained on PDA as before. Simultaneously, the spores were transferred to PDA and continuous observation was made on the sporulation, pigmentation, conidial structures and other parameters and compared with the original cultures of the isolates and thus Koch's postulates were proved.

4.8.1.2. Assessment of yield loss

The assessment of yield loss was carried out mainly based on yield comparisons between infected and healthy plants or between plants with different disease severities using, single

plants; between resistant and susceptible varieties, between healthy plants and plants where disease damage has been simulated by the removal of essential plant organs, such as the flag leaf on a cereal plant (Cooke, 2006).Percent yield loss (%YL) on the seven finger millet varieties in terms of grain weight was calculated as follows (Mousanejad *et al*., 2010).

$$\% \text{ YL} = \frac{\text{Yield in intensive protected plot} - \text{Yield in particular Treatment}}{\text{Yield in intensive protected plot}} \times 100$$

4.9. *In vitro* evaluation of *Trichoderma* and *Pseudomonas* isolates against the isolated pathogens.

The *In vitro* evaluation of three bioagents *viz. T. harzianum* (AAUT1) and *T. viride* (AAUT2) and *Pseudomonas fluorescence* obtained from Mycology Laboratory, Department of Microbial, Cellular and Molecular Biology, Addis Ababa University were evaluated against the test pathogens for percent inhibition of radial mycelial growth on PDA using dual culture method (Rao, 2003) of *T. harzianum* and *T. viride*. Four mm diameter mycelial disc was cut from an actively growing culture the pathogen isolates and placed on the surface of fresh PDA medium at the center of the Petri plates and Four mm diameter mycelial disc from the periphery of 10-day old culture of the two antagonists was placed on the plate at four locations, approximately 3 cm from the center.

Similarly, *Pseudomonas fluorescence* isolate was assessed for potential antagonistic activity against the pathogen on PDA using dual culture technique (Rangajaran *et al.,* 2003). Four mm diameter mycelial disc was cut from an actively growing pathogen culture and placed on the surface of fresh PDA medium combined with sucrose at the center of the Petri plates. A loopful of 48 hr old actively growing *Pseudomonas fluorescence* isolate was placed opposite to the fungal disc and streaking the *Pseudomonas fluorescence* isolate on the plate at four locations, approximately 3 cm from the center. The plates inoculated with the pathogen without *Pseudomonas fluorescence* and *Trichoderma* species were used as a control.

All *in vitro* tests of antagonism were performed three times. All plates were incubated at, $27^{o}C \pm 1$ for 7 days. Visual observations of growth inhibition were recorded every two days and the final measurements were recorded at the 7th days of incubation. The degree of antagonism was determined by measuring the radial mycelial growth of coculture of the pathogen (radial mycelial growth reduction) and the biocontrol agents in relation to the

growth of the control and percentage of inhibition was calculated by using the following equation (Riungu *et al*., 2008).

I% =(C-T)/C x100.Where:

I = percentage inhibition of pathogen by antagonists

C= radial growth measurement of the pathogen in the control plates and

T= radial growth of the pathogen in the experimental plates

4.10. *In vitro* evaluation of fungicides

Sancozeb and Curzate fungicides were obtained from Mycology Laboratory Research, Addis Ababa University for *in vitro* evaluation conditions. Two fungicides were tested by the food poisoning method at 200,400, 600, 800 and 1000 ppm concentrations. The fungicides concentration were prepared as follows, if the formulated product (fungicide).Stock concentrations of the fungicides used were: Curzate 50% (Cymoxanil and cupper oxychloride), and Sancozeb 80% WP (mancozeb 80% a.i and inert 20%). Therefore, Curzate 200 ppm (0.35 g), 400 ppm (0.7g), 800 ppm (1.4 g) and 1000 ppm (1.75 g) were added in a liter of solvent. For the preparation of Sancozeb (Mancozeb 80% WP) 0.64 g, 1.28 g, 2.56 g and 3.2 g were used for 200, 400, 800 and 1000ppm, respectively and were dissolved in a liter of sterilized distilled water (Nene and Thapliyal, 1993). The fungicides were added to the autoclaved Potato dextrose agar (PDA) medium to prevent denaturation of the fungicides, cooled to 45°C with the amount of 2 ml per plate of each containing 20 ml of the test medium. Triplicate culture plates were used to test each isolate at each concentration. PDA medium without fungicide served as control. After solidifying the medium, 4mm disc of a pure culture of test fungal isolates was placed at the center of Petri-dishes. There were three replications of each treatment. Inoculated plates were incubated at, 27±1° C for 7 days. The growth of isolates was determined by measuring colony diameters in two perpendicular directions on each culture plate in darkness at, 27±1° C. The relative growth reduction for each rate of fungicide was calculated as before (Riungu *et al*., (2008).

4.11. Compatibility Studies of *Trichoderma* and Fungicides

The compatibility of the biocontrol agents and fungicide was tested as before. The combined use of *Trichoderma* species and fungicides were applied by the method of (Nene and Thapliyal, 1993). In this technique, the growth medium was poisoned with fungal toxicants.

The fungicides concentrations of 100, 200 and 400 ppm for Curzate and Sancozeb were prepared and added to the autoclaved PDA medium after having cooled to 45°C. Triplicate culture plates, each containing 20 ml of the test medium was poured and after solidification of the medium, the tests *Trichoderma* species were inoculated. Potato dextrose agar plates without *Trichoderma* species and fungicides were used as a control. The growth of *Trichoderma* species at 100, 200 and 400 ppm fungicides combination were determined by measuring mycelia growth diameters and percentage inhibition of radial growth was calculated following the formula suggested by (Rita and Tricita, 2004):

$L = (C - T)/C \times 100$ Where,

L is mean inhibition per cent of radial mycelial growth;

C is radial growth measurement of the test *Trichoderma* species in control;

T is radial growth of the test *Trichoderma* species in combination with fungicides.

4.12. Statistical Analysis

The statistical analysis of growth characteristics of isolates at different media, temperature, and pH and mean comparisons of isolates based on different parameters were conducted using one-way ANOVA procedures of SPSS statistical analysis software version 21. Differences between treatments were determined by using least squared means comparisons with ($P<0.05$). Mean comparisons of each treatment were performed by using Duncan's multiple range test ($\alpha = 0.05$).

5. RESULTS

5.1. Collection and isolation of different isolates of the pathogen

The fungal isolates were assigned *Pyricularia grisea* (*Pg*) isolates 1- 26. For intensive characterization, only nine isolates of *P. grisea* were selected based on geographical location of the origin of the isolate and growth characteristics of the test pathogen and designated as *Pg*.003, *Pg*.007, *Pg*.015, *Pg*.025, *Pg*. 034, *Pg*. 046, *Pg*.058, *Pg*.080 and *Pg*.085 (Table 1).

5.2. Cultural, morphological, physiological and biochemical characteristics of the test pathogen

5.2.1. Conidial characteristics of the test pathogen isolates

5.2.1.1. Septation, Shape, size and color of conidia

Description of morphological characteristics of microconidia and macroconidia indicated that the nine isolates were grouped into *P. grisea*, which coincided with their cultural characterization. There existed variation in septation and the way they are septated. The majority of the conidia were found to have 3 septations. Conidia with one and two septation were rarely observed in all isolates. The conidia were found to show variations in septation and are indicated in Figure 4. In all isolates, the shape of the conidia was the same with typically pyriform conidia, base rounded, apex narrowed, 2-4 celled, middle cells was broader than others in all isolates (Figure.4). The aerial mycelium was white to gray and black to gray. Though closely resembling one another in shape and color, distinct differences in spore size were seen among the isolates. Some of them were very long and narrow, while some were fairly broad. The isolate *Pg.*003 showed the maximum size of conidial length about 37.01-40.01µm (average 40.0µm), and the minimum size of conidial length was noticed by the isolates *Pg.*080 of 25.52-38.00µm (average 27.54µm). Maximum conidial widths were observed by isolates of *Pg*.080 with 12.13-14.52µm (Average 14.28µm) (Table 4). The mycelia of the *P. grisea* isolates, in general, were observed to be highly branched at a wider angle, repeatedly septated, superficial and bear conidia at the tip or side of the conidiophores (Figure.5). Sporulation was abundant with maximum fruiting bodies at the center of the plate as compared to that of the periphery mycelium growth of the test pathogen. Based on the morphology of the test fungus the pathogen was identified as *P. grisea* isolates. The different size and shapes of conidia and conidial mass were indicated in (Figure.6).

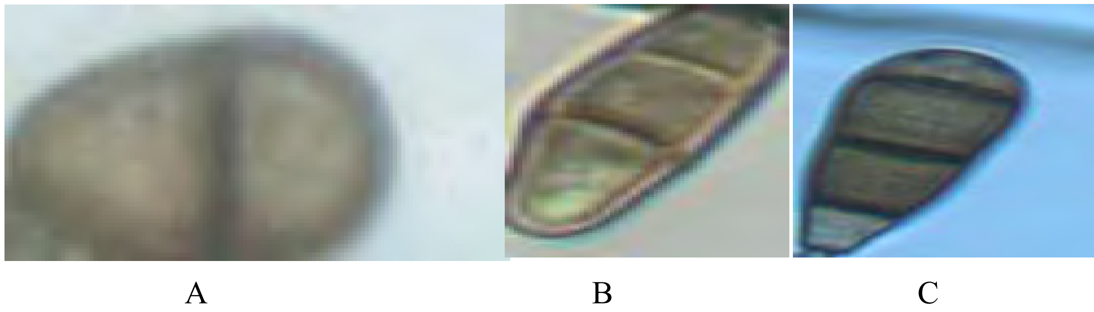


Figure 4. Microphotograph showing conidia of *P. grisea* (A) with one septation, (B) with two septation and (C) with three septation (40x).

Pg.003 *Pg*.007 *Pg*.15
Pg.25 *Pg*.34 *Pg*.46
Pg.58 *Pg*.80 *Pg*.85

Figure 5.Microphotograph showing conidial mass of *P. grisea* with different shapes and size.

Figure 6. Microphotograph showing conidia and conidiophores of *P. grisea* (20, 40 & 100x).

Table 4.Conidial size of nine *P. grisea* isolates after three weeks of incubation at 27±1° C.

SI. No.	Isolate	Range(µm)	Average (µm)
1	*Pg*.003	37.01-40.01 x 10.82-14.02	40.0 x 13.10
2	*Pg*.007	35.24-40.00 x 13.09-14.47	39.60 x 13.23
3	*Pg*.015	33.32-39.93 x 11.07-13.24	36.67 x 12.40
4	*Pg*.025	32.87-37.88 x 12.90-14.12	35.23 x 13.80
5	*Pg*.034	32.27-39.42 x 09.54-13.70	36.65 x 11.04
6	*Pg*.046	34.33-39.49 x 11.46-14.87	37.01 x 13.49
7	*Pg*.058	33.25-37.34 X10.10-11.53	35.73x11.31
8	*Pg*.080	25.52-38.00x12.13-14.52	27.54 x 14.28
9	*Pg*.085	30.50-39.00x12.50-14.99	35.29x10.01

5.2.2. Cultural characteristics and Sporulation pattern isolates on different solid media

The colony of *P. grisea* isolates showed significant differences in their growth rate and slight variations in color on the four mycological media. Growth was initially slow but increased with time on all culture. Variation in colony characters *viz*., colony pigmentation, type of growth and colony margin among the isolates of *P. grisea* are presented in Table 5.With regard to their colony colors, isolates imparted gray, greyish black, black and buff white colours on the different growth media. Sporulation of the nine isolates was observed on host seed extract + 2% sucrose agar, oat meal agar, PDA and Richard's agar medium (Table 5).

5.2.2.1. Host seed extract + 2% sucrose agar

All the isolates were grayish-black color whereas *Pg*. 080, *Pg*.046 and *Pg*.058 have shown gray color and *Pg*.007 and *Pg*.034 has showed black color. Colony margin of all the isolates

was smooth. Except *Pg*. 080 for which sporulation was negligible, all the isolates of *P. grisea* showed good growth. Abundant sporulation of all the isolates was noticed in host seed extract + 2% sucrose agar (Appendix 3A). The isolates *Pg*.085 (neck blast) and *Pg*.015 (leaf blast) have shown the raised mycelium with concentric ring pattern (Appendix 4).

5.2.2.2. Oatmeal agar

Colony colour of all the isolates was usually grayish-black colored with good growth whereas, *Pg*.025 and *Pg.* 003 (neck blast) showed white tinged gray and *Pg.* 080 (head blast) off-white color and medium growth. All the isolates of *P. grisea* showed the raised mycelial growth with smooth colony margin. Except *Pg*. 080 for which sporulation were negligible, abundant sporulation of all the isolates was noticed in oatmeal agar (Appendix 3B).

5.2.2.3. Potato Dextrose Agar

Colonies were dark gray colored in *P. grisea* isolates *Pg.*003, *Pg.*007, *Pg.*034, *Pg.*046 and *Pg.*058. However isolates *Pg.*015, *Pg.025, Pg.*080 and*Pg.*085 have shown gray color. Except for the isolate *Pg*.003, *Pg*.046 and *Pg*.080 almost all the isolates showed good mycelial growth on PDA. The isolates *Pg*.003, *Pg*.034, *Pg*.058 and *Pg*.080 have showed irregular colony margin whereas other isolates were with regular and smooth colony margin. Except for *Pg*.015, *Pg*.034, *Pg*.046 and *Pg*.080, all the isolates have shown concentric ring pattern on PDA. Though the isolate *Pg*.015, *Pg*.034, *Pg*.046 and *Pg*.080 sporulation was negligible on PDA medium. However, the isolates *Pg*.003, *Pg*.007, *Pg*.025 and *Pg*.058 and *Pg*.085 were sporulated moderately (Appendix 3D).

5.2.2.4. Richards's Agar Medium

All the *P. grisea* Isolates have shown gray color except the isolate *Pg*.007 and *Pg*.080 which showed grayish-black and off-white respectively. All the Isolates have shown smooth colony margin with good mycelial growth. With regard to sporulation, though the isolate *Pg*.080 showed profused mycelial growth, sporulation was less on Richard's agar medium. However, abundant sporulation was noticed by *Pg*.007, *Pg*.015, *Pg*.034 and *Pg*.085 isolates. The isolate *Pg*.003, *Pg*.025, *Pg*.046 and *Pg*.058 showed moderate sporulation (Appendix 3C)

Table 5. Effect of different culture media on colony characters development and sporulation pattern of *P. grisea* isolates, incubated at 27±1°C for 10 days.

Media	Colony characters								
	Isolates								
	Pg.003	*Pg*.007	*Pg*.015	*Pg*.025	*Pg*.034	*Pg*.046	*Pg*.058	*Pg*.080	*Pg*.085
Host Seed Extract +2% Sucrose agar	Grayish-black color Smooth colony margin Good growth	black color Smooth colony margin Good growth	Grayish black color Smooth colony margin Good growth	Grayish black color Smooth colony margin Good growth	black color Smooth colony margin Good growth	Gray color Smooth colony margin Good growth	Gray color Smooth colony margin Good growth	Gay color Smooth colony margin Medium growth	Grayish-black color Smooth colony margin Good growth
Sporulation	++	+++	+++	+++	+++	++	++	-	+++
Oatmeal agar	White tinged Gray color Smooth margin Good growth	Grayish black color Smooth margin Good growth	Grayish black color Smooth margin Good growth	White tinged Gray color Smooth margin Good Growth	Grayish black color Smooth margin Good growth	Grayish black color Smooth margin Good growth	Grayish black color Smooth margin Good growth	Off-white color Smooth colony margin Medium growth	Grayish black color Smooth margin Good growth
Sporulation	+++	+++	+++	+++	+++	+++	+++	-	+++
Potato dextrose agar	Grayish-black color Irregular margin Medium growth	Grayish-black color Smooth margin Good growth	Gray color Smooth margin Good growth	Gray color Smooth margin Good growth	Grayish-black color Irregular margin Good growth	Grayish-black Smooth colony margin Medium growth	Grayish-black color Irregular colony margin Good growth	Gray color Irregular colony margin Medium growth	Gray-black color Smooth margin Good growth
Sporulation	+	++	-	++	-	-	++	-	++
Richards's agar	Gray Color smooth margin Good growth	Grayish black color Smooth margin Good growth	gray color Smooth margin Good growth	Gray color Smooth margin Good growth	Gray color Smooth margin Good growth	Gray color Smooth margin Good growth	Gray color Smooth margin Good growth	off-white color Smooth margin medium growth	Gray color smooth margin Good growth
Sporulation	++	+++	+++	++	+++	++	++	-	+++

+++= Abundant, ++ = Moderate, += Fair, - = No sporulation

5.2.1.2. Effect of different culture media on mycelia growth of Pathogen

The results indicated in Table 6 revealed that there was a significant difference between isolates, media and their interaction among the four media used. The maximum mean radial mycelial growth was recorded by neck blast isolate (*Pg.*085) (85.5 mm) significantly followed by the leaf blast isolate (*Pg.*015) (81.4mm) and the head blast isolate *Pg.*080 with 59.4 mm, showed the least radial growth.

All isolates showed consistently better growth on oatmeal agar followed by Host extract + 2% sucrose with maximum mean radial growth of (81.9 mm) which was followed by Richards' agar medium (79.1 mm). Least mean radial growth was observed in Potato dextrose agar (66.4 mm). The utilization of media by the isolates was varied. Radial growth of isolates (*Pg.*085) (88.0mm) on oatmeal agar and (*Pg.*085 and *Pg.*003) (87.0 mm) on Host extract + 2% sucrose agar medium were significantly superior to that of the other isolates and least mycelial growth was observed by isolates of *Pg.*080 with 52.0mm on PDA(Table 6).

Table 6. Evaluation of different culture media for the mycelial growth of *P. grisea* isolates.

SI No.	Isolates	Colony diameter (mm)				
		Media				
		OMA	HSEA	RA	PDA	Mean±SD
1	*Pg.*003N	83.3±3.2^{ab}	87.0±2.6^{a}	72.3±5.0^{c}	64.6±4.1^{cde}	76.8±10.2^{cd}
2	*Pg.*007L	80.6±1.5^{b}	86.3±3.0^{a}	81.3±4.0^{ab}	71.0±4.0^{bc}	79.8±6.3^{bc}
3	*Pg.015L*	84.0±3.5^{ab}	86.6±2.2^{a}	82.3±3.5^{a}	73.0±2.0^{b}	81.2±5.9^{b}
4	*Pg.*025N	82.0±2.6^{b}	83.0±2.6^{ab}	75.0±4.0^{bc}	73.3±5.0^{b}	78.2±4.9^{c}
5	*Pg.*034L	85.0±2.0^{ab}	84.±4.0^{ab}	84.0±2.5^{a}	59.0±5.0^{de}	78.0±12.6^{c}
6	*Pg.*046S	85.0±4.1^{ab}	79.0±2.0^{b}	85.6±3.5^{a}	57.6±1.5^{ef}	76.9±13.2^{cd}
7	*Pg.*058L	85.0±3.6^{ab}	80.6±3.0^{b}	85.0±4.0^{a}	66.0±3.0^{bcd}	79.1±9.2^{b}
8	*Pg.*080H	63.0±3.6^{c}	63.6±1.1^{c}	59.0±4.0^{d}	52.0±1.0^{f}	59.4±5.3^{d}
9	*Pg.*085N	88.0±0.0^{a}	87.0±2.0^{a}	86.6±2.5^{a}	80.6±6.5^{a}	85.5±3.3^{a}
Mean±SD		81.9±7.5^{a}	81.9±7.5^{a}	79.5±9.1^{ab}	66.4±9.2^{c}	77.3±7.4

Each value is an average of three replicates ±standard deviation. Values in bold were compared within their respective column and row. Data followed by the same letter are not significantly different (p<0.05), according to Duncan's multiple range test, Alpha=0.05, CV=19.3

5.2.3. Physiological variability

5.2.3.1. Temperature

All the *P. grisea* isolates were showed different responses to the different incubation temperatures (Table 7). The variation in mycelial growth among the isolates of the test pathogen at different temperatures was found to be significant. The isolates of *Pg*.007, *Pg*.025, *Pg*.034 and *Pg*.085 showed maximum diameter of (74.7mm, 74.7mm, 77.3mm and 79.0mm respectively) at 30°C, which was significantly superior to other temperature level as shown in Table 7. The optimum temperature range for the maximum growth of the isolates of *P. grisea* was found to be 20°C to 30°C. The mycelial growth of all the isolates was decreased at 40°C. Sporulation was negligible at the highest temperature (40°C) for all isolates, but the isolates showed fair to abundant sporulation at 15°C, 20°C, 25°C, 30°C and 35°C except for the isolates, *Pg*.034, *Pg*.046 and *Pg*.058 on which sporulation was not observed at 15°C (Appendix 2).

Table.7.Effect of different temperature on mycelial growth of *P. grisea* isolates.

No	Isolate	Mean diameter						mean± SD
		Temperature (°C)						
		15	20	25	30	35	40	
1	*Pg*.003	18.0±1.0^{e}	38.0±1.0^{e}	61.3±0.7^{d}	63.0±2.0^{d}	41.3±1.5^{d}	13.3±0.6^{b}	39.1±20.9^{e}
2	*Pg*.007	29.0±1.0^{cd}	49.0±1.0^{cd}	71.7±1.5^{bc}	74.7±1.5^{bc}	44.3±1.5^{c}	NG*±0.0^{d}	44.7±27.8^{c}
3	*Pg*.015	27.7±0.6^{d}	47.7±0.6^{d}	70.3±0.6^{c}	73.0±2.0^{c}	45.0±1.0^{c}	NG*±0.0^{d}	43.9±27.4^{c}
4	*Pg*.025	30.3±0.6^{bc}	50.3±0.6^{bc}	73.3±1.5^{b}	74.7±2.5^{bc}	43.3±0.6^{cd}	14.7±0.6^{a}	47.7±23.6^{b}
5	*Pg*.034	32.0±1.0^{a}	52.0±1.0^{ab}	74.0±1.0^{b}	77.3±2.1^{ab}	50.3±2.5^{b}	NG*±0.0^{d}	47.6±28.7^{b}
6	*Pg*.046	27.3±2.1^{d}	47.3±2.1^{d}	59.7±0.6^{d}	61.0±1.0^{d}	37.3±0.6^{e}	12.0±2.0^{c}	40.7±19.1^{d}
7	*Pg*.058	28.0±1.0^{d}	48.0±1.0^{d}	61.7±2.1^{d}	64.0±1.7^{d}	38.3±0.6^{e}	11.3±0.6^{c}	41.9±20.3^{d}
8	*Pg*.080	13.7±1.5^{f}	35.7±0.6^{f}	41.0±1.0^{e}	48.7±2.5^{e}	32.3±1.5^{f}	10.7±0.6^{c}	30.3±15.1^{f}
9	*Pg*.085	33.0±1.0^{a}	52.7±1.5^{a}	76.3±2.1^{a}	79.0±2.0^{a}	54.7±1.5^{a}	NG±0.0^{e}	49.3±29.5^{a}
Mean±SD		26.5±6.3^{e}	46.7±5.7^{c}	65.5±10.7^{b}	68.4±9.6^{a}	43.0±6.6^{d}	6.9±6.4^{f}	42.8±23.4

Observation on mean colony diameter was recorded ten days after incubation.

Means with the same letters are not significantly different .NG=no growth; Alpha = 0.05, CV= 24.1*

5.2.3.2. pH (Hydrogen) ion concentration:

The various pH levels on the growth of the isolate have indicated that their interactions were found to be significant (Table 8). The results of the present investigation showed that

maximum mean mycelial growth (484.2mg) at pH 6.5 was significantly superior to other pH levels (table 8). Hence, at pH 6.5 all the isolates exhibited maximum mean dry mycelial weight indicated it as optimum pH. The mean mycelial growth of all the isolates significantly increased from pH 3.0 to pH 6.5 which further started declining. Maximum dry mycelial weight was recorded by Neck blast isolate, *Pg*. 003 with 521mg at pH 6.5 whereas least growth was obtained by Head blast (*Pg*.080) isolate with 92.33 mg at pH 3.0.

Table 8. Effect of different pH values on dry mycelial weight of *P. grisea* isolates.

S. NO	pH	Dry mycelial weight (mg)									
		Pg.003	*Pg*.007	*Pg*.015	*Pg*.025	*Pg*.034	*Pg*.046	*Pg*.058	*Pg*.080	*Pg*.085	Mean
1	3.0	188±2.1^{f}	162.6±1.1^{d}	306.6±1.5^{a}	283.7±2.5^{b}	263.3±2.6^{d}	242.6±2.5^{e}	304.3±1.5^{ab}	92.3±1.5^{i}	302.6±2.0^{b}	238.5±81.3^{i}
2	3.5	201.6±1.1^{g}	267.6±1.1^{e}	351.7±1.1^{a}	311.0±1.7^{c}	302.6±0.5^{d}	256.6±1.1^{f}	327.3±0.5^{c}	152.3±1.5^{h}	339.3±1.1^{b}	249.1±86.3^{h}
3	4.0	267.3±1.5^{h}	315.6±1.5^{f}	361.6±1.5^{c}	369.2±3.0^{b}	343.6±2.0^{e}	274.0±1.0^{g}	352.6±1.1^{d}	157.6±1.1^{i}	376.0±2.0^{a}	313.0±83.1^{f}
4	4.5	347.0±1.7^{f}	425.3±1.5^{a}	372.3±2.5^{d}	378.0±1.1^{c}	360.7±1.5^{e}	293.7±1.5^{g}	373.7±1.5^{d}	213.0±1.7^{h}	411.7±1.5^{b}	352.7±79.3^{e}
5	5.0	397.6±1.5^{f}	492.3±1.1^{a}	444.0±2.0^{c}	403.6±1.5^{d}	368.3±1.1^{g}	330.0±1.0^{h}	398.6±1.5^{e}	262.3±1.5^{i}	446.6±1.1^{b}	394.0±80.9^{d}
6	5.5	411.3±0.5^{e}	494.2±1.5^{a}	462.0 ±2.0^{b}	431.0±1.1^{c}	379.0±1.0^{f}	378.6±1.5^{f}	421.0±1.0^{d}	367.6±1.5^{g}	461.0±1.0^{b}	422.8±87.7^{c}
7	6.0	496.0±1.7^{b}	497.7±1.1^{a}	484.3±1.5^{bc}	462.7±1.1^{c}	403.6±1.1^{f}	423.7±0.6^{e}	458.3±1.5^{d}	394.6±1.5^{g}	479.7±1.5^{cd}	455.6±77.1^{b}
8	6.5	521.0±1.7^{a}	499.3±1.1^{ab}	498.7±1.5^{ab}	491.3±1.5^{c}	449.0±2.0^{f}	465.3±1.1^{e}	481.3±1.1^{d}	447.7±1.5^{g}	501.3±1.5^{ab}	484.2±64.6^{c}
9	7.0	302.0±1.7^{h}	414 .3±1.5^{e}	448.0±2.0^{c}	486.3±1.1^{a}	335.6±1.5^{ef}	320.6±1.5^{g}	336.6±1.5^{f}	422.6±1.5^{d}	482.6±0.6^{b}	494.2±97.7^{g}
10	7.5	313.6±1.1^{e}	401.6±0.6^{ab}	301.3±1.5^{f}	398.0±1.0^{d}	301.6±1.5^{f}	291.0±1.1^{g}	228.6±1.5^{h}	388.0±1.0^{c}	467.6±1.5^{a}	289.2±93.5^{g}
11	8.0	297.0±1.7^{e}	319.6±1.1^{c}	283.6±1.1^{f}	382.3±1.1^{a}	298.3±1.5^{d}	271.0±1.0^{g}	202.3±1.1^{i}	225.3±1.1^{h}	381.0±1.7^{ab}	295.6±70.2^{f}
Mean±SD		331.1±81.3^{g}	390.0±72.6^{d}	392.2±89.6^{c}	400.0±68.6^{b}	346.0±83.7^{f}	322.1±58.9^{h}	353.1±82.8^{e}	284.0±81.3^{i}	422.0 ±84.5^{a}	360.4±57.3^{d}

Means with the same letters are not significantly different. Alpha =0.05, CV = 25.2

5.2.4. Biochemical studies

5.2.4.1. Effect of carbon sources on the mycelial growth of *P. grisea* isolates

The response of the isolates to the different sugars was variable. Dextrose supported maximum mycelial growth in isolate *Pg*.085 (86.6mm) and least was observed by isolate *Pg*. 080 (67.0mm). Sucrose supported maximum mycelial growth in isolate *Pg*.007 (81.3mm) and least growth was observed in isolate *Pg*.080 (58.3mm). Maltose supported least mycelial growth by isolates *Pg*.080 (60.3mm). Similarly fructose supported maximum mycelial growth in isolate *Pg*.007 (83.3mm) and least growth was observed in isolate *Pg*.080 (58.0mm) (Table 9). Effect of different Carbon sources on mycelial growth of *P. grisea* isolates have shown on (Appendix 5).

Table9. Effect of different carbon sources on mycelial growth of *P. grisea* isolates.

SI No.	Isolate	diameter (mm)				
		Dextrose	Maltose	D-fructose	Sucrose	Mean ±SD
1	*Pg*.003	85.0±1.0^{c}	80.0±1.0^{c}	73.3±0.6^{d}	71.0±1.0^{e}	77.3±6.4^{d}
2	*Pg*.007	87.6±1.5^{b}	86.6±1.5^{a}	83.3±1.1^{a}	81.3.±0.6^{a}	84.7±2.9^{a}
3	*Pg*.015	87.6±1.5^{b}	84.3±1.5^{b}	78.0±1.7^{c}	79.0±1.0^{b}	82.2±4.5^{c}
4	Pg.025	88.0±1.0^{a}	81.6±0.6^{c}	79.6±0.6^{bc}	77.3±1.5^{cd}	82.3±4.7^{c}
5	*Pg*.034	88.3±1.1^{a}	81.0±1.0^{c}	79.6±2.1^{bc}	78.0±1.1^{c}	81.7±4.5^{cd}
6	*Pg*.046	87.3±2.1^{b}	85.0±1.0^{ab}	81.3±0.6^{ab}	79.3±1.5^{b}	83.2±3.6^{b}
7	*Pg*.058	83.6±1.5^{d}	80.3±0.6^{c}	74.3±0.6^{d}	75.6±0.6^{d}	78.4±4.3^{d}
8	*Pg*.080	67.0±1.0^{e}	60.3±0.6^{d}	58.0±1.7^{e}	58.3±1.5^{f}	60.9±4.1^{e}
9	*Pg*.085	88.6±0.6^{a}	86.6±1.1^{a}	78.0±1.7^{c}	78.0±1.7^{c}	82.8±5.6^{c}
Mean ±SD		84.8±6.7^{cd}	80.6±7.8^{b}	76.2±7.3^{c}	75.3±6.8^{d}	79.2±4.4

Alpha =0.05, CV= 7.9

5.2.4.2. Nitrogen utilization

The results of the utilization of four different nitrogen sources KNO_3, $NaNO_3$, L-Glycine, and L-Arginine by each isolated of *P. grisea* were recorded in table 10. The response by *P. grisea* isolates to exogenous supply of nitrogenous compounds was variable among the isolates and $NaNO_3$ proved to be the best source of nitrogen for all the isolates followed by L-glycine. L-arginine supported higher mycelial growth than KNO3.Generally sodium nitrate and L-glycine represented good sources of nitrogen (Table 10).

Table 10. Effect of different nitrogen sources on mycelial growth of *P. grisea* isolates.

No	Isolate	diameter (mm)				
		$NaNO_3$	KNO_3	L-arginine	L-glysine	Mean±SD
1	*Pg*.003-N	$81.3±1.1^{d}$	$74.3±1.0^{e}$	$74.3±0.6^{e}$	$77.7±1.5^{d}$	$76.9±3.3^{e}$
2	*Pg*.007-L	$87.0±1.0^{b}$	$87.0±1.0^{a}$	$88.3±0.6^{a}$	$88.6±0.6^{a}$	$87.7±0.8^{a}$
3	*Pg*.015-L	$84±1.0^{c}$	$82.7±0.5^{c}$	$84.3±1.1^{c}$	$83.3±1.1^{c}$	$83.5±0.7^{d}$
4	*Pg*.025-N	$81.0±1.0^{d}$	$73.6±0.6^{e}$	$76.3±0.6^{d}$	$78.7±0.5^{d}$	$77.4±3.1^{e}$
5	*Pg*.034-L	$88.3±0.6^{ab}$	$85.0±1.1^{ab}$	$86.3±0.5^{b}$	$86.6±0.6^{b}$	$86.5±1.3^{b}$
6	*Pg*.046-S	$86.3±0.6^{b}$	$78.6±1.1^{d}$	$84.7±1.5^{c}$	$85.0±1.7^{bc}$	$83.6±3.4^{d}$
7	*Pg*.058-L	$88.0±1.7^{ab}$	$84.0±1.0^{bc}$	$84.3±1.5^{c}$	$86.3±0.7^{b}$	$85.6±1.8^{c}$
8	*Pg*.080-H	$69.6±1.5^{e}$	$65.3±1.3^{f}$	$66.3±1.1^{f}$	$68.3±0.6^{e}$	$67.4±1.9^{f}$
9	*Pg*.085-N	$83.8±5.8^{a}$	$86.7±0.6^{a}$	$87.7±0.6^{ab}$	$88.0±0.7^{a}$	$87.9±0.9^{a}$
	Mean ±SD	$83.8±5.8^{a}$	$79.7±7.1^{d}$	$81.4±7.1^{c}$	$82.6±6.4^{b}$	81.8±1.7

Values with the same letters are not significantly different, Alpha =0.05, CV=8.

5.3. Pathogenicitytest

Pathogenicity test showed that the disease symptoms, dark brown was developed on the leaves after the 7th days of inoculation and increased up to 60 days. However, in the case of wild finger millet varieties, it was increased when it was young and tried to be resisted by, 90 days after planting or 36 days after inoculation (Appendix 6). There was the significant difference amongst varieties in their susceptibility to the test pathogen and among the tested isolates of *P. grisea*. When these infected leaves were removed from the plant and re-isolate the pathogen, a group of conidiophores and conidia of the fungus were observed which were similar to the original culture isolated from the field. Disease incidence and severity of the six isolates on the different varieties are indicated in Table 11. The highest mean disease incidence was on variety Wama (susceptible) (73.6%), where as the lowest was displayed on AAU-EIU-46 (34.3%). It is evidenced that the highest mean percentage disease incidence and severity was observed by isolate *Pg*.007 (73.6% and 17.6%, respectively) which was significantly different from the remaining isolates. The lowest mean percentage disease incidence and severity was observed by *Pg*.034 (45.0% and 14.2% respectively) on the seven finger millet varieties (data not shown).The isolates have shown variation in their virulence on a particular host species that differentially respond to different isolates (Appendix 9).

Table.11 Percentage disease incidence and severity on seven varieties inoculated with six *P. grisea* isolates under green house condition.

Isolates			003	007	015	025	034	085	Mean ±SD
Finger millet varieties	AAU-ELU-65	Severity (%)	16.4±1.2^{a}	14.3±0.6^{a}	11.6±1.4^{a}	12.1±1.6^{a}	5.8±1.1^{b}	8.5±2.1^{b}	11.4±3.2
		Incidence (%)	38.3±1.6^{a}	35.2±0.7^{abc}	37.2±1.7^{abc}	33.8±01.0^{c}	36.0±2.0^{abc}	35.3±2.1^{abc}	36.0±2.0^{d}
	AAU-ELU-46	Severity (%)	7.9±0.6^{b}	9.0±1.2^{b}	11.6±1.5^{a}	7.0±1.1^{b}	7.9±1.0^{b}	17.9±1.3^{a}	10.2±2.1^{f}
		Incidence (%)	35.4±1.0^{a}	34.2±1.3^{a}	36.0±1.7^{a}	31.3±1.1^{b}	35.5±0.6^{a}	33.7±0.6^{a}	34.3±2.0^{d}
	AAU. EIU-15	Severity (%)	13.7±2.3^{a}	16.9±1.2^{a}	14.8±2.0^{a}	19.5±1.1^{a}	17.9±1.2^{a}	17.4±1.4^{a}	16.7±3.5^{e}
		Incidence (%)	47.3±1.0^{c}	57.4±1.4^{a}	51.3±1.0^{b}	44.2±0.7^{d}	46.0±2.0^{cd}	56.7±0.6^{a}	50.5±5.3^{c}
	AAU-ELU-8	Severity (%)	17.4±2.1^{a}	16.9±0.6^{a}	15.3±1.5^{a}	13.2±1.3^{a}	16.9±1.4^{a}	18.0±0.7^{a}	16.4±4.3^{e}
		Incidence (%)	48.7±1.2^{d}	60.5±1.6^{a}	53.7±1.0^{c}	46.4±1.5^{e}	45.0±1.0^{e}	55.9±1.0^{b}	51.7±5.7^{c}
	Susceptible	Severity (%)	23.3±2.0^{a}	23.8±0.5^{a}	24.3±0.6^{a}	22.7±1.2^{a}	21.1±0.7^{a}	22.2±1.4^{a}	22.9±2.8^{e}
		Incidence (%)	87.7±1.0^{a}	86.0±1.1^{a}	75.0±0.4^{b}	64.0±0.3^{c}	55.0±1.4^{d}	75.0±1.1^{b}	73.6±11.8^{a}
	Tolerant	Severity (%)	20.1±0.6^{a}	22.2±.2.2^{a}	20.6±1.3^{a}	21.1±2.4^{a}	18.0±2.1^{b}	18.5±1.2^{b}	20.1±2.6^{e}
		Incidence (%)	81.6±1.5^{a}	71.6±1.7^{b}	66.3±0.7^{c}	50.6±0.4^{f}	53.3±0.6^{e}	62.2±1.0^{d}	64.3±11.0^{b}
	Resistant	Severity (%)	19.0±0.7^{b}	20.6±1.2^{a}	15.8±12^{b}	16.4±1.1^{b}	12.1±1.4^{b}	14.3±1.0^{b}	16.4±4.1^{e}
		Incidence (%)	73.5±1.5^{a}	68.0±1.6^{b}	61.6±1.0 ^{c}	49.4±0.5^{e}	50.8±1.0^{e}	58.7±0.6^{d}	60.2±8.9^{b}

5.4. Yield loss caused by *P. grisea* isolates

There was significant difference among the varieties in their percentage yield loss to the pathogen and among the grain weight infected by the isolates (Table 12). The highest percent of yield losses was recorded by isolate *Pg*.015 (67.0%) on the cultivar variety susceptible (Wama) and by the isolate *Pg*. 025 (56.2%) in case of wild finger millet varieties (ACC.AAU.EIU-15). From the overall mean value of yield losses in cultivar finger millet variety were superior to the tested wild finger millet varieties (Table 12).

Table.12. Yield losses caused by *P.grisea* isolates on the seven finger millet varieties under green house condition.

Isolates			003	007	015	025	034	085	Mean ±SD	Control
yield (g/pot)/ Finger millet varieties	AAU-ELU-65	YL (%)	4.9±1.6^{b}	28.4±12.6^{a}	3.9±1.2^{c}	2.8±2.2^{d}	3.2±2.1^{c}	4.2±2.1^{b}	7.9±1.4^{d}	9.4±0.5^{a}
		Infected	8.9±0.2^{a}	6.7±1.2^{b}	9.3±0.4^{a}	9.1±0.2^{a}	9.1±0.2^{a}	9.0±0.2^{a}	8.7±1.0^{d}	
	AAU-ELU-46	YL (%)	1.6±0.7^{e}	3.2±1.4^{c}	11.0±5.5^{b}	2.4±1.2^{d}	1.6±1.0^{e}	17.0±1.2^{a}	6.3±6.0^{d}	8.2±1.2^{b}
		Infected	8.1±0.1^{a}	7.9±0.1^{a}	7.3±0.4^{b}	8.0±0.1^{a}	8.1±0.1^{a}	6.8±0.1^{b}	7.7±0.5^{d}	
	AAU. EIU-15	YL (%)	31.5±4.9^{d}	44.4±3.2^{c}	17.6±2.8^{f}	56.2±1.4^{a}	23.8±3.2^{e}	49.1±1.8^{b}	37.1±14.5^{b}	10.8±0.8
		Infected	7.4±0.5^{c}	6.0±0.3^{d}	8.9±0.3^{a}	4.7±0.2^{e}	8.2±0.4^{b}	5.5±0.2^{d}	6.8±1.6^{d}	
	AAU. ELU-8	YL (%)	45.5±4.1^{a}	47.4±1.6^{a}	42.3±3.5^{a}	21.6±1.9 ^{c}	28.7±1.9^{c}	35.3±9.7^{b}	37.1±10.1^{b}	10.8±0.1^{a}
		Infected	5.6±0.4^{b}	6.2±1.0^{b}	6.3±0.4^{b}	8.5±0.2^{a}	7.7±0.2^{a}	7.5±0.4^{a}	7.0±1.1^{d}	
	Susce ptible	YL (%)	52.6±0.0^{b}	65.9±1.3^{a}	67.0±1.6^{a}	48.9±4.2^{c}	44.6±4.2^{c}	39.3±1.9^{d}	53.0±10.0^{a}	9.4±1.2^{a}
		Infected	4.5±0.0 ^{b}	3.2±1.1^{c}	3.1±0.2^{c}	4.8±0.2^{b}	5.2±0.2^{a}	5.7±0.2^{a}	4.4±1.0^{d}	
	Resist ant	YL (%)	64.5±5.8^{a}	22.6±10.0^{b}	9.4±2.6^{d}	18.4±9.3^{c}	4.3±2.6^{e}	6.8±1.2^{d}	20.5±17.^{c}	7.8±0.5^{b}
		Infected	2.8±0.4^{d}	6.0±0.8^{c}	7.1±0.2^{ab}	6.4±0.7^{bc}	7.4±0.2^{a}	7.5±0.1^{a}	6.2±1.7^{d}	
	Tolera nt	YL (%)	58.2±6.7^{b}	64.5±10.5^{a}	61.4±5.2^{a}	23.9±2.2^{d}	42.4±0.4^{c}	44.9±5.4^{c}	49.2±15.6 ^{a}	9.5±2.0^{a}
		Infected	4.0±0.6^{c}	3.4±1.1^{c}	3.7±0.5^{c}	7.2±0.2^{a}	5.5±1.0^{b}	5.2±0.5^{b}	4.8±1.5^{d}	
Mean ±SD			36.9±26.3^{a}	39.5±23.3^{a}	33.6±21.0^{a}	24.9±14.0^{b}	21.2±12.5^{b}	28.1±15.7^{b}	30.1±17.8	9.4±0.4

5.5. *In vitro* evaluation of antagonistic activity of *Trichoderma* species and *P. fluorescence* against the test pathogen.

Significance difference was observed in the percentage mycelial growth inhibition among the three biocontrol agents (table 13). The advancing hyphae of *Trichoderma* species covered the entire medium in the Petri plates, suppressing the growth of *P. grisea* isolates. *T. viride* showed maximum mycelial growth inhibition (77.9% and 75.2%) on *P. grisea* isolates (*Pg*.015 and *Pg*.058), respectively. The minimum percent of mycelial growth inhibition (61.3%) of *T. viride* was observed on *Pg*.046. *T. harzianum* showed maximum mycelial growth inhibition (74.9%) on *Pg*.003 and minimum mycelial growth inhibition (57.4%) on *Pg*.080. Similarly, antagonistic activities of *P. fluorescence* against the isolates of *P. grisea* were clearly showed by limited growth of fungal mycelium in the inhibition zone surrounded by *P. fluorescence*. *Pseudomonas fluorescence* have showed maximum mycelial growth inhibition on isolate *Pg*.085 (73.5%), followed by *Pg*.015 (67.0%), *Pg*.003and *Pg*.058 (66.5 and 66.1% respectively). Least percent of mycelial growth inhibition of *P. fluorescence* was

observed on isolate *Pg*.046 (63.6%). The control plates without *T. viride*, *T. harzianum* and *P. fluorescence* were grown and the Petri plates were covered by the *P. grisea* isolates.

Table 13. In vitro evaluation and testing of *T.harzianum*, *T. viride* and *P. fluorescen*ce against mycelial growth of *P. grisea* isolates.

Isolates	Control (mm)	*T. harzianum*		*T. viride*		*P. fluorescence*	
		MG (mm) Mean ± SD	% Inhibition Mean±SD	MG (mm) Mean±SD	% Inhibition Mean±SD	MG (mm) Mean ±SD	% Inhibition Mean±SD
Pg.003	71.33	$18.0{\pm}1.7^{d}$	$74.9{\pm}2.9^{a}$	$20.3{\pm}0.6^{cd}$	$71.4{\pm}1.1^{c}$	$17.6{\pm}2.1^{a}$	$65.1{\pm}3.1^{c}$
Pg.007	72.66	$22.6{\pm}2.1^{c}$	$68.8{\pm}2.3^{bc}$	$24.0{\pm}1.0^{ab}$	$66.9{\pm}1.1^{cde}$	$17.0\ {\pm}2.6^{a}$	$66.5{\pm}4.1^{bc}$
Pg.015	72.33	$26.0{\pm}1.7^{b}$	$63.0{\pm}1.4^{de}$	$16.0{\pm}1.7^{de}$	$77.9{\pm}1.9^{ab}$	$16.6{\pm}2.8^{a}$	$67.0{\pm}3.5^{b}$
Pg.025	81.00	$28.6{\pm}0.6^{a}$	$65.8{\pm}2.5^{cd}$	$26.3{\pm}1.5^{a}$	$67.4{\pm}2.2^{cd}$	$19.0\ {\pm}2.6^{a}$	$65.2{\pm}7.1^{c}$
Pg.34	75.66	$23.3{\pm}1.5^{bc}$	$69.1{\pm}1.5^{bc}$	$21.6{\pm}1.1^{bc}$	$71.3{\pm}1.8^{bc}$	$19.3\ {\pm}1.1^{a}$	$64.4{\pm}1.4^{d}$
Pg.46	69.00	$27.3{\pm}0.6^{b}$	$60.3{\pm}1.4^{ef}$	$26.7{\pm}1.5^{a}$	$61.3{\pm}3.0^{e}$	$19.6\ {\pm}1.5^{a}$	$63.6{\pm}7.2^{cd}$
Pg.58	67.33	$17.6{\pm}1.1^{d}$	$72.3{\pm}2.9^{ab}$	$16.6{\pm}2.5^{de}$	$75.2{\pm}4.0^{ab}$	$18.0{\pm}1.7^{a}$	$66.1{\pm}3.4^{c}$
Pg.80	62.66	$25.6{\pm}2.1^{bc}$	$57.4{\pm}2.9^{f}$	$22.3{\pm}3.0^{bc}$	$64.2{\pm}5.7^{de}$	$19.6{\pm}0.6^{a}$	$64.5{\pm}2.0^{d}$
Pg.85	69.66	$21.3{\pm}2.3^{c}$	$69.3{\pm}1.6^{bc}$	$19.0{\pm}4.0^{cde}$	$72.8{\pm}4.4^{ab}$	$19.6{\pm}3.5^{a}$	$73.5{\pm}9.3^{a}$
Mean±SD	71.2	$23.4{\pm}4.0^{c}$	$66.7{\pm}5.7^{b}$	$21.4{\pm}4.1^{d}$	$69.8{\pm}5.7^{a}$	$18.5{\pm}2.2^{e}$	$66.1{\pm}5.0^{b}$

Key: SD= standard deviation of mean, Alpha= 0.05

5.6. *In vitro* evaluation of fungicides against the test pathogen

Both Curzate and Sancozeb fungicides showed varied levels of antifungal activity. The highest percentage mycelial growth inhibition was recorded on isolate *Pg*.085 (90.2%) at 1000PPM and the lowest percentage mycelial growth inhibition was recorded on isolate *Pg*.034 (40.0) at 200PPM. There was significant difference among the concentrations (200-1000 PPM) of both the fungicides on mycelial inhibition of isolates on the growth media (Table 14). However, no significant difference of inhibition was recorded from Sancozeb fungicides with regard to concentration ranges from 200- 1000 PPM in all isolates, even maximum inhibition was recorded at 1000PPM (figure 7). Relatively high percentage of inhibition of 65.0-90.2% and 82.9-92.4% were recorded by 200-1000 PPM of Curzate and Sancozeb, respectively, see figure 7 for Sancozeb and Appendix 7 for Curzate.

The response of individual isolates to the different fungicide was shown on Table 15. The highest mean percentage of inhibition of mycelial growth of the isolate was displayed on isolate *Pg*.015 ranging from 62.5% with Curzate and 92.1% with Sancozeb. The isolate,

Pg.015 was found to be relatively sensitive to Sancozeb, while was the most resistant to curzate.

Table.14. Evaluations of different concentration of fungicides on mycelial growth of *P. grisea.*

Isolates	Concentration (PPM)								
		Mycelial growth inhibition (%) by Curzate				Mycelial growth inhibition (%) by Sancozeb			
	Control	200	400	800	1000	200	400	800	1000
Pg.003	71.3	43.0±1.1^{f}	38.3±1.0^{h}	83.2±1.0^{b}	88.3±1.1^{a}	77.1±1.1^{d}	81.4±1.0^{c}	86.0±0.0^{d}	89.8±0.0^{ab}
Pg.007	44.6	57.1±0.6^{c}	63.4±4.1^{d}	76.9±1.1^{e}	82.9±0.6^{d}	74.0±1.1^{f}	86.2±0.6^{a}	93.8±0.0^{a}	90.1±0.0^{ab}
Pg.015	72.3	62.1±1.5^{b}	43.8±0.6^{g}	79.8±1.5^{d}	88.1±0.6^{ab}	82.9±0.6^{a}	86.0±1.0^{a}	91.1±0.0^{ab}	91.2±0.0^{ab}
Pg.025	81.0	50.2±1.5^{d}	54.3±2.5^{f}	82.3±0.6^{c}	89.7±1.1^{a}	78.2±1.1^{c}	86.9±0.6^{a}	90.8±0.1^{ab}	92.4±0.0^{a}
Pg.034	75.6	40.0±1.5^{g}	65.2±0.6^{c}	74.4±0.6^{f}	85.4±1.0^{c}	76.8±0.0^{d}	86.7±1.0^{a}	87.4±0.1^{c}	90.9±0.0^{ab}
Pg.046	69.0	57.1±1.1^{c}	75.9±0.6^{a}	79.2±0.6^{c}	85.0±0.6^{c}	81.7±0.6^{a}	78.2±0.6^{d}	90.1±0.6^{ab}	91.2±0.6^{ab}
Pg.058	67.3	44.1±0.6^{e}	59.8±1.7^{e}	75.8±0.6^{e}	88.7±0.6^{a}	82.1±1.0^{a}	82.3±0.6^{b}	89.6±0.1^{a}	86.9±0.0^{b}
Pg.080	62.6	49.3±1.7^{d}	65.9±1.5^{c}	74.4±0.6^{b}	89.9±0.6^{a}	65.9±0.6^{e}	86.7±1.1^{a}	84.9±0.0^{e}	88.9±0.0^{b}
Pg.085	71.6	65.0±1.5^{a}	74.4±1.5^{b}	84.2±1.5^{a}	90.2±0.0^{a}	80.0±0.6^{b}	82.8±0.6^{b}	88.7±0.0^{b}	89.3±0.0^{ab}

Table 15. The mean percent of mycelial growth inhibition of fungicides against *P. grisea* isolates on PDA medium after 7 days of incubation at 27 ±1°C.

Isolates	Fungicides		*Mean*
	Curzate	Sancozeb	
Pg.003	63.2±18.6^{b}	91.0±7.7^{a}	77.1±19.6^{a}
Pg.007	67.7±7.1^{b}	81.4±10.5^{a}	74.5±9.6^{a}
Pg.015	62.5±18.1^{b}	92.1±6.3^{a}	77.3±20.9^{a}
Pg.025	69.2±16.0^{b}	91.2±8.5^{a}	80.2±15.5^{a}
Pg.034	66.2±14.6^{b}	90.6±8.5^{a}	78.4±17.2^{a}
Pg.046	74.3±8.3^{b}	89.6±7.9^{a}	81.9±10.8^{a}
Pg.058	67.1±10.3^{b}	91.2±6.6^{a}	79.1±17.0^{a}
Pg.080	69.8±10.7^{b}	88.0±9.9^{a}	78.9±12.8^{a}
Pg.085	79.6±7.8^{a}	90.5±7.4^{a}	85.0±7.7^{a}
Mean	68.8±5.2^{b}	89.5±3.2^{a}	79.2±3.0

Means with the same letter are not significantly different, Alpha=0.05, CV=10.7

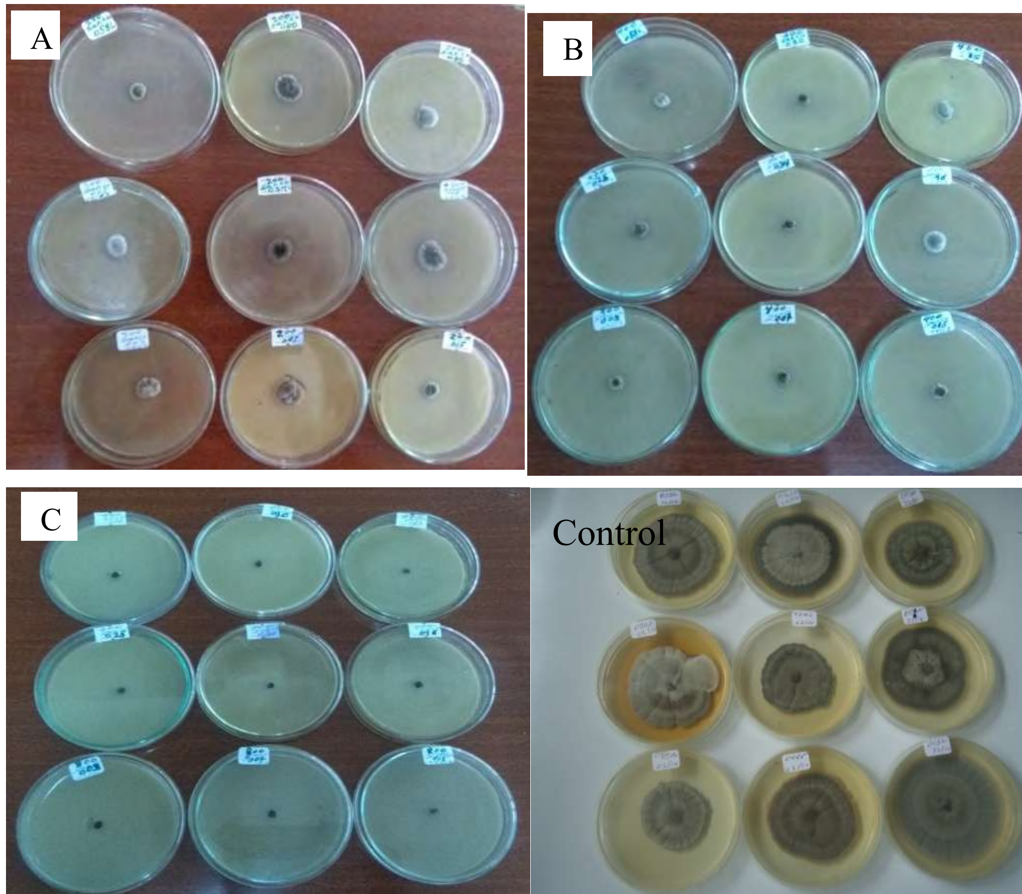


Figure 7. Percentage mycelial growth inhibition of *P. grisea* isolates by Sancozeb ® 80%W (A at, 200, B= 400, C= 800PPM).

5.7. Selection of selective fungicides and fungicides resistant antagonists for compatibility studies.

In Curzate and Sancozeb, the lower concentrations of 100 and 200 ppm, they well tolerated with both *Trichoderma species.* The highest (99.8%) was recorded for Sancozeb fungicides when combined with *T. viride* and 87.3% of compatibility was recorded at a concentration of 100 ppm when *T. harzianum* is combined with the same fungicides (table 17). But, in the case of Curzate the highest compatibility (99.4%) was recorded when combined with *T. viride* and 89.4% with *T. hazianum* (table 16) (Appendix 8).

Table 16. Screening of Trichoderma species for tolerance to Curzate at different concentration after seven days of incubation at 25°C.

Concentration of fungicides	*T. harzianum*		*T. viride*		Mean±SD
	Growth (mm)	% compatibility	Growth (mm)	% compatibility	
100	89.4±0.9	97.2	91.5±0.6	99.4	46.1
200	72.2±0.6	78.5	83.4±0.9	90.6	46.6
400	63.9±0.8	69.5	72.1±1.1	78.4	47.0
800	32.5±0.4	35.3	32.9±1.4	35.7	48.6
1000	22.5±1.0	27.7	26.7±0.4	29.0	48.1
Control	92.0±1.2	100	92.0±0.7	100	46.0
Mean±SD	62.1±4.9	68.0±3.4	66.4±2.8	72.2±5.2	45.9

Each value is an average of three replicates ± Standard deviation. Means followed by the same letters within a column are not significantly (p<0.05) different, according to Duncan's multiple range test.

Table 17: Screening of *Trichoderma species* for tolerance to Sancozeb at different concentration after seven days of incubation at 25°C.

Concentration(ppm)	*T. harzianum*		*T. viride*		
	Growth (mm)	% compatibility	Growth (mm)	% compatibility	Mean±SD
100	87.3±0.57	94.9	91.9±0.6	99.8	46.1
200	69.0±0.9	75.0	73.9±0.9	80.3	46.8
400	48.2±0.6	52.4	47.8±1.2	51.9	47.9
800	25.1±0.7	27.2	29.3±0.6	31.8	48.8
1000	21.4±0.8	23.3	23.1±0.3	25.1	48.9
Control	92.0±0.0	100.0	92.0±0.0	100	46.0
Mean±SD	57.1±4.3	62.1	59.6±4.8	64.8	47.4

each value is an average of three replicates ± Standard deviation. Means followed by the same letters within a column are not significantly (p<0.05) different, according to Duncan's Multiple range test

6. DISCUSSION

Wild finger millet blast disease is the most important disease that found, where *E. coracana* is cultivated as a major crop and where *E. africana* grows as aweed with or adjacent to this crops, in the areas like east Wellega, west Wellega, Assosa, Beles and Chagni, and west Gojjam zones. Blast infected plants in the field were easily identified by the blackish velvety accumulation, dark brown on the leaf and neck part of the plant and some of the infected seeds were shrivelled.

Based on the morphology of the test fungus the pathogen was identified as *P. grisea* isolates. The morphological characters of *P. grisea* revealed that, the conidiophores of the isolates were found to be slender, straight, gray, grayish-black or dark-brown, smooth bearing clusters of conidia which are typically pyriform with 2-4 cell (Mew and Gonzales, 2002 ; Getachew *et al*., 2013). The variation among the isolates was also evidenced by the existence of difference in conidial septation that ranged from having one septation up to those with 3 septations. Conidia with septation of 2 to 3 occurred in all the isolates and 1-3 septations rarely observed (Mew and Gonzales, 2002; Meena, 2005). The pathogen, *P. grisea* with similar characters of the present study was also reported from seeds and different parts of rice seedlings by Meena (2005) and from seeds and different parts of finger millet seedlings by Getachew *et al*. (2013).

Among the nine isolates tested in the present study, the morphological variability in respect to conidia length and width were also observed to be significant. However, no variation with respect to conidial shape was noticed. The isolate, *Pg*.003 showed the maximum size of conidial length about 37.01-40.01µm (average 40.0µm) and maximum conidial width were observed by isolates of *Pg*.080 with 12.13-14.52µm (Average 14.28µm) which was superior to others in Table 4. Nishikado (1927) indicated that *P. grisea* conidia size ranged from 14 to 40 µm long and from 6 to 13 µm wide (usually 19 to 23 µm long and from 7 to 9 µm wide) with small basal appendage. Harish *et al*. (2007) also reported that, the isolates of *P. grisea* varied in size (length and width) of the conidia, measuring 15.66-35.43 x 6.35-12.90µm. Getachew *et al*. (2013) stated that the different environmental conditions under which the various strains were growing exert an important influence upon the form and size of the conidia produced.

Cultural studies carried out in this study showed variation for the growth and sporulation of isolates. Sporulation of each *P. grisea* isolate on the 10th day of incubation at room temperature (27 ± 1°C) on four different media (host extract + 2% sucrose agar, oat meal agar, potato dextrose agar and Richards's agar) showed significant differences between isolates of *P. grisea* with respect to colony characters like type of growth, color of colony and colony margin. From these features, in colony color (gray, grayish-black, black and buff white) on different media, conidial shape and septation they could be categorized in to the genus Pyricularia as reported by Thakur, (2011). Sporulation was abundant with maximum fruiting bodies at the center of the plate as compared to that of the periphery mycelium growth of the test pathogen.

Among the four media used in the present study; all the *P. grisea* isolates were showed good growth and sporulation on media of oatmeal agar (OMA). Afshana *et al*. (2011) and Bandyopadhyay *et al*. (2009) who found that OMA media was suitable and good for growth and sporulation of blast isolates and Khadka *et al*. (2012) found that, among the different media used, OMA media was found to be the best for sporulation of the blast isolates from both rice and finger millet. The highest colony diameter was recorded on OMA (88.0mm) by isolate *Pg*.085, neck blast and the lowest colony diameter was observed on PDA (52.0) by isolate *Pg*.080, head blast. It was due to OMA has microelements as components which are essential for good growth of *Pyricularia* species as indicated by Meena (2005). This can be an indication that the pathogen has preference for the different media components for good growth, which could be related to specificity for host (Viji *et al*., 2001). The findings were contradicted with (Mijan and Hossain, 2000) who observed that among the non synthetic media, potato dextrose agar supported maximum radial growth (85.00 mm), next was host extract + 2% sucrose agar medium (80.33 mm) followed by oat meal agar (75.00 mm) for the isolates from the pear millet. It might be due to different sensitivity of the isolates isolated from different host to different component of media.

Other than the sizes as well as shape of the spores and colonies of filamentous fungi are also the most important factors in fungal identification. The results fromcolony characteristics of *Pg* isolates on different media, on OMA media showed grayish-black, White tinged Gray, off-white and smooth colony margin and also showed both irregular and smooth colony margins of some isolates on PDA medium. Similarly, Meena (2005) also reported that the colony characteristics of *P. grisea* isolates on OMA media showed grayish-black and entire colony margin and also showed both irregular and entire colony margins of some isolates on

PDA medium. Bandyopadhyay *et al*. (2009) was also reported that the OMA media produced off-white, good and regular mycelial growth. This study is also found in agreement with that of (Hossain *et al*., 2004) in rice blast isolates of the color of colony showed gray and black-gray for the finger millet isolates on OMA medium. In this study the mycelium of the isolates in culture was septate and highly branched. Mijan and Hossain (2000) noticed that mycelium in cultures was first hyaline in color, then changed to olivaceous, septated and branched. Smooth to irregular margin, medium to good growth of the pathogens was observed. According to Meena (2005) the difference in colony color arises from the difference in sporulation capacity of *P. grisea* on different media. The color difference observed in this study between the sectors might be due to the stages of the spores on the different sectors of growth.

Each fungus has its temperature range for the growth and sporulation as reported by Agrios (2005). All the isolates have shown different responses to the different incubation temperatures. In this study the highest growth was recorded at an incubation temperature of $30^{o}C$ followed by $25^{o}C$ for all the isolates and the lowest growth was recorded and sporulation was negligible at the highest temperature $40^{o}C$.The optimum temperature range for the maximum growth of the isolates of *P. grisea* was found to be 20°C to 30°C. Getachew *et al*. (2013) found that different *P. grisea* isolates exhibited maximum growth at 30^{0}.

In this study there was a considerable variation among the dry mycelial weight of the different isolates on different pH concentrations. The maximum mean dry mycelial weight was observed at pH 6.5 (484.2mg) followed by pH 6.0 (455.6mg). Meena (2005) and Getachew *et al*. (2013) reported that the growth of all the isolates significantly increased from pH 3.0 to pH 6.5 which further started declining as well. And Mijan and Hossain (2000) indicated that, growth of *P. grisea* increased with increase in pH from 3.5 to 6.5 and the pathogen showed maximum growth at pH 6.5. The different pH ranges showed significant difference on growth of *P. grisea*. In (Meena, 2005; Getachew *et al.,* 2013*)* maximum growth of *P. grisea* isolates was found at an optimum pH of 6.5. It is possible to link with acid soil distributed in Western and north Western Ethiopia if the pathogen can stay for several years in soil with plant debris. The pH of the soil is <5.5 in Western and north-western Ethiopia which attributes for the existence of the pathogen. The variation among the findings might be due to the difference in the sensitivity of the different isolates. It is probable that if nutrient requirements are satisfied most fungi will grow well over a broad pH range on the acid side of neutrality, pH 5.5 -7 as observed in this study.

Significant difference in utilization of carbon sources by *P. grisea* isolate wich indicating there by biochemical differences among the isolates as studied by Hossain *et al.* (2004) and Getachew *et al*. (2013) Among the carbon sources tested in, dextrose was the most preferred carbon source as evidenced by higher mean diameter (84.8 mm) followed by maltose (80.6mm). Tripathi (2006) showed that maltose better used as the carbon source for the mycelial growth and sporulation. And as Getachew *et al.* (2013) dextrose supports mean diameter of 88.4mm and maltose 84.8mm which showed that dextrose was the most preferred for *P. grisea* isolates. The maximum mean mycelial growth was recorded by leaf blast isolate (*Pg.*007) (84.7mm), and minimum growth was observed by the head blast isolate (*Pg.*080) (60.9mm). As indicated by Getachew *et al*. (2013) who reported that the least mycelial growth was observed by the head blast.

NaNO3 (83.8mm) was the best nitrogen source for *P. grisea* which was significantly superior to the three of the nitrogen sources, which were differently preferred by all the isolates which indicate the variations among the isolates for nutrient utilization. Getachew *et al*. (2013) stated that, on synthetic media, nitrate Nitrogen was better for the growth of *P. grisea* which accounts 88.5mm mean diameters. Similarly (Mijan and Hossain , 2000) reported that, sodium nitrate used in place of ammonium salts in a synthetic medium, significantly improved the growth and that these variations may be to some extent be related to differences in physiological or biochemical properties among the nine isolates of the pathogen.

The *in vivo* evaluation of the pathogenicity of the isolates resulted in causing blast disease in all wild and cultivar varieties tested though the incidence of the disease was differed amongst the isolates and on the different varieties. The typical symptoms (Black or dark brown spots) appear on leaves, neck, stem and head. The leaves lesions are typically spindle-shaped, wide in the center and pointed toward either end. Symptoms usually develop a grayish center, with a brown margin on older lesions. Symptoms with similar characters were observed in the study of Sreenivasaprasad (2005) and Getachew *et al*. (2013). When lesions on the leaves of susceptible lines expand rapidly and tend to coalesce; leading to complete drying of infected leaves and the fungus may also attack the neck causing neck blast. When a neck is infected, all parts above the infected node may die as of Sreenivasaprasad (2004). In this study, the apparent susceptibility of the finger millet varieties to leaf infection appeared to differ. Varieties of *E. coracana* subsp. *africana* were less affected by leaf blast than cultivated finger

millet. An isolate obtained from different tissues of Wild finger millet were able to infected both cultivated and wild types to a variable degree among varieties as well as different tissues of individual, with some isolates being as aggressive as some of the isolates from finger millet. From the molecular analysis Takan *et al*. (2004) suggested that the pathogen harbored by the wild millet can serve as an inoculum source that underlining the potential of finger millet wild relative and weeds to serve as inoculum sources for the blast, *P. grisea*. Takan *et al*. (2012) reported that at least eight haplotypes were common to cultivated and wild millets, further emphasizing the importance of wild hosts in pathogen epidemiology. *P. grisea* isolate *Pg*.007 caused the most severe infection followed by isolate Pg. 003, but severity score was least for isolate *Pg*.034, as the isolate *Pg*.11 showed the highest disease score on all the three finger millet varieties used in the study of Getachew *et al*. (2014) . The isolates, *Pg*.007 and *Pg*. 003, has caused the highest mean disease incidence (58.9%) and (58.8%) respectively, on the seven finger millet varieties. Isolate *Pg*.025 was less virulent when compared to other isolates of *P. grisea*.

The difference of disease incidence the seven finger millet varieties inoculated at this stage indicated that there was difference in varietal reaction to the blast pathogen and aggressiveness of the pathogens as well. In this study the wild *Eleusine*, AAU-EIU-46 and AAU-EIU-65 variety was better in their resistance to the pathogen. The varieties AAU-EIU-8 and ACC. AAU. EIU-15 of the wild *Eleusine* was susceptible which accounted up to 51.7% and 50.7% disease incidence, respectively. But the cultivar variety, wama was the most susceptible to all the isolates. The most aggressive isolates, in general, were aggressive on all the seven varieties tested and, similarly, the least aggressive isolates produced low levels of disease on all the varieties. Similarly, Adipala (1989), in Uganda has indicated that some isolates of blast from weeds will also infect finger millet, which implies the need to removal alternate host for better disease management. Weed control, particularly wild millet, which is very common and difficult to distinguish from the crop at early stages, is a key issue in blast disease management as recommended by Sreenivasaprasad *et al*. (2005).

Grain yield loss was higher with the highly virulent isolates of *Pg*.007 *Pg*. 003 and *Pg*.015 (with 39.5, 36.9 and 33.6%) values, while the less virulent isolate, *Pg*.034 resulted in least mean yield loss of 21.2% on the seven finger millet varieties. This loss in grain yield was greater in Cultivar than in Wild finger millet. The highest and the lowest mean seed weight after infected by the six *P. grisea* isolates was obtained by AAU-EIU-65 and ACC.BKFM0031 varieties with 9.3g/pot and 2.8g/pot respectively. The highest percent of

yield losses was recorded by isolate *Pg*.015 (67.0%) on the cultivar variety susceptible (Wama). The size and quality of the seed also showed variations among the seven finger millet varieties infected by the six *P. grisea* isolates. The seed size of Wama variety was so small and empty as compared with the other. The work of Takan *et al*. (2004) in Kenya and Uganda was revealed that yield losses of 10% to 80% were reported on improved varieties. Similarly Ahmad *et al*. (2011) reported that losses due to blast disease may range up to 90% depending up on the component of the plant infected. Overall, there was a trend for grain yield to decrease as the incidence was increased from early leaf infection to late finger development. The test in this study indicated that all the isolates were pathogenic to all tested finger millet varieties with some level of variation in severity.

Growth of *P. grisea* was inhibited *in vitro* with three microbial antagonists. It was suggested that, because the pathogen growth may be inhibited due to the production of antimicrobial compounds, metabolite or exudates that diffused through the medium and reached to the pathogen surface, or due to competition for space, nutrients as reported by Bikila (2015). Both *T. harzianum* and *T. viride* overgrew throughout the medium in Petri plates and occupied all the spaces and competed for nutrients hence hindered the growth of the test *P. grisea* isolates. Since the growth of *Trichoderma* species was fast, the clear inhibition zones were not formed in all incubated Petri plates. The isolates *Pg*.015 (77.9%) and *Pg*.058 (75.2%) have shown higher percent of inhibition by *T. viride*. Harish *et al*. (2007) indicated that T*richoderma* species have an inhibitory effect on *Pyricularia* species.

Pseudomonas fluorescence isolate treatments reduced the mycelial growth of the nine *P. grisea* isolates on PDA. These might be due to producing secondary metabolites which inhibited growth of *P. grisea*. The antimicrobial activity of *P. fluorescence* isolate was reported against numerous fungi. The present study showed that *P. fluorescence* was succeded inhibiting the growth of nine *P. grisea* isolates.The mean mycelial growth inhibition by *P. fluorescence* on all *P. grisea* isolates was 66.1%. Rangajaran 2003; Srivastava and Shalini, 2008) reported that *Pseudomonas fluorescence* was shown to have effective antifungal activity against different plant pathogenic fungi.

Evaluation of the efficacy of the two different fungicides on the blast under *in vitro* condition, were undertaken by mycelium growth inhibition against wild finger millet pathogenic fungi. All the isolates were found to respond differently to the tested fungicides. Both fungicides were reduced mycelial growth and finally inhibited the mycelia growth with high concentration. Percich *et al*. (1997) reported the fungicide Sancozeb as very effective to

control fungal blast disease caused by *P. grisea* on wild rice. Isolate *Pg*.015 was found to be the most sensitive to Sancozeb and was the least sensitive to Curzate fungicides. The fungicide Sancozeb caused the highest, up to 92.4% inhibition on the isolates. Harish *et al*. (2007) reported that Mancozeb (0.2%), main component of Sancozab (Agrios, 2005), was observed to be the most effective, which significantly reduced the spore germination of *Pyricularia oryzae*. The difference in the activity of the fungicides in the laboratory condition was an indication for the difference in the control ability or response of the isolates to the chemicals (Bhojya, 2013). The different rates of effect of Curzate and Sancozeb fungicide used for this test were not statistically significantly different though there was difference in their inhibition percent; the highest rates have higher inhibition effect on the mycelial growth of the isolates.

From *In vitro* evaluation on the effectiveness of biological agents and fungicides on the mycelia growth of *P. grisea* isolates, fungicides were most effective for the control of the blast disease than biological agents and among the biological control agents, fungal species were most effective than bacterial species. One of the advantage of using *Trichoderma* species as biocontrol agent is its ability to be partners for pesticides in integrated disease management (Roberti *et al*., 2006).

From the in vitro screening of selective fungicides and fungicides resistant antagonists for compatibility studies, incorporation of Curzate and Sancozeb in growth medium did not affect the growth of *Trichoderma spp.* Instead, fungicides favoured the growth of antagonistic fungi at lower concentrations of 100, 200 and 400ppm and hence they are effective in managing plant pathogens. Similarly Monte (2001) reported that the combination of bioagents with fungicides would provide similar disease suppression as achieved with higher fungicide use. And even, when the fungicidal concentrations were increased to 600ppm, the antagonists tolerate the fungicides to some extent. Tolerance of antagonists to the fungicides was reduced slightly at higher concentrations of 800 and 1000 ppm compared to control.

The highest percentage of compatibility (99.8%) was recorded for Sancozeb fungicides when combined with *T. viride* and (99.4%) was recorded for Curzate fungicides when combined with *T. viride.* The present study was found in agreement with Bikila (2015) who reported that *T. viride* is more compatible than *T. harzianum*. Using combinations of fungicides with bioagents at lower concentration were decrease the resistance activity of the blast disease and may help in the relative cost of the formulations as reported by Thoudan and Dutta (2014).

The percent of compatibility decreased with an increase in the concentration of fungicide. Reduced amount of fungicide can stress and weaken the pathogen and render its propagules more susceptible to subsequent attack by the antagonist (Hjeljord and Tronsmo, 1998).

7. CONCLUSIONS AND RECOMMENDATIONS

7.1. CONCLUSIONS

The study was carried out by identifiying and characterizaterizing of *P. grisea* isolates, its pathogenicity, the effect of biocontrol and fungicides against the pathogens and compatibility of the bioagents could provides a basis for the blast management strategy in Ethiopia. The following conclusion has been concluded from this study.

- The morphological, cultural, and physiological features of the isolates showed that the isolates were belongs to the genus *Pyricularia* with distinct differences in spore size;
- Brown to pale olive conidia and gray, grayish-black, buff white colony color
- Better growth and sporulation was on oat meal agar and within a range of temperature 20°C to 30°C and pH 3.0 to 6.5.
- From biochemical characters of the isolate, dextrose and $NaNo_3$ was better utilized by all the isolates.
- All the isolates of *P. grisea* were able to infect the seven finger millet varieties and Varietal susceptibility to the pathogen has been identified. Variety wama of the cultivar finger millet were found susceptible to all isolates of *P. grisea*, isolated from the wild finger millet.
- The most virulent isolates, Pg.007 and *Pg*.003 have showed the highest finger millet yield reduction under green house condition.
- *In vitro* bio-efficiency of biological agents on the mycelia growth of the isolates indicated that the fungal bioagents were more effective than bacterial antagonists.
- The compatibility test showed that Sancozeb and *T. viride were* compatible. *Trichoderma* isolates were tolerant to lower concentration of 100, 200, 400ppm of Curzate and Sancozeb.
- Weed management should be taken in to consideration to manage the blast disease. Hence, it implicates the need for proper crop rotation and removal of alternate hosts.

7.2. RECOMMENDATIONS

As this study was the first to study cultural, morphological, physiological, biochemical and pathogenisity test of the blast, *Pyricularia grisea* on its alternate host, wild finger millet at *in vitro* and *in vivo* conditions. This study gave hint to the future research intervention as follows:

- ❖ Further studies must be conducted on the characterization, virulence mechanism and pathogenicity of the blast pathogen isolates from other regions. Hence could help in breeding for blast resistant varieties that could be stable over several locations.
- ❖ Field experiments must be carried out to further confirm the effectiveness of fungal biocontrol and bioagents compatibility in combination with lower concentration of chemical fungicides against the blast.
- ❖ Farmers and development agents should be trained in the management of the blast disease by using resistant cultivar, proper crop rotation, and field application of biocontrol agents.

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APPENDICES

Appendix 1**. Composition and preparation of different growth media.**

Richards's agar (Ainsworth, 1981)

Sucrose ($C12H22O11$) ………………………………. 50.00 g

Potassium dihydrogen phosphate ($KH2PO4$)……….. ..5.00 g

Potassium nitrate ($KNO3$) …………………………... 10.00 g

Magnesium sulphate ($Mgso4$ $7H2O$) ……………….. .2.50 g

Ferric chloride ($Fecl3$, $6H2o$) ……………………….... 0.02g

Agar – agar ……………………………………………20.0g

Distilled water (to make up)…………………………. 1000ml

All the above ingredients except potassium dihydrogen phosphate and agar-agar dissolved in 450 ml of distilled water. Agar-agar was melted separately in 500 ml of distilled water and was mixed with the above solution. The volume was made up to 950 ml. Potassium dihydrogen phosphate was dissolved in 50 ml of distilled water. The two solutions were sterilized at, 121^{0}C for 15 min and subsequently mixed together.

Host extract + 2 per cent sucrose agar (Srivastava *et al*., 2009).

Sucrose …………………………………………………20 g

Finger millet seed……………………………………100g

Agar – agar ……………………………………………20 g

Distilled water (to make up) …………………………1000 ml

100g of wild finger millet seeds were boiled in 500 ml of water for 30min at 100^{0}C. Then the extract was filtered through the muslin cloth and mixed with the sucrose. Agar – agar was melted in 500 ml of water and mixed thoroughly. The volume was made up to 1000 ml with distilled water and sterilized at, 121^{0}C for 15 min.

Oat meal agar(Ainsworth (1981)**)**

Oat flakes ……………………………………………30.0 g

Agar – agar …………………………………………..20.0 g

Distilled water (to make up) ………………………..1000 ml

First oat flakes were boiled with 500 ml of distilled water for 30 min. and filtered through muslin cloth. Agar – agar was melted in 500 ml water separately. Both the solutions were mixed thoroughly and sterilized.

Potato dextrose agar (Tuite, 2013)

Potato ……………………………………………………………… 200 g
Dextrose……………………………………………………….. .. .20 g
Agar – Agar ……………………………………………………….. .20.g
Distilled water (to make up)…………………………………………… 1000 ml

First, potatoes were peeled off and cut into small pieces. Then they were boiled and extract was filtered through the muslin cloth. The dextrose was dissolved in the solution. Later biotin and thiamine were added to the solution.

Malt Extract Agar (MEA)

Malt extract ………………………………………………………...........30g
Dipotassium hydrogen phosphate ……………………………………..2.0g
Ammonium chloride ………………………………………………...1.0g
Citric acid 1 N …………………………………………………………15.0ml
Agar ……………………………………………………………………….12g
Distilled water …………………………………………………………1000.ml

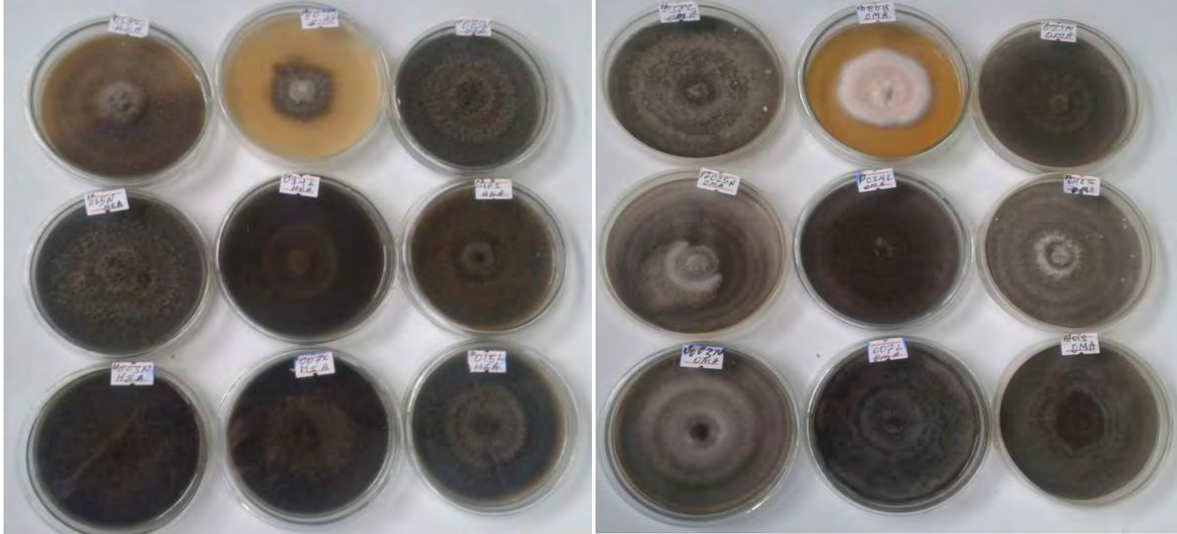
Appendix 2. **Growth characters of *P. grisea* isolates at different temperature levels.**

No	Tem. C)	Growth characters								
		Pg.003	*Pg*.007	*Pg*.015	*Pg*. 025	*Pg*. 034	*Pg*.046	*Pg*. 058	*Pg*.080	*Pg*.085
1	15	White Grey colony brown conidiophore Medium growth Smooth margin 2-3 septate	White Grey colony Grey conidiophore Medium growth Smooth margin 2-3 septate	White Grey colony brown conidiophore Medium growth Smooth margin 2-3 septate	White Grey colony Grey conidiophore Medium growth Smooth margin 2-3 septate	White Grey colony Grey conidiophore Poor growth Smooth margin 2-3 septate	White Grey colony Grey brown conidiophore Poor growth Smooth margin 2-3 septate	White Grey colony Grey conidiophore Poor growth Smooth margin 2-3 septate	White Grey colony Grey conidiophore Medium growth Smooth margin 2-3 septate	White Grey colony Grey conidiophore Medium growth Smooth margin 2-3 septate
	Sporulation	++	++	++	++	-	-	-	++	++
2	20	White Grey colony brown conidiophore Good growth Irregular margin 2-3 septate	Grey colony Grey Conidiophore Good growth Smooth margin 2-3 septate	White Grey colony brown conidiophore Good growth Smooth margin 2-3 septate	White Grey colony Grey conidiophore Good growth Smooth margin 2-3 septate	Grey colony Grey conidiophore Medium growth Smooth margin 2-3 septate	White Grey colony Grey conidiophore Medium growth Irregular margin 2-3 septate	Grey colony Grey conidiophore Medium growth Smooth margin 2-3 septate	Grey colony Grey conidiophore Good growth Smooth margin 2-3 septate	White Grey colony Grey conidiophore Good growth Smooth margin 2-3 septate
	Sporulation	+++	+++	+++	+++	++	++	++	+++	+++

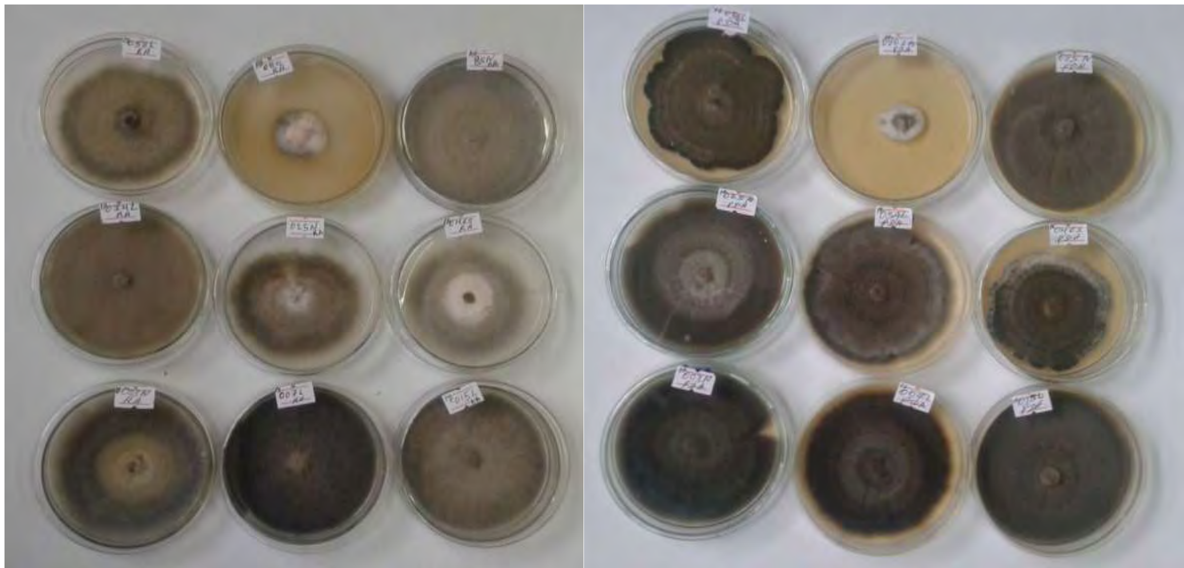
3	25	Grey colony Grey conidiophore Good growth Irregular margin 2-3 septate	Greyish black colony Grey conidiophore Good growth Smooth margin 2-3 septate	Black colony Grey conidiophore Good growth Smooth margin 2-3 septate	Black colony Grey conidiophore Good growth Smooth margin 2-3 septate	Black colony Grey conidiophore Good growth Smooth margin 2-3 septate	Greyish black colony Dark brown conidiophore Medium growth Smooth margin 2-3 septate	Grayish Black colony Grey conidiophore Good growth Smooth margin 2-3 septate	Grayish Black colony Grey conidiophore Good growth Irregular margin 2-3 septate	Grey colony Grey conidiophore Good growth Smooth margin 2-3 septate
	Sporulation	+++	+++	+++	+++	+++	++	+++	+++	+++
4	30	Grayish black colony Grey conidiophore Irregulrmargin Good growth 2-3 septate	GrayishBlack colony Grey conidiophore Good growth Smooth margin 2-3 septate	Grayish Black colony Grey conidiophore Good growth Smooth margin 2-3 septate	Grayish Black colony Grey conidiophore Good growth Smooth margin 2-3 septate	Grayish black colony Grey conidiophore Good growth Smooth margin 2-3 septate	Grey colony Grey conidiophore Good growth Irregular margin 2-3 septate	Grayish Black colony Grey conidiophore Good growth Smooth margin 2-3 septate	Grayish Black colony Grey conidiophore Good growth Irregular margin 2-3 septate	Grey colony Grey conidiophore Good growth Smooth margin 2-3 septate
	Sporulation	+++	+++	+++	+++	+++	+++	+++	+++	+++
5	35	Brown Grey colony Dark brown conidiophore Good growth Smooth margin 2-3 septate	Grayish Black colony Grey conidiophore Medium growth Smooth margin 2-3 septate	Grayish brown colony Greyish white conidiophore Medium growth Smooth margin 2-3 septate	White tinged Grey colony Grey conidiophore Good growth Smooth margin 2-3 septate	Grayish Black Grey colony Dark Grey conidiophore Good growth Smooth margin 2-3 septate	White Grey colony Grey conidiophore Medium growth Irregular margin 2-3 septate	White Grey colony Grey conidiophore Mediumgrowth Smooth margin 2-3 septate	Grey colony Grey conidiophore Medium Growth Smooth margin 2-3 septate	Grayish Black colony Grey conidiophore Medium growth Smooth margin 2-3 septate
	Sporulation	+++	++	++	+++	+++	++	++	++	++
6	40	Poor	No growth	No growth	Poor	No growth	Poor	Poor	Poor	No

		growth			growth		growth	growth	growth	growth
	Sporu lation	-	-	-	-	-	-	-	-	-

Appendix 3. **Effect of different culture media on mycelial growth of *P. grisea* isolates**

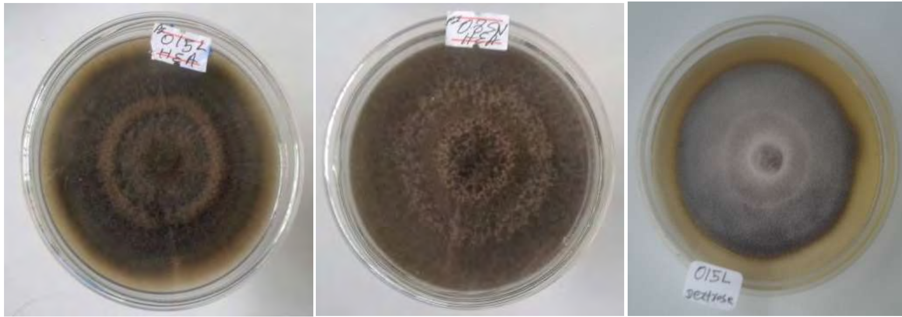


A. **Host Seed Extract Agar** **B. Oat Meal Agar**



C. Richard's Agar Medium **D. Potato Dextrose Agar**

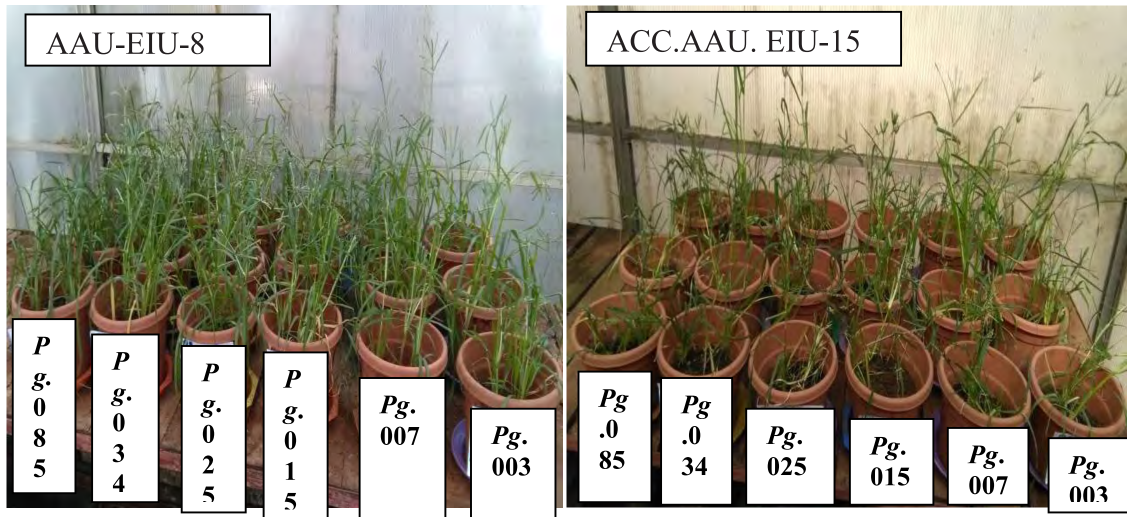
Appendix 4. **Concentric ring pattern of seed and leaf blast *P.grisea* isolates.**

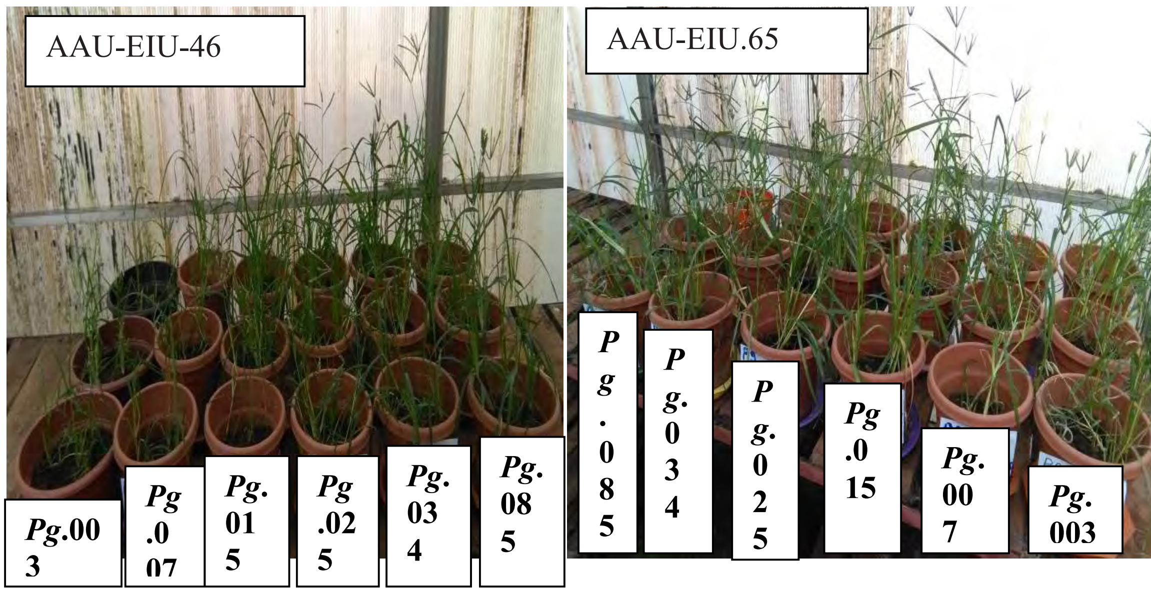


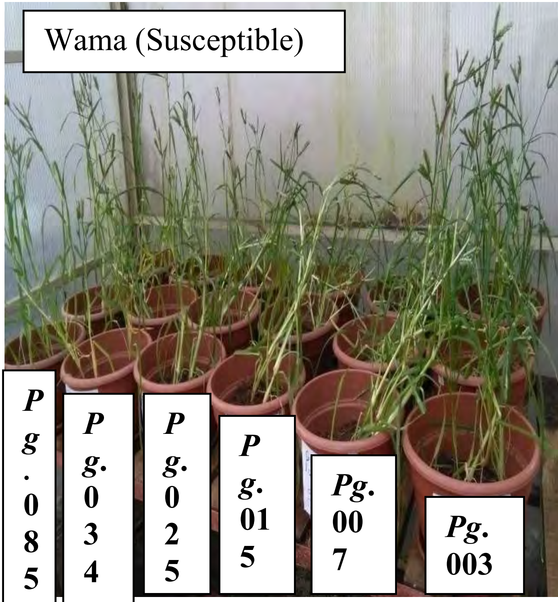
Appendix 5**. Effect of different Carbon sources on mycelial growth of *P.grisea* isolates.**

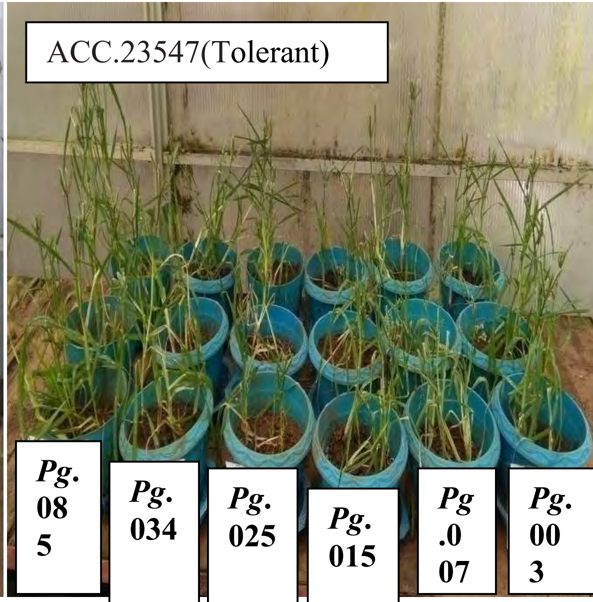
D=Dextrose; F=Fructose; M=Maltose; S=Sucrose

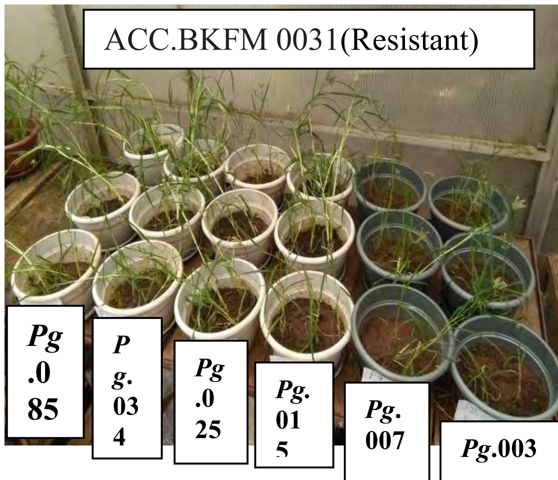
Appendix 6.**Finger millet seedlings showing blast symptoms following inoculations.**






Wama (Susceptible)
Pg.085
Pg.034
Pg.025
Pg.015
Pg.007
Pg.003


ACC.23547(Tolerant)
Pg.085
Pg.034
Pg.025
Pg.015
Pg.007
Pg.003


ACC.BKFM 0031(Resistant)
Pg.085
Pg.034
Pg.025
Pg.015
Pg.007
Pg.003

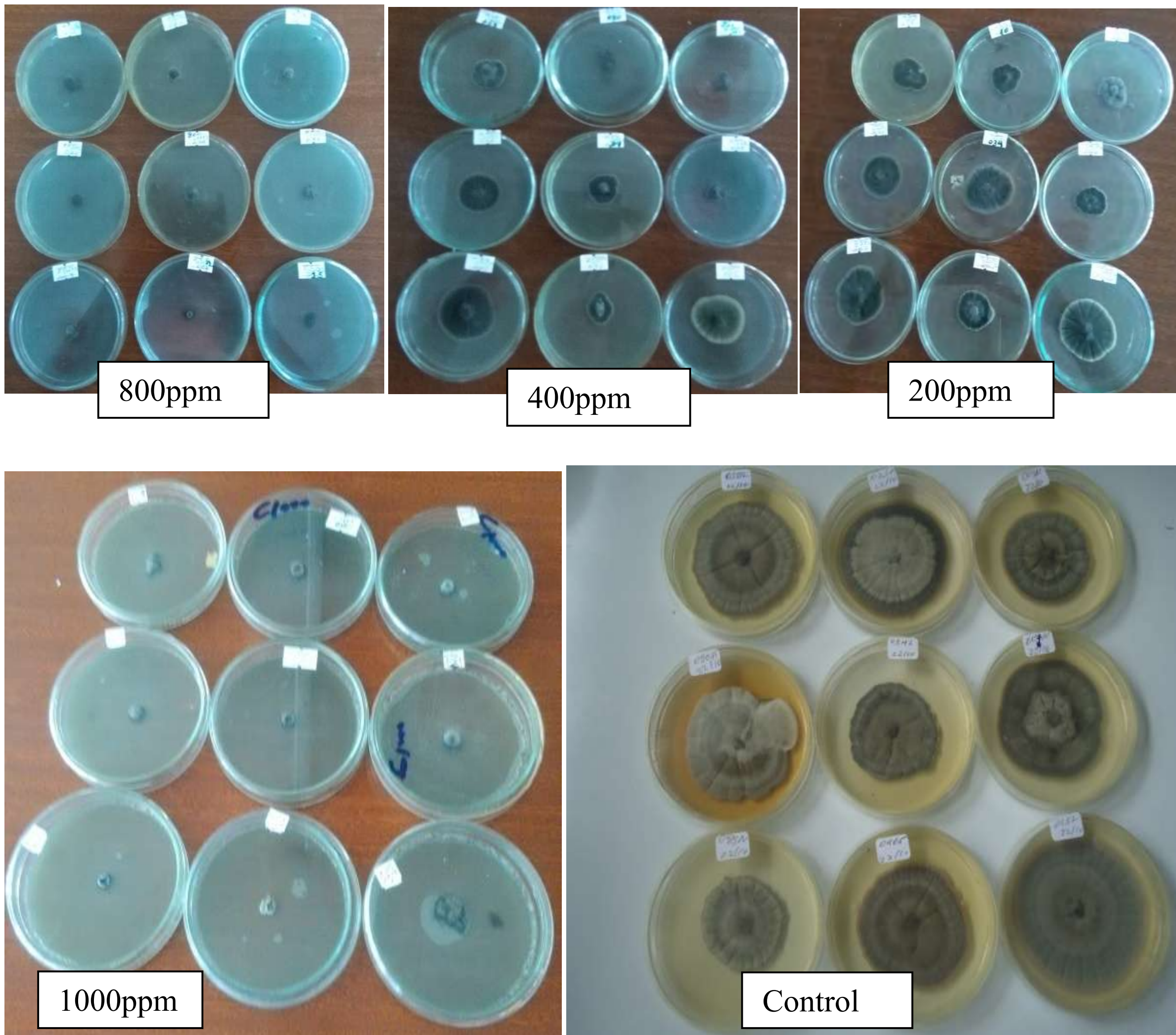
Head blast symptom

Leaf blast symptom

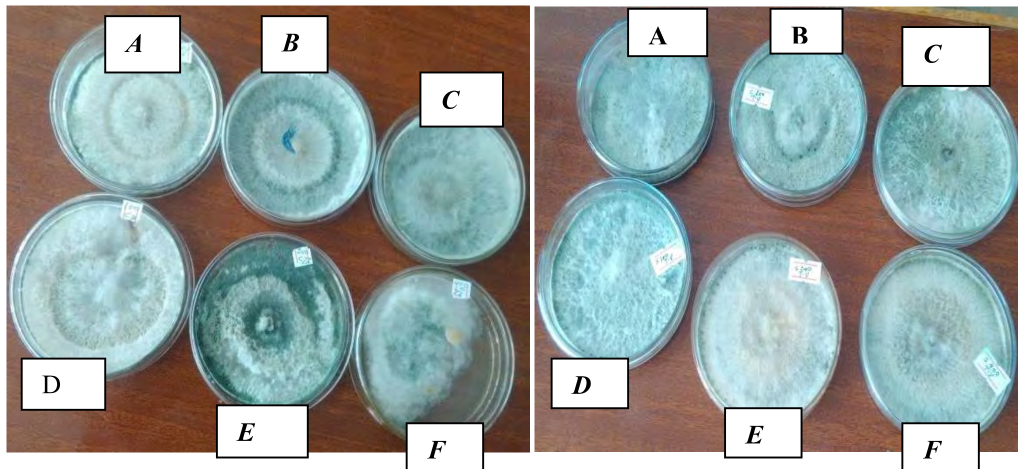
Stem blast symptom

CFM = Cultivar Finger millet WFM = Wild finger millet

Appendix 7. **Percent mycelial growth inhibition of *P. grisea* isolates by Curzate fungicides at different concentration.**



Appendix8. **Percentage Compatibility of *Trichoderma species* with sancozeb and curzate at different concentration after seven days of incubation at 25°C.**



T. harzianum

A=Sancozeb, 100ppm D=Curzate, 100ppm

B=Sancozeb, 200ppm E= Curzate, 200ppm

C=Sancozeb, 400ppm F= Curzate, 400ppm

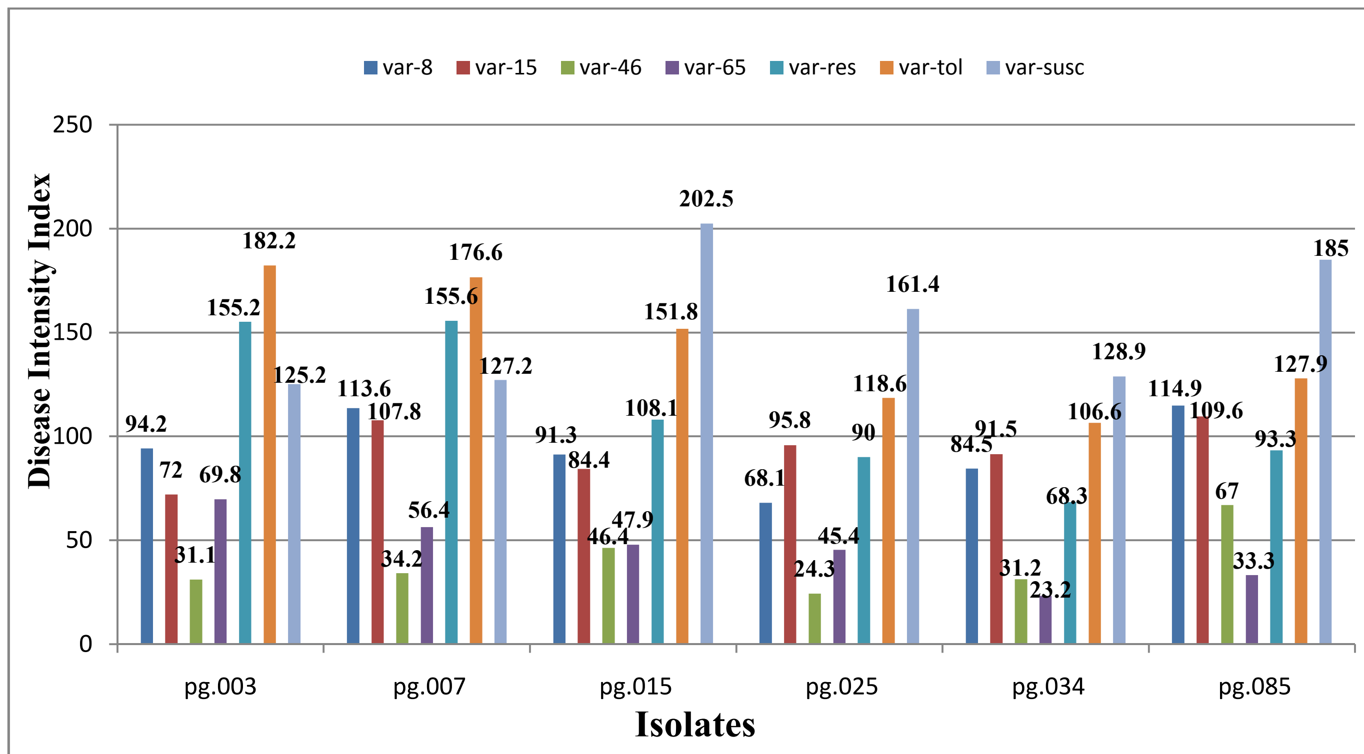
T. verdie

A= Curzate, 100ppm D= Sancozeb, 100ppm

B= Curzate, 200ppm E= Sancozeb, 200ppm

C= Curzate, 400ppm F= Sancozeb, 400ppm

Appendix 9. **Disease intensity index.**



Declaration

I, the undersigned, declared that this is my own original work, has not been presented for a degree to any other university and that all sources of materials used for the thesis have been fully acknowledge. I also confirm that this work has not been submitted anywhere else for the same purpose.

Fantaye Ayele Dadi

Signature ____________________

Date ________________

This thesis has been submitted for examination with our approval as University advisors.

Kassahun Tesfaye (Ph.D)

Signature __________________

Date ________________

Tesfaye Alemu (Ph.D)

Signature _________________

Date _______________