CHARACTERIZATION OF SOIL FUNGI CAPABLE OF *STRIGA* SEED BANK DEPLETION UNDER *IN VITRO* CONDITIONS

By: Mamo Bekele Bedada

A Thesis submitted to the Department of Microbial, Cellular and Molecular Biology School of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology (Applied Microbiology)

Major Advisor: Fassil Assefa (PhD)

Co-advisor: Taye Tessema (PhD)

June, 2020

Addis Ababa, Ethiopia

Declaration

I declare that this thesis entitled **"Characterization of Soil Fungi Capable of *Striga* Seedbank Depletion under *In Vitro* Condition"** is submitted to the School of Graduate Studies of Addis Ababa University for the Master's Degree in Applied Microbiology. I would like to prove through my signature below that it is my own independent work and has not earlier been submitted elsewhere by me or anybody else. All authors of the references cited in the current study were properly acknowledged.

Candidate	Signature	Date
Mamo Bekele	_______________	___/____ /______

ADDIS ABABA UNIVERSITY

COLLAGE OF NATURAL AND COMPUTATIONAL SCIENCES

DEPARTMENT OF MICROBIAL, CELLULAR AND MOLECULAR BIOLOGY

This is to certify that the thesis prepared by Mamo Bekele, entitled "**Characterization of Soil Fungi Capable of *Striga* Seed Bank Depletion Under *In Vitro* Conditions"** and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Biology (Applied Microbiology) fulfills with the regulations of the University and meets the accepted standards with respect to originality and quality.

Approved by Examining Board	**Signature**	**Date**
Dr. Fassil Assefa (Advisor)	___________	_______________
Dr. Taye Tessema (Co-Advisor)	____________	_______________
Dr. Diriba Muleta (External Examiner)	____________	_______________
Dr. Asnake Desalegn (Internal Examiner)	____________	_______________
Dr. Adey Feleke (Chair of the Department)	____________	_____________

Dedication

To my grandmother and grandfather who passed away during my course study, who lit the candle of knowledge in my life.

Acknowledgement

I would like to pay special thanks, warm appreciation to my major advisor Dr. Fassil Assefa from the Department of Microbial Cellular and Molecular Biology, Addis Ababa University for his, guidance in the research and preparation of this thesis. I would also like to express my gratitude to my co-advisor, Dr. Taye Tessema from the EIAR working as national coordinator for PROMISE project, whose comments and suggestions were indispensible for the study.

My appreciation also goes to Mr. Getahun Mitiku a Ph.D student at Leiden University, The Netherlands, for his special support. I would like to give my special thanks to Professor Jos M. Raaijmars and Dr. Desalegn Etalo, who are working in The Netherlands Institute of Ecology, whose comments made enormous contribution to my thesis research work. I also wish to thank one of my best friends Mr Getachew Yilma for his immense support and collaboration in the laboratory activities.

My strong gratitude goes to all staff members of Holeta National Agricultural Biotechnology Research Center for creating good working conditions, while I was conducting the research at the Center. I extend my special thanks to staff members of Pawe Agricultural Research Center as well.

I would like to thank EIAR for giving me the chance to continue my postgraduate study at Addis Ababa University and PROMISE project, all of them shared the financial issue of my thesis work and other expenses during the MSc study.

I would like to thank all members of Addis Ababa University, especially the staff of the Department of Microbial, Cellular and Molecular Biology, my instructors, librarians, my colleagues and true friends for their encouragement and friendly criticisms, which directly or indirectly have contributed to my success.

Lastly, I would like to express my thanks to my beloved wife Menbere-Mariam Regasa and my daughter Sipara Mamo for their patience and understanding while I was totally devoted to this work at the expense of their conveniences. It is impossible to mention all who helped me with all their hearts, but to all of you I say thank you very much.

Table of Contents

List of Figures viii

List of Tables ix

List of Appendixes x

List of Abbreviation/ Acronyms xi

Abstract xii

1. INTRODUCTION 1
   1.1. Objectives 3
      1.1.1. General objectives 3
      1.1.2. Specific Objectives 3

2. LITERATURE REVIEW 3
   2.1. 1. Biology of parasitic weed, *Striga* 4
   2.2. 2. Distribution of *Striga* species in Ethiopia 5
   2.3. *Striga* life cycle and mechanism of host infection 6
   2.4. Sorghum production in Ethiopia 7
   2.5. Impacts of *Striga* on sorghum production 9
   2.6. *Striga* weed control methods 11
      2.6.1. Cultural control of *Striga* 11
      2.6.2. Chemical control of *Striga* 11
      2.6.3. Use of *Striga* resistant varieties 12
      2.6.4. Biological control of *Striga* seed 12

3. MATERIALS AND METHODS 17
   3.1. Study area descriptions 17

3.2. The study design and soil sampling methods 20
   3.3. Soil physico-chemical properties analysis 20
   3.4. Assessment of *Striga* seedbank depleting soil 20
   3.5. Isolation of major fungi associated with *Striga* seeds decay 22
   3.6. Identification and characterization of fungi capable of depleting *Striga* seedbank 22
   3.7. Screening of fungal isolates for *Striga* seeds germination inhibition 23

3.8. Physiological and biochemical based characterization.......... 24
3.8.1. Effects of different liquid media on mycelium dry weight of fungal isolates 24
3.8.2. Effect of hydrogen ion concentration (pH) on mycelial dry weight of fungi isolates 24
3.8.3. Effect of temperature on mycelial dry weight of different fungal isolates 24
3.9. Evaluation of *Striga* seeds germination inhibition using fungi isolates in the presence of host crop 25
3.10. Evaluation of fungi isolates on *Striga* seed mortality using tetrazolium test 25
4. DATA ANALYSIS 26
5. RESULTS AND DISCUSSION.......... 27
5.1. Soil physicochemical properties of major *Striga* seed decaying soil 27
5.2. Assessment of *Striga* seedbank depleting soils from teabag experiment 28
5.3. Isolation and characterization of fungal isolates decaying *Striga* seeds.......... 30
5.4. Screening of *Striga* seedbank depleting fungi in absence of host plant 35
5.5. Physiological and biochemical studies 37
5.5.1. Effect of different liquid media on mycelium dry weight of different fungi isolates.......... 37
5.5.2. Effect of hydrogen ion concentration (pH) on the mycelial growth of fungi isolates 38
5.5.3. Effect of varying temperatures on mycelial growth of fungi isolates.......... 41
5.6. Evaluation of fungal isolates on *Striga* seed germination inhibition in the presence of host crop.. 42
5.7. Effects of fungal isolates on *Striga* seed mortality.......... 44
6. CONCLUSION AND RECOMMENDATIONS 47
6.1. Conclusion.......... 47
6.2. Recommendations 47
7. REFERENCES 48
8. APPENDIXES.......... 61

List of Figures

Figure 1: Illustrated Striga life cycle 6
Figure 2: Areas with heavy Striga infestation in Ethiopia 10
Figure 3: Different fungal isolates on different media and conidia structures 32

List of Tables

Table 1: Distribution of Striga infestation in four regions of Ethiopia 5

Table 2: GPS coordinates and other accessory information of soil sampled areas 18

Table 3: Soil physicochemical properties of the different soil samples collected from northern parts of Ethiopia 27

Table 4: Striga seeds decaying soils from teabag experiment in (%) and their respective soil collection site 29

Table 5: Cultural and microscopic characteristics of the *Striga* decaying fungi on different media 31

Table 6: Different isolates with respective collection site and at genus identification 33

Table 7: Diversity of Striga decaying fungi isolated from different sampling sites of Ethiopia 35

Table 8: Screening of fungal isolates in the presence of synthetic stimulant GR-24 for their ability to inhibit Striga seeds germination using paper disc method 36

Table 9: Effects of different liquid media on mycellium dry weight of different fungal isolates 38

Table 10: Effect of hydrogen ion concentration (pH) on the mycelial growth of Fungi isolates on Potato destrose broth medium 40

Table 11: Effect of varying temperatures on mycelial growth of fungi isolates on the PDB medium 42

Table 12: Effects of fungal spores on Striga seed germination inhibition at different incubation periods ... 44

Table 13: Effects of fungi isolates on Striga seeds mortality at different incubation period in percentage .. 46

List of Appendixes

Appendix 1. Assessment of *Striga* seed decaying soil teabag experiment result………………..61
Appendix 2. Tea bag experiment trial photo during soil sample evaluation in the laboratory …62
Appendix 3. Seed trap methods for purposive/targetic fungi isolation from soil sample……….62
Appendix 4. Slide culture technique procedure for microscopic fungal identifications:………..62
Appendix 5. Striga seed germination inhibition and mortality test……………………………...62
Appendix 6. Different fungi isolate morphology on different media and conidia on PDA …….63
Appendix 7. Screening of *Striga* seedbank decaying fungi isolates …………………………...64

List of Abbreviation/ Acronyms

ACC	Aminocyclopropane-1-Carboxylic Acid
AVG	Aminoethoxy-Vinyl-Glycine
CSA	Central Statistical Agency
CV	Coefficient of Variation
DOI	Days of Incubation
DdH2O	Double Distilled Water
DMBQ	Dimethoxy Benzoquinone
EIAR	Ethiopian Institute of Agricultural Research
GPS	Geographical Position System
GR- 24	Growth Regulator
IPM	Integrated Pest Management
MCPA	Methyl Chlorophenoxy Acetic Acid
MSD	Minimum Significance Difference
PARC	Pawe Agricultural Research Center
PGPR	Plant Growth Promoting Rhizobacteria
PDA	Potato Dextrose Agar

Abstract

Striga hermonthica (Del.) Benth is an obligate hemi-parasitic plant which severely threatens cereal production. Therefore, the use of soil-borne antagonists for *Striga* seedbank depletion is one of the useful control methods suggested for integrated pest management (IPM). The objectives of this study were to preliminary screen and evaluate effective soil fungi that are capable of decaying *Striga* seed using seed trap method. From the soil samples showing seed decay, fungi were isolated and characterized using standard methods. The effect of fungal spores to inhibit *Striga* seeds germination was also tested using synthetic stimulant GR-24 and was further studied using sorghum variety *Teshale*. Besides, effects of fungal spore on *Striga* seeds viability were also studied. Soil samples collected from Tahtay Adiabo (E22) showed the highest *Striga* seed decay (19.8%) whereas soil samples collected from Raya-Kobo (E46) showed the lowest activity (3.6%). A total of 43 fungal isolates were collected from the soil of which 20 isolates decayed more than 50% of the *Striga* seeds. Genus *Fusarium* was the dominant and only ten further characterized. All isolates were showed maximum mycelium dry weight at 25°C and different mycelium dry weight at different hydrogen ion concentration (pH). Effects of fungal isolate on *Striga* seeds germination inhibition using sorghum variety *Teshale* as test crop revealed that there were significant ($p<0.05$) differences among fungal isolates. The results indicated that inoculation of the *Striga* seeds with isolate (F-254) significantly ($p<0.05$) have showed the highest percentage of *Striga* seed germination inhibition of 74.4%, 82.8% and 92.8% of all the fungal isolates upon 15, 21 and 30 days of incubation, respectively. In terms of *Striga* seed viability analysis, the highest number of *Striga* seed mortality (70%, 86% and 92%) were obtained when the *Striga* seeds treated with isolate (F-254) at 15, 21 and 30 days of incubation, respectively which were statistically significant ($p<0.05$) difference from all fungal isolates. However, the lowest results (16.4%) and (29.6%) were obtained from isolate (F-301) at 15 and 21 days of incubation while 39.6 ±8.98 was recorded at 30-DOI with isolate (F-040). This study therefore provides preliminary information on the existence of potential fungal isolates as candidates for biocontrol against *Striga* seeds in the soil that could be further tested in the management of *Striga* seedbanks.

Keywords: - Biocontrol, Haustoria, Strigolactone, Sorghum, Viability

1. INTRODUCTION

Sorghum is one of the most important staple food crops next to teff and maize in many regions of the country, covers an area 14 of the 18 major agro-ecological zones in Ethiopia (MOA, 2014). It provides more than one third of the cereal diet and is almost entirely grown by subsistence farmers to meet needs for food, income, feed and brewing. It is the second most important crop for injera quality next to teff (Adugna Asfaw, 2012).

However, parasitic weed *Striga* has been one of the more intractable agricultural problems seriously limiting productivity of sorghum in Ethiopia. *Striga hermonthica* is a hemiparasitic weed that contributes to 30-90% yield loss (Mrema *et al.,* 2017). However, complete yield losses depending upon infestation level, crop types and species, particularly in the savanna areas in western, central, eastern and southern Africa (Gebisa Ejeta, 2007). It is one of the most economically important parasitic plants in modern agriculture globally than insects, birds and plant diseases (Musyoki *et al.,* 2015).

In Sub-Saharan Africa, it reduces food production of the subsistence farmers in Sahel, Burkina Faso, Cameroon, Ethiopia, Ghana, Mali, Nigeria and Sudan. *Striga* infested lands in Africa is estimated at 7.9 million ha, 3 million of which are in east and central Africa, 3.5 million in western Africa, and 1.4 million in southern Africa (Mbuyi *et al.,* 2017). Because, i) it attacks the host plant under the ground before the flowering stem of the parasite appears above the ground and cause considerable sorghum yield loss annually in Ethiopia (65% - 100%) (Gebisa Ejeta *et al*., 1993), ii) its prolific seed production (100,000 – 500000 seeds) and survival of *Striga* species remain viable for up to 20 years (Parker and Riches, 1993; Smith and Webb, 1996), iii) its intimate life cycle with that of its host and the fact that its seeds germinate only in the presence of a host (Aflakpui *et al.,* 2008), iv) *Striga* seedbank refilling will cause ride of cereal crop farm land.

Although the exact distributions and coverage of *Striga* spp. is not known, it is estimated that over 50% area covered by sorghum, i.e., about one million hectare is infested by *Striga* species in Ethiopia (Taye Tesema and Gebisa Ejeta, 2019). General assessment survey report from the four government states in Ethiopia indicated that 114,488 ha in Amhara (Amhara BoANRD, 2016), 149,691 ha in Tigray (Girmay Shinun, 2017), 61,254 ha in Oromia (Ayana Mirkana,

2015) and 97,360 ha in Benishangul Gumuz region are highly invaded by *Striga* species (Benishangul Gumuz BoANRD, 2016). In SNNP region, 62 villages were reported to be infested with *Striga* species (Sisay Lemawork, 2017). Hence, there is a strong need for novel, effective, affordable and durable control strategies to reduce soil seedbank.

There are different mechanisms to control this noxious weed; chemical, biological and cultural. Biological control is considered as a potential cost effective, safe and environmentally beneficial means of reducing *Striga* seedbank and weed population to a level below the economic threshold (Charudattan, 2001) as compared to other control methods. The use of soil-borne antagonists or diseases of *Striga* seeds, suicidal germination and volatile organic compound production by different microbial species are important components of feasible biological control option that meet the target of reducing *S. hermonthica* parasitism and narrowing crop yield loss within the season of application (Berner *et al.,* 1995).

Among the microorganisms, fungi are very successful in decaying of parasitic seedbank and enhance sorghum production in Sudan (Abbasher, 1994), in Ghana (Abbasher and Sauerborn, 1995), in Kenya (Nzioki *et al.,* 2016), in Burkina Faso, Mali, Niger (Ciotola *et al.,* 1995; Abbasher *et al.,* 1998) and in Nigeria (Marley *et al.,* 1999). Though there are diverse fungal genera in soil, few species of *Fusarium*, *Aspergillus* and *Penicillium* are the most *Striga* seedbank depleting microbes (Lendzemo and Kuyper, 2001). Rebeka Gebrestadik, (2013) has also stated that *Fusarium oxysporum* was the best biocontrol of *Strata* in Ethiopia.

Despite the various reports on the existence of fungal species that are capable of suppressing germination of *Striga* seeds in many parts of Africa, there is information gap regarding the importance of fungi to control *Striga* seeds in Ethiopia. Consequently, the objective of this work was to characterize fungi associated with *Striga* seeds depletion using seed trap methods.

1.1. Objectives

1.1.1. General objectives

The general objective of this study was to isolate and characterize fungi from *Striga* infested sorghum growing areas that could deplete *Striga* seedbank under laboratory conditions with a potential for biological control of the weed and enhance sorghum production.

1.1.2. Specific Objectives

The specific objectives of the current study were to:

- To assess soil which could decay striga seedbank
- To isolate and identify fungi associated with *Striga* seedbank depletion
- To evaluate *Striga* seedbank depleting fungal isolates under *in-vitro* laboratory condition

2. LITERATURE REVIEW

2.1. Striga weed

Weeds are one of the most biological constraints which cause yield losses and perturb food security and human welfares in the world. In Sub-Saharan Africa, *S. hermonthica* is a major constraint to cereal production including sorghum, finger millet, rice, teff, sugar canes. It is causing yield losses worth US$ 9 billion (Gebisa Ejeta, 2007). It infests more than 50 million hectares of farmland with intensifying dissemination, which makes it one of the serious threats to food security (Westwood *et al.,* 2010). During the life cycle below ground, it forms haustorium, attaches and penetrates the host root cortex cells and sucking nutrients, minerals and water cause final damage of host.

Even though much research has been done to control *Striga* weeds, the life cycle of parasitic weeds and its host interaction make it difficult to control. *Striga* plants have a high reproductive capacity: a single plant can produce 100,000-500,000 very tiny (0.15-0.30 mm in diameter) seeds, which are easily dispersible and the seeds stay more than 20 years in the soils without germination until they recognize signals to chemical stimulated by host plant (Parker and Riches, 1993).

Depletion of the persistent seeds using soil-applied chemicals to stimulate germination has received much attention while the researches for microorganisms selective for seed decay are at infant stages. Limited studies indicate that microorganisms that are associated with weed seeds can contribute to seedbank depletion through attraction to seeds by chemotaxis and production of enzymes and/or phytotoxins to kill seeds prior to germination (Compant *et al.,* 2013).

To achieve success, more in-depth research on microbial factors affecting weed seedbanks is required. The knowledge of microbial-related seed-bank processes and the concept of useful (micro-) biological processes, including seed decay, germination stimulant (suicidal germination) by microbes in weed management strategies is promising (Abbasher and Sauerborn, 1995).

2.1. 1. Biology of parasitic weed, *Striga*

Striga is a hemi-parasitic weed that parasitizes sorghum, millet, sugarcane, maize, rice and more of graminaceous species. The genus *Striga* contains 41 species which are found in Africa and some parsts of Asia (Cochrane *et al.,* 1997). Gobena Daniel *et al.* (2017) have also reported that, five of the most economically important *Striga* species, i.e. *Striga hermonthica*, *Striga asiatica, Striga forbesii, Striga aspera, and Striga gesneriodes*, affect the production of sorghum (*Sorghum bicolor*), finger millet *(Eleusine coracana),* maize *(Zea mays),* sugarcane *(Sacharum officinarum) and* cowpea *(Vigna unguiculata)* resulting in annual losses over 1 billion USD in cereal productivity in Africa (Gurney *et al.,* 2003; Elzein and Kroschel, 2004; Amusan *et al.,* 2008).

Striga can grow up to 80 cm with hairy, hard quadrangle shaped and fibrous stem, narrow leaf, and spike-shaped raceme inflorescence bearing up to 60 flowers for the terminal and 10-20 for the lateral inflorescence with bright pink, rose-red, white, or yellow color (Musselman, 1980). *Striga* has been given the common name of "witchweed" because it attaches itself to the roots of the host plant thus depriving it (the host) of water and nutrients (Berhane Sibhatu, 2016). *Striga* does not only release phytotoxins to the host plants as it germinates, but also drain photosynthates from the hosts plants causing the crop to wither and produce low yields.

Striga spends most of its life cycle underground and develops above ground for stem formation and flowering. It stands for the principal biological constraints of crop production than any other

biological pests such as insects, birds, or plant diseases in Sub- Saharan Africa (Musyoki *et al.,* 2015). It represents the largest challenges to food security affecting livelihoods of over 300 million peoples in 25 African countries and the problem is getting worse (Khan *et al.,* 2014).

2.2. 2. Distribution of *Striga* species in Ethiopia

Striga is abundant in most of sorghum growing areas especially where soil fertility and moisture stress are limiting factor. It causes the crops to be witched while it is invisible underground which results in stunting, wilting and chlorosis. Of the devastating *Striga* species of the world, the two worst species *(S. hermonthica and S. esiatica*) are represented in Ethiopia and cause widespread losses to major cereals productions. Yield losses of 65 to 70% are common in sorghum growing areas and arable lands are often abandoned because of the prohibitive parasites populations (Parker and Riches, 1993).

Although the exact distributions and coverage of *Striga* spp. is not known, it is estimated that over 50% area covered by sorghum, i.e., about one million hectare is infested by *Striga* species in Ethiopia. General assessment survey report from the four government states in Ethiopia, however, indicated that 114,488 ha in Amhara, 149,691 ha in Tigray, 61,254 ha in Oromia and 97,360 ha in Benishangul Gumuz region area highly invaded by *Striga* species (Table 1). In SNNP, 62 kebeles were reported to be infested with *Striga* species (Sisay Lemawork, 2017)

Table 1: Distribution of Striga infestation in four regions of Ethiopia

Region	**Area of Striga infested**	**References**
Tigray	149,691	Girmay Shinun, 2017
Amhara	114,488	Amhara BoANRD, 2017
Benishangul Gumuz	97,360	Benishangul BoANRD, 2016
Oromia	61,254	Ayana Mirkana, 2015
Total	422,793	

2.3. *Striga* life cycle and mechanism of host infection

The presence of parasitic hosts and their secondary metabolites have great role in their interaction and regulation for the life cycle of the parasitic weeds (Figure 1). These secondary metabolites are involved in signaling, for example in the induction of parasite germination and the formation of the haustorium, and in plant defense against the parasite (e.g. phytoalexins) (Bouwmeester *et al.,* 2003). Cotton can also serve as false trap crop to produce germination stimulant strigol from its root exudates and used for suicidal germination of parasitic *Striga* seed (Sato *et al.,* 2005).

Though non host crop produce germination stimulant, the strigolactones are known to be the most potent stimulant, eliciting germination at high concentrations in seeds of many *Striga* species (Bouwmeester *et al.,* 2003). This chemical firstly produced by host plants mainly to induce colonization by symbiotic arbuscular mycorrhizal fungi in the rhizosphere. However, different plant species and different cultivars of the same species determine the amounts and composition of strigolactones production (Xie *et al.,* 2013). A crystal structure of a highly sensitive strigolactone receptor from *Striga* revealed a larger binding pocket than that of the *Arabidopsis* receptor, which could explain the increased range of strigolactone sensitivity. Thus, the sensitivity of *Striga to* strigolactones from host plants is driven by receptor sensitivity.

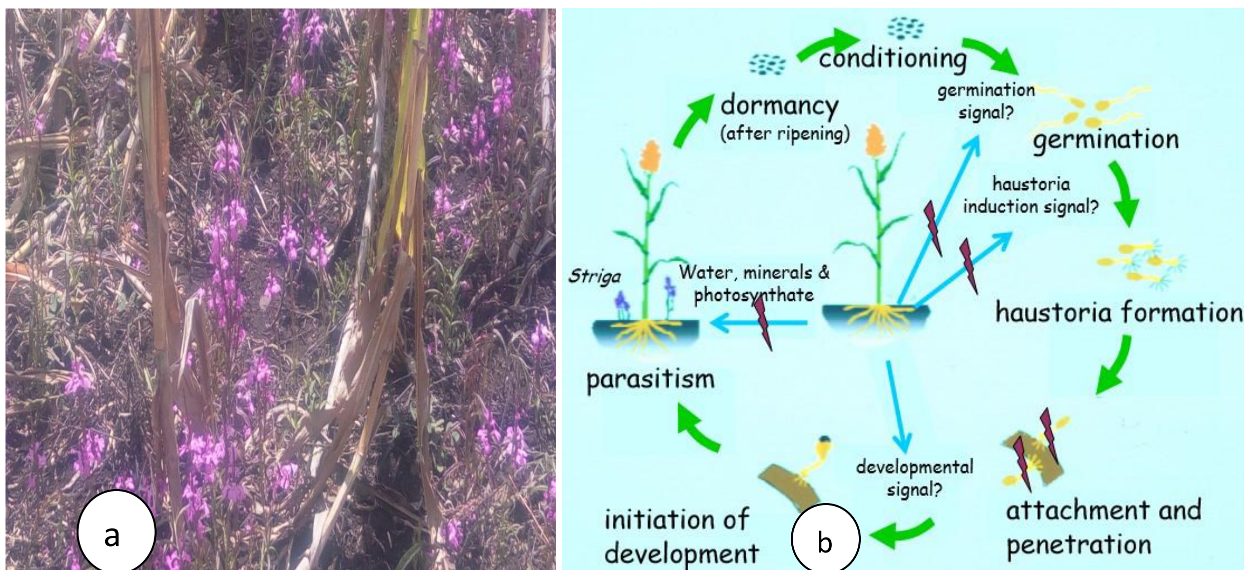


Figure 1: Illustrated Striga life cycle: a) Striga plant by author b) Striga Life cycle by (Gebisa Ejeta *et al.,* 1993)

Subsequent to germination, the parasites attach to a host through a specialized organ known as the haustorium and regulate the development of this organ through the recognition of chemical factors from host plants (Berner, 2007). The haustorium factor, 2, 6-dimethoxy-p-benzoquinone (DMBQ), is a product of lignin oxidation and decarboxylation of phenolic acids found in plant cell walls described as one of the best exogenous haustorial inducing factors obtained from sorghum root extracts (Chang *et al*., 1986).

The level of host recognition in *Striga* is mediated through their enzymatic digestion of the host root surface. Degradation of surface components liberates quinonoid compounds, such as 2,6-DMBQ, which in turn trigger haustorial development. Some chemicals released by plant roots for defense against other competing plants, parasites or pathogens have also been found to trigger parasite seed germination. Examples of these chemicals are sorgoleone and hydroquinone sorghum xenognosins (SXSg). The chemicals are distinct from strigolactones but can trigger *Striga* germination (Keyes, *et al* 2000).

After attachment the haustorium invades the host epidermal cells, penetrates into the root cortex and proceeds to form a xylem-to-xylem connection with the host and then starts to acquire the hosts nutrients and water (Dorr, 1997). The penetration of haustorium cells into host tissue (xylem and/or phloem system) is carried out mechanically by pressure on the host endodermal cells and by hydrolytic enzymes. Subsequently adventitious roots are often produced, shoot development follows and *Striga* eventually emerges above ground and matures within a few weeks and sets seeds (Musselman, 1980).

2.4. Sorghum production in Ethiopia

Ethiopia is the center of origin and diversity for both cultivated as well as wild relatives of sorghum ([*Sorghum bicolor* (L.) Moench]). Thus Ethiopias' germplasm contribution to the international gene pool has been huge. It is a known source for various economically important traits such as high lysine sorghum, good grain quality, resistance to diseases and insect pests, and stays green (Taye Tesema and Gebisa Ejeta, 2019).

Sorghum is cultivated in all regions in Ethiopia in altitude that ranges from 400 to 2500m above sea level. It is cultivated mostly at lower altitude along the western, south western, north western

north eastern and central parts of the countries. Sorghum is grown in 14 of the 18 major agro-ecologies of Ethiopia. The main sorghum growing areas are grouped into four major traditional agro-ecologies such as dry lowland, humid lowlands, intermediate altitudes and highland sorghum growing agro-ecologies (EIAR, 2014).

Dry lowland agro-ecology is the vast majority sorghum growing area of the country and mostly characterized by erratic rain-fall, low soil fertility and fragile ecosystem. In humid lowland sorghum growing areas, the altitude range is the same as the dry lowland but moisture level and humidity is very high. The intermediate altitude sorghum growing agro-ecology (16000-1850m) is characterized by high moisture and humidity, while the high elevation sorghum growing areas have altitude ranges from 1850-2500 masl and receive high annual rainfall (EIAR, 2014).

Out of the total grain crop area covered by cereals, i.e., 12,727,191.21 hectares, 14.38% (1,829,662.39 hectares) is covered with sorghum, ranking third next to tef and maize. Similarly, out of the total national grain production, cereals contribute to 87.97% (277,638,380.98 quintals) with sorghum shares amounting up to 15.92% (50,243,680.72 quintals) of the grain production. This makes sorghum the fourth ranking grain crop produced in the country next to maize, tef and wheat (CSA, 2019). It was produced by around five million small-scale farmers and its yield increased from 13.6 quintals/ha while cropped area increased from 1.28 million ha to 1.83 million ha during the same period.

Oromia, Amhara, Tigray and SNNP regions are the major producers of sorghum, covering more than 91% of the total area and production (CSA, 2015). In Oromia, sorghum covers 719,399.7 hectare of land. It is the third major food crop next to maize and tef and the fourth in production next to maize, wheat and tef with an average yield of 25.22 quintals/ha. Its major challenges are lack of resistant variety, proper management systems, post-harvest loss and unfavorable environmental conditions. Estimated losses due to *Striga* infestation are ranged from 40 to 100% (Ayana Mirkana, 2017).

In SNNP, sorghum is the fourth both in area of cultivation (150,000) ha and production volume (2,500,000 quintal of grain). Regional average production is 20 q/ha (Sisay Lemawork, 2017). In Tigray, more than 399, 2009.5 ha of land covered by sorghum though its productivities is limited by different abiotic and biotic factors. Among the biotic *Striga* is the most devastating pests that

may cause up to 100% losses and its enormity is magnified in less fertile and dry areas (Girmay Shinun, 2017).

In Ethiopia, sorghum provides more than one third of the cereal diet and is almost entirely, grown by subsistence farmers to meet needs for foods , income , feeds, traditional brewing, and construction, purposes (EIAR, 2014). It is also the preferred grain to complement tef in making enjera among the rural and urban poor. The grain is used for the preparation of other traditional foods and beverages liked tela and areke. Besides, usages as grain, the stover of sorghum has several roles in many cases in the country even more than the grain does in some cases. It is used as livestock feed, fuel wood, construction materials and several other uses. Sorghum is also known to be strongly tied to the livelihood of the people particularly in the dry lowlands and highland sorghum growing areas where livestock feed is very scarce. It is also a vital source of cash via its versatile purposes and products (Taye Tesema and Gebisa Ejeta, 2019).

2.5. Impacts of *Striga* on sorghum production

Striga is a severe biotic problem in major cereal growing areas in Ethiopia (Gebisa Ejeta *et al.,* 2002). The major sorghum growing areas in all regions of Ethiopia are highly infested by *Striga* and up to 65% yield loss of sorghum was observed (Figure 2) (Tesso Tesfaye *et al.,* 2007). In the North Eastern and Western parts of Ethiopia, severe yield losses were recorded in sorghum genotypes because of *Striga* infestation (Wondimu Bayu *et al.,* 2001; Mesfin Abate *et al.,* 2014). As indicated above, high *Striga* infestation is present in sorghum based production systems of the country. Hence, *Striga* is threatening the livelihoods of sorghum growing resource poor farmers in all regions of the country.

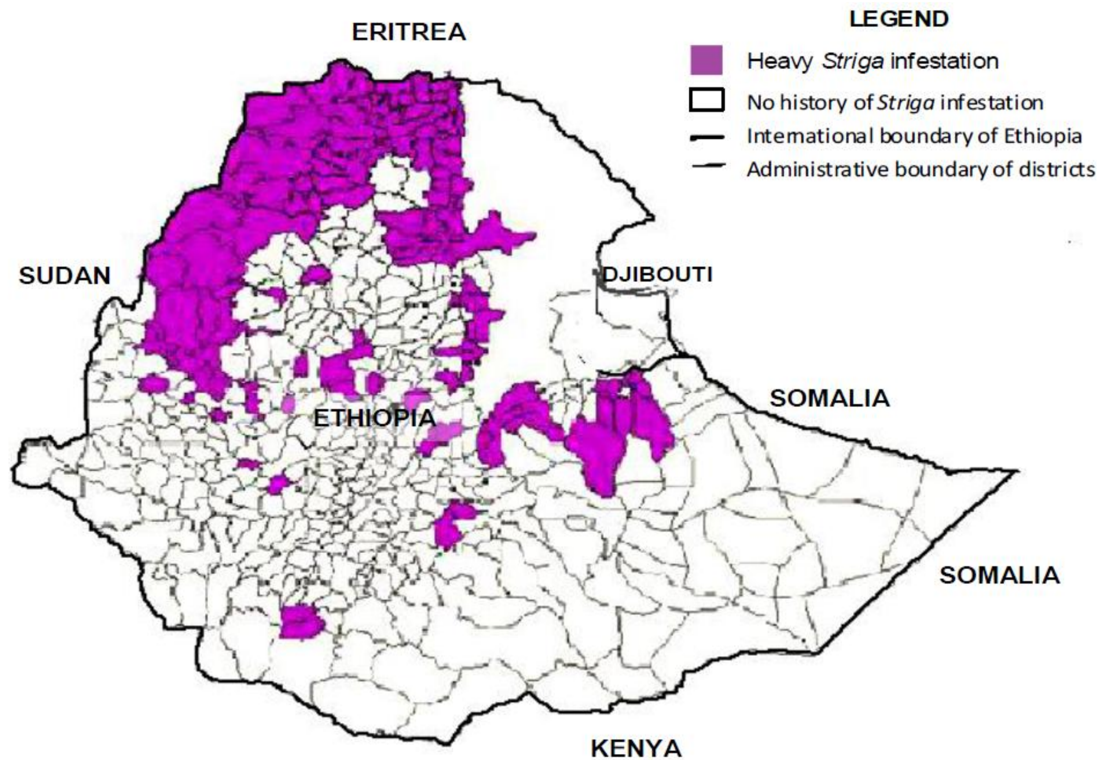


Figure 2: Areas with heavy Striga infestation in Ethiopia (Tesso Tesfaye *et al.,* 2007)

Prolific seed production and prolonged viability of the seeds result in a rapid buildup of huge seedbanks in infested sorghum fields, which are eventually abandoned because the field becomes unsuitable for sorghum production (Gebisa Ejeta, 2007). Although these parasites are able to develop green leaves and have intact chloroplast genomes, they exhibit only low rates of photosynthesis and therefore they need to deprive most of organic matter from their hosts. Moreover, these parasitic plants exhibit high rates of transpiration and consequently they obtain a large amount of water and minerals from their hosts (Musselman, 1980). These facts result in the parasites causing yield losses from a few percent to complete crop failure depending on crop species, crop variety and severity of infection.

When nitrate is assimilated by host plant, there is rapid transfer of nitrogen to parasite and parasite have approximately equal amount of nitrate and amino acid mostly glutamine and asparagines, infection alter free amino acid profiles (Pageau, *et al.,* 2003). Frost, *et al.,* (1997) reported that infected sorghum plant by *Striga* has less shoot and root biomass, smaller leaf area, lower stomata conductance and steady state photosynthesis with having twice abscisic acid concentration than uninfected sorghum crops. *Striga* infested plants failed to flower and water

use efficiency and reduced to 72% when compared to the control treatment (Gebremedhin Woldewahid *et al.,* 2000).

2.6. *Striga* weed control methods

In developing countries, weeds continue to be one of the most important contributing factors to yield loss in agricultural production. Significant weed seedbank increments through prolific seed production, competition for water and nutrients, cost associated with seed cleaning are the main weed to contribute for crop yield losses. There are several options for the control of *Striga* in sorghum though there are no single, all-inclusive and ultimately effective methods widely applied. The available strategies include the use of resistant sorghum varieties, crop rotation practices, intercropping with pulse crops, manipulation of planting time, deep planting, use of trap crops, application of organic and inorganic fertilizers, use of herbicides and biological control agents (Hearne, 2009). There are basic weed management methods including cultural, genetic, chemical and biological methods that have been studied and applied in particular scenarios (Gebisa Ejeta *et al.,* 2007; Labrada, 2010).

2.6.1. Cultural control of *Striga*

Among the cultural control methods, the most commonly practiced are hand weeding, use of trap and cash crops, crop rotation, intercropping, and multi-year fallowing. Crop rotation, intercropping, alley cropping with perennial legume shrubs along with integrated use of resistant varieties, fertilizer, 2,4-D, and hand pulling significantly improved land and crop productivity along with effective control of *Striga* (Fasil Reda and Verkleij, 2007).

2.6.2. Chemical control of *Striga*

There are two categories for the chemical control of *Striga*; germination stimulants and herbicides (pre and post-emergences). Chemicals capable of inducing *Striga* seed germination; for example ethylene, ethephones, strigol, and strigol analoges can be used as control strategy. These are known to induce *Striga* seed germination in the absence of *Striga* host crops known as suicidal germination. These strategies have a key role in depleting *Striga* seedbanks. The

drawback of this strategy is related to cost associated with the use of ethylene and accessibility of the technology to smallholder subsistence farmers (Xie *et al.,* 2010; Yoneyama *et al.,* 2011).

Among the post emergence herbicides tested, 2, 4-D has been the most selective and widely used option. Similarly, the 2-methyl-4-chlorophenoxy acetic acid (MCPA), a compound closely related to the 2, 4-D, has also been showed to be effective when mixed with bromoxynil. The use of glufosinate oxyfluorfen, the combination of urea and dicamba and the combined use of chlorsulfuron and dicamba were also shown to be effectively control *Striga* (Satish *et al.,* 2012)

The limitation of both pre and post emergence herbicides is related to their inabilities to prevent crop yield loss. This occurs because, at the time this chemicals affect *Striga*, the impact on the host crop already occurred. In addition, these are relatively expensive and often inaccessible methods to subsistence farmers (Labrada, 2010).

2.6.3. Use of *Striga* resistant varieties

Host plant resistance is often the cornerstone up on which an integrated *Striga* management program is built. It is regarded as the most feasible and attractive method for *Striga* control. However, the current sources of resistance are quantitative in nature and yield losses can still be significant at high *Striga* levels. Genetic resistance needs to be verified in each environment, as it is probable that resistance may be *Striga* biotype specific (Ransom *et al.,* 2017). Hence integrating the genetic resistance with other control measures is the smartest option possible for both the effectiveness of control as well as for the increased durability of resistance genes (Rodenburg *et al.,* 2017). Moreover, adequate knowledge related to the host-parasitic interaction at the different growing stages of both plants is required, in addition to better understanding of the complex genotype by environment interaction.

2.6.4. Biological control of *Striga* seed

The biological control of *Striga* with microbial resources can occur at any of the stages of *Striga* life cycle, from germination to seed set. During this time, crucial exchange of signal molecules and biochemical cross talk occur between plant-associated microbes and *Striga* (Cardoso *et al.,* 2011). Microbes can suppress *Striga* either by preventing seed germination or enhancing it in the absence of host plant, thus leading to suicidal germination.

In addition, microbes can be deployed at distinct stages of *Striga* lifecycle (germination, haustorium formation and attachment). Moreover, other microbial based methods are directed to improvement of field conditions, plant- growth promotion, enhanced fertility and direct pathogenesis (Pal and Brian, 2006; Rubiales *et al.,* 2012). A diverse set of soil microorganisms including bacteria, filamentous fungi and yeast are capable of producing physiologically active metabolites that have known effects on sorghum plant-growth, development and health. A better understanding of this tripartite association system may allow seeking effective complementary strategies of Strata control (Rubiales *et al*., 2012).

So far, several fungal genera including *Alternaria* spp. *(A. alternate, A. chlamydospora), Aspergillus* spp*., Fusarium* spp. *(F. equiseti, F. oxysporum)* (Abbasher *et al.,* 1998) and bacteria spp. (*Acetobacter, Agrobacterium, Arthobacter, Azospirillum, Azotobacter, Bacillus, Klebsiella, Pseudomonas* and *Xanthomonas)* (Ahonsi *et al.,* 2002; Keyes *et al.,* 2000) pathogenic to *S. hermonthica* were identified from *Striga* infested areas across the world. Complete suppression of weed is unfeasible by using any mechanism and biological weed control strategy relatively reduces the establishment of weed population to below the economic threshold (Hayelom Berhe, 2014).

Microbes used as bio-control in two approaches; classical approach which is characterized by the importation, introduction and release of natural enemy from the same geographical origin of the weed into an area. In this approach, the natural enemy bacteria or fungi pathogen is allowed to self-perpetuate, survive and establish, thus providing long-term weed control over several years. The second approach is inundative through repeated application of inoculums. The pathogens are often indigenous, artificially mass-produced and applied during the growing season. Weed control is short term when compared to classical biological approach and the microbes are not expected to persist in the environment (Charudattan and Dinoor, 2000).

2.6.4.1. Bacteria as *Striga* control strategies

The exploitation of microbes capable of inducing *Striga* suicidal or ineffective germination has long been practiced (Cardoso *et al.,* 2011; Rubiales *et al.,* 2016). For instance ethylene produced by microbes stimulates *Striga* germination, even in the absence of suitable host. The life span of germinated seeds with no access of host plant is short days to weeks. Hence strategy can assist

the reduction of the *Striga* seedbank in soils, thus relieving the plant from future attacks. For instance, the Genus of *Pseudonomonus* is an effective producer of ethylene, thus capable of stimulating *Striga* germination in the absence of sorghum plants (Babiker *et al*., 1993).

Members of the bacterial genera of *Acetobacter, Agrobacterium, Arthobacter, Azospirillum, Azotobacter, Bacillus, Klebsiella, Pseudomonas,* and *Xanthomonas* are known to produce phytohormones and related lipophylic compounds. These metabolites were previously reported to have the potential for inhibiting *Striga* seed germination, affecting radicle growth and cell differentiation (Keyes *et al.,* 2000). In addition, hydrogen cyanide (HCN) is a potent inhibitor of cytochrome C oxidase and other several metalloenzymes. HCN affects sensitive organisms by inhibiting the synthesis of ATP mediated by cytochrome oxidase and it is highly toxic to aerobic microorganisms, even at picomolar concentrations.

2.6.4.2. Fungi as Striga biological control

Either directly through inoculation of soils with effective pathogenic strains or indirectly by managing soil fertility that enhance native seedbank decaying microbes, seed depleting microorganisms have a defined potential for management of weed seedbank (Muller-Stover *et al.,* 2016). *Fusarium nygamai* and *Fusarium semitectum* reduce *Striga* emergence and germination in the soil and improved sorghum performance in Sudan when sorghum seed inoculated with those inoculums spores. This inhibition is because fungi spore attack *Striga* germ tubes (Abbasher and Sauerborn, 1995).

In Ghana, two *Fusarium oxysporum* isolates were found to reduce *Striga* emergence by 88 and 98 percent and *Fusarium semitectum* 76 percent with an increase of 26 percent of sorghum yield (Abbasher and Sauerborn, 1995). Fungal mycelium of *Fusarium nygamai* penetrated the *Striga* seeds in soils and parasitize inside and host specific reduce *Striga* biomass by 10-50 percent and increase maize vigor and yield.

Few studies showed that fumonism B1, which is produced by *Fusarium nygamai* isolated from *Striga hermonthica*, has an herbicidal effects on *Striga* spp. in particular when applied at post-emergence. In addition, fusarenon X, nivalenol, deoxynivalenol, T-2 toxin, HT-2 toxin, diacetoxyscirpenol, and neosolaniol produced by *Fusarium* spp. were shown to be effective in

inducing detrimental impact on *Striga* panicle development and maturity. Other *Fusarium* species were also shown to have the potential to *Striga* control, e.g., *F.equisetii* (Hess *et al.,* 2008; Saurborn *et al.,* 2007).

Considering the mechanisms through which the bio-control of plant weeds occur is serious to the ultimate improvement and wider use of bio-control technique. The majority of bio-control agents are specific in action; they do not pollute the environment through residues and are more proper (Heydari and Pessarakli, 2010). Sicuia *et al.* (2015) have reported that fungal isolates especially *Aspergillus* species produce high extracellular enzymes which inhibit *Striga* seed germination. In addition, they also produce antimicrobial and bioactive metabolites such as surfactin, iturin and fengycin characterized by well-established *in vitro* activity. The close genetic affiliation to genus *Aspergillus* by most of the screened fungal isolates cultured from *Striga* suppressive soils underlines their role in *S. hermonthica* suppression (Nzioki *et al.,* 2016).

2.7. Formulation and application methods of fungal biocontrol agent

Formulation of a bioherbicide is the key for successful biological control and can be defined as the mixing of the biologically active propagule with inert carriers and other adjuvants, to give a product, which can be effectively delivered to the target weed (Rhodes, 1993). *F. oxysporum* PSM-197 was identified as a potential candidate for *Striga* control completely inhibited *Striga* growth when grown on sorghum grain and incorporated in the soil as pre-emergence treatment under controlled conditions in Nigerian Savannah (Marley *et al*. 1999). In field trials, chlamydospore-rich powder of *F. oxysporum* M12-4A substrate reduced *S. hermonthica* emergence by 92 per cent. Complete inhibition of *S. hermonthica* emergence occurred when sorghum seeds were coated with the inoculum using arabica as adhesive material (Ciotola *et al*. 2000).

F. nygamai was tested for its ability to control *Striga* in maize under Ivory Coast's field conditions using liquid and solid medium (fermented sorghum grains) and adopting two methods of application (foliar application and soil treatment) in the first season. Both liquid and solid inoculums were also applied in maize as pre- and post-emergence application. In the second season, *F. nygamai* was formulated as dry powder or as granules applied into the planting hole which was found to be the most effective application method Sauerborn *et al*., (1996b).

In Sudan, a *Fusarium* isolate - named Abuharaz isolate - was obtained from diseased *Striga* plants and proved to be effective in controlling *S. hermonthica* when it was propagated on autoclaved sorghum grains, *Striga* emergence and *Striga* biomass were significantly reduced by 42 to 68 per cent and 43 to 80 per cent respectively (Mohamed, 2002) in the field condition.

The efficacy of *F. oxysporum* PSM-197 in controlling *Striga* under field conditions was evaluvated (Marley and Shebayan, 2005). It was applied in four different methods. These included: placement in a 15cm wide band at a rate of 0.5 kg over a 10 m ridge, broadcast application of 2.5 kg per plot, spot application of 5 -10 g per planting hole and surface placement of 10g over the sown seed covered with soil. Overall, the spot application gave the best results in reducing *Striga* emergence with about 90 per cent, while the broadcast application provided the lowest reduction of *Striga* emergence with 75 per cent, which was still significant compared to the control (Marley and Shebayan, 2005).

Two greenhouse experiments were conducted in Burkina Faso to study the efficacy of 14 *Fusarium* isolates to control *S. hermonthica*. This study concluded that all *Fusarium* isolates tested were able to reduce the *Striga* shoots, *Striga* vigor and dry biomass. As a result, sorghum dry biomass and grain yield were improved. The authors suggested that inoculum should be incorporated in the top 5 cm of the soil and not below 10 cm depth because the first *Striga* shoot to emerge in the field were observed within the 10 cm radius (Yonli *et al*., 2006).

3. MATERIALS AND METHODS

3.1. Study area descriptions

The study was conducted in Northern parts of Ethiopia such as seven districts of Amhara regions (Kewot, Jile Tumuga, Artumfusrsi, Xumakarsi, Kobo, Wuchale, Raya Azebo) and from four Tigray regions districts (Haftayhumera Abergele, Taytay Adiabo, Alamaxa) wich was supported by GPS coordinates (Table 2). The altitude ranges from 619 to 1664 m above sea level (Table 2). The study area were known by main sorghum growing areas which are grouped into two major traditional agro-ecologies such as dry lowland and intermediate altitudes sorghum growing agro-ecologies. Dry lowland agro-ecology is the vast majority sorghum growing area of the country and mostly characterized by erratic rain-fall, low soil fertility and fragile ecosystem (EIAR, 2014). There are three regionally recognized seasons in the study area. The first is the main rainy monsoon season which lasts from June to September (locally called Kiremti), the second is the dry season from October to February and the third is pre-monsoon hot season from March to May Fasil Reda, (2010).

Table 2: GPS coordinates and other accessory information of soil sampled areas

	Collection_site					GPS coordinate		
code	**Region**	**Zone**	**Woreda**	**Kebele**	**Alt(m)**	**Longitude**	**Latitude**	**S.C**
E01	Amhara	Oromia	Kewot	Abayatir	1394	09.54.49.3	04.00.53.4	0
E02	Amhara	Semenshoa	Kewot	Abayatir	1386	09.55.25.3	040.00.45.4	0
E03	Amhara	Oromia	Jilexumuga	Karakore	1664	10.27.33.3	039.56.12.4	103
E04	Amhara	Oromia	Jilexumuga	Merewa	1416	10.17.43.2	033.58.08.6	199
E05	Tigray	WestTigray	Haftayhumera		668	14.11.35.9	036.46.35.2	4
E06	Tigray	Tembein	Abergele	Yechela	1629	13.13.58.3	038.59.33.2	13
E07	Amhara	Oromia	Arxumafulsi	Chefadire	1457	10.31.17.9	039.55.36.4	1
E08	Amhara	Oromia	Xumakarsi	Jarakichini	1456	10.30.54.0	039.56.51.3	17
E09	Tigray	Tembein	Abergele	Yechela	1587	13.15.26.2	038.59.36.5	4
E10	Tigray	WestTigray	Haftayhumera	Maykedira	668	13.58.13.6	036.34.35.7	0
E11	Tigray	Tembein	Abergele	TitayHagum	1478	13.25.49.3	038.59.46.6	61
E12	Amhara	North wolo	Kobo	Gobiye	1607	11.59.29.2	039.38.20	116
E13	Tigray	WestTigray	TahtayAdiabo	Qushet	1041	14.34.34.4	037.44.25.7	0
E14	Tigray	Rayaazebo	Alamaxa	Alamaxa	1468	12.29.49.7	039.37.30.3	124
E16	Amhara	Dese	Wuchale	Chisabalima	1617	11.29.02.4	039.07.14.6	0
E17	Amhara	Oromiya	Jilexumuga	Merewa	1476	10.19.33.3	033.53.06.3	11
E18	Tigray	WestTigray	Haftayhumera	Maykedira	625	14.10.32.7	036.39.22.5	30
E19	Amhara	North shoa	Kewot	Abayatir	1373	09.51.11.0	040.01.42.3	2
E20	Tigray	Tembein	Abergele	Yechela	1455	13.30.09.8	039.02.25.8	0
E21	Tigray	Rayaazebo	Alamaxa	Xumuga	1501	12.20.18.4	039.35.17.5	0
E22	Tigray	WestTigray	TahtayAdiabo	Gemhalo	1040	14.32.12.4	037.46.36.0	78
E23	Amhara	Semenwolo	Kobo		1503	12.06.09.3	039.36.56.6	28
E24	Amhara	Semenshoa	Kewot	Mariye	1263	09.59.21.4	033.55.26.1	68
E25	Tigray	WestTigray	TahtayAdiabo	Simret	1079	14.24.22.8	037.52.11.6	47

E26	Tigray	WestTigray	Haftayhumera		619	14.14.46.7	036.39.46.2	53
E27	Tigray	Rayaazebo	Alamaxa	Alamaxa	1513	12.21.53.3	039.34.36.2	11
E28	Tigray	WestTigray	TahtayAdiabo	Qushet	1040	14.39.40.1	037.45.37.8	0
E29	Tigray	WestTigray	Haftayhumera	Maykedira	635	14.10.27.4	036.36.11.3	3
E30	Amhara	Oromia	Arxumafulsi	Hulatukuye	1460	10.33.46.8	039.55.50.5	0
E31	Tigray	WestTigray	Haftayhumera		649	14.12.57.7	036.43.58.1	0
E32	Amhara	Dese	Wuchale	Golbo	1565	11.27.07.3	039.37.01.0	42
E33	Amhara	Northwolo	Rayaazebo	kebele08	1441	12.06.00.3	039.38.52.7	0
E34	Tigray	WestTigray	Haftayhumera	Maykedira	621	14.0457.6	036.34.23.2	0
E35	Tigray	WestTigray	Haftayhumera	Maykedira	642	14.02.11.9	036.34.2.21.7	56
E36	Tigray	Rayaazebo	Alamaxa	Alamaxa	1494	12.25.46.4	039.35.05.7	28
E37	Amhara	Semenshoa	Kewot	Debrinajegol	1281	09.56.29.4	033.57.52.4	22
E38	Amhara	Oromia	Jilexumuga	Merewa	1421	10.17.38.8	033.58.03.3	18
E39	Tigray	WestTigray	Haftayhumera		628	14.14.14.6	036.40.52.5	64
E40	Amhara	Oromia	Xumakarsi	Jarakichini	1457	10.30.54.2	039.58.47.7	5
E41	Amhara	Dese	Wuchale	Golbo	1565	11.26.54.3	039.07.00.05	13
E43	Amhara	Dese	Wuchale	Golbo	1571	11.26.34.7	039.36.59.2	6
E46	Amhara	North wolo	Rayaazebo	kebele07	1425	12.05.26.2	039.39.26.8	2

3.2. The study design and soil sampling methods

Purposive sampling was conducted to select the study area, and simple random sampling was employed to collect soil samples from from the rhizhosphere of *Striga* plants and sorghum within a diameter of about 5-km along the road side by a spade washed with 70% ethanol followed by 1% of commercial bleach between samplings to avoid soil microbial contamination. Hence, a total of 46 soil samples were collected from sorghum growing regions where there are natural striga infestations in October 2018. From each location per *Striga* infestation, about 3-kg soil samples were collected from the top layer (0-30) cm where there are high microbial activities of five representative field sites per *Striga* infestation sorghum agro-ecology. For each of the field soils the codes were given as (E01, E02, E03, and E04...E46).

3.3. Soil physico-chemical properties analysis

For the physico-chemical analysis of the soils, 1 kg of soil was taken from each composite soil sample in a separate plastic bag and transferred to Soil Chemistry Laboratory of Holeta Agricultural Research Center. Then, soil samples were air dried and ground as well as sieved to (2 mm) to remove stones and plant debris. For each soil sample, soil texture, organic carbon, pH, available P, total N, were analyzed by using standard procedures for each parameter. Soil particle size distribution (percentage of clay, silt and sand) in the soil was analyzed by Boycouos hydrometer method (Bouyoucos, 1962). Potentio-metric method using a glass-calomel combination electrode was used to measure pH of the soils in water suspension in a 1:2.5 (soil: liquid ratio) (Van Reeuwijk, 1992). The cation exchange capacity was determined from the ammonium acetate saturated samples through distillation and measurement of ammonium using the modified Kjeldhal procedure. The (Walkley and Black., 1934) wet digestion method was used to determine soil OC content. Total N was analyzed using the Kjeldahl digestion, distillation and titration method as described by (Black, 1965). Available P was determined using the standard Bray-II extraction method (Bray and Kurtz, 1945).

3.4. Assessment of *Striga* seedbank depleting soil

The *Striga* seeds were surface sterilized by 70% ethanol for one minute, followed by 1% of NaOCl for five minutes and then thoroughly washed with distilled water (5-times) for removal of commercial bleach. Then, the seeds were spread at the center of five petri dishes containing

whatman No.1 filter papers. Equal volume of distilled water (5 ml per petri dish) was added and the seeds were incubated at 30°C for 14-days for preconditioning.

About 250 g of soil samples was added in Petri dishhaving 12 cm diameter and 3 cm depth. About 350 mg preconditioned *Striga* seeds were filled into tea bags having pore size of 90 µm and buried in Petri dishand moistened at field capacity with distilled water. There were four tea bags in each Petri dishas one experimental unit with three replications for all soil samples in completely randomized design. *Striga* seeds, buried in sterilized river sand were used as control. There was soil layer below and top of the tea bag during burying the samples, and the buried *Striga* seeds were incubated at 30°C for six weeks.

After four and six weeks, one tea bag from each Petri dishfrom all replications was exhumed by pulling the threads tied to the tea bag. Two pieces of 9-cm diameter sterile filter papers were placed in individual sterile petri dish. Three small filter paper disks having diameter of 2.5 cm were placed on the top of the moist filter papers that were laid inside the petri dishes.

Samples of 50 *Striga* seeds were placed on discs in Petri dishon filter paper and 50 µl (3ml of 0.1ppm) of synthetic stimulant GR-24 (StrigoLab, Italy) was added to each disc and 1.5 ml of distilled water was added surrounding filter paper ring and sealed with aluminum foil. Seeds were incubated at 28°C for 72 hours. The seed viability was determined by adding 5 ml containing 3000 ppm of triphenyl tetrazolium chloride. The petri-dishes were incubated as stated above for eight days. The seed samples were observed under stereomicroscope (SZ series, China) magnification (40X) fitted with digital camera (AmScope FMA050, China). Therefore, three categories of seeds (viable, germinated and decayed) were scored and finally, the percentage of germinated, viable, and decayed seed was determined from these laboratory experiments according to Van-Mourik *et al.* (2016). Based on the outcome, the most ten *Striga* seeds decaying soil samples were screened and used for major fungal isolates associated with *Striga* seeds decay.

$$Striga\ seed\ decayed\ percentage = \frac{number\ \ of\ decayed\ striga\ seed}{total\ seeds} x100$$

$$Striga\ \ seed\ germination\ percentage = \frac{number\ of\ \ germinated\ \ striga\ seed}{Total\ seeds} x100$$

$$Striga\ seed\ viability\ percentage = \frac{number\ of\ viable\ \ striga\ seed}{total\ seeds} x100$$

3.5. Isolation of major fungi associated with *Striga* seeds decay

Culturable fungi capable of decaying *Striga* seeds were isolated following the procedure of seed trap method (Ahonsi *et al.,* 2002). Based on the bioassay results, isolation was carried out from ten soil samples showing higher seed decay. After the *Striga* seeds were exhumed, the highest decayed seeds were picked and surface-disinfected using 70% ethanol for one minutes and for three minutes in aqueous 1% NaOCl, finally thoroughly cleaned with distilled water and (10-20 seeds per plate) transferred onto Potato dextrose agar (Sisco Research Laboratories Pvt. Ltd.26, Navketan Ind., M. C. Rd., Andheri (E), Mumbai 93, India) (PDA:-20 g/L dextrose, 15 g/L agar, and 4 g/L potato starch), supplemented with 50 ppm streptomycin with three replication and incubated at 25°C until visible colonies were obtained.

Colonies with differentiable morphologies from all petri dishes were picked using needle individually and transfered to Potato dextrose agar medium and incubated at the same temperature for further purification. The pure isolates were coded preserved in normal refrigerator for immediate use and at -80°C in 50% glycerol for long term preservation.

3.6. Identification and characterization of fungi capable of depleting *Striga* seedbank

The fungal isolates were identified on the basis of macroscopic analysis on different media: MEA Maltose (12.75 g/L), glycerol (2.75 g/L), peptone (2.35 g/L) and agar (15 g/L) PDA and CDA (Czapex dox agar):- sodiaum nitrate 2gm, potassium chloride 0.5gm, magnesium glycerophosphate 0.5gm, ferrous sulfate 0.01, potassium sulphate 0.35, sucrose 30gm and agar 12gm, Sisco Research Laboratories Pvt. Ltd 26, Navketan Ind., M. C. Rd., Andheri (E), Mumbai 93, India.). The macroscopic variables analyzed included obverse and reverse colony color according to illustrated manual of Singh, (1991). Microscopic characteristics of the fungal isolates were examined using slide culture techniques. Aseptically, with a pair of forceps, a sheet of sterile filter paper in a petri dish was placed and was followed by placing a sterile U-shaped glass rod on the filter paper. The filter paper was completely moistened by 4ml of sterilized distilled water and the agar block was cut with the help of sterile surgical blade and put on agar

bock (6x6 mm) and was inoculated the side of the agar block with fungal spores to be grown followed by flaming the cover slide and was placed on agar block on Petri dish. Then it was incubated at 25°C for 7-days and the cover slip was carefully removed from the agar block and rinsed with a drop of 95% alcohol as a wetting agent and gently lower the cover slip onto a small drop of lactophenol cotton blue (cotton blue 0.05g/L, phenol crystals 20g/L, glycerol 40ml/L, lactic acid 20ml and distilled water 20ml) and the slide was left overnight to dry and later observed under microscope magnification power of (X40) and the fungal conidia structure, shape and hyphae structure was seen Leck Astrid, (1999). These techniques allowed the fungal cultures to be identified at the genus level.

3.7. Screening of fungal isolates for *Striga* seeds germination inhibition

Standardization of Fungal inoculums

Fungal isolates were grown on PDA medium for 14-days to allow sporulation. The spores were extracted with fifteen milliliters of sterile distilled water, filtered using an 80 µm filter sieve to standardize spore concentration to 10^{6} spore ml^{-1} using hemocytometer. About fifty sterilized *Striga* seeds per disc were carefully spread on discs in separate petri dishes over layered with double Whatman No.1 filter paper and replicated five times in completely randomized design. Ten milliliters of standardized fungal spore (10^{6} spore ml^{-1}) were inoculated to wet the filter papers to cause *Striga* seed decaying according to Kroschel *et al.,* (1996).

The petri dishes were sealed with parafilm and incubated at 25°C in the dark for a month. The *Striga* seeds were then moistened with the concentration of 50 µl (3ml of 0.1ppm) of synthetic stimulant GR-24 in petri dishes and incubated at 30°C in the dark for 48-hours. The petri plates were observed under a magnification power of (40X) stereomicroscope fitted with AmScope FMA050 camera and numbers of non *Striga* seed germinated were counted. The *Striga* seed germination inhibition was determined according to Neondo, (2017) with little modification. Based on the result, the top ten isolates were selected for further evaluation.

$\text{GI}\,\% = \frac{n}{N}\text{x}100$, Where GI = percentages of *Striga* seed germination inhibition, n= number of *Striga* seed germination inhibited and N represent total number of *Striga* seed tested.

3.8. Physiological and biochemical based characterization

3.8.1. Effects of different liquid media on mycelium dry weight of fungal isolates

Mycelium dry weights of fungal isolates were studied on three cultures, *i.e.* Potato dextrose broth (PDB), Czapek dox broth (CDB), Malt extract broth (MEB). The 100 ml of culture media was prepared in 250 ml conical flasks and final pH was adjusted to 6.5. They were later inoculated aseptically with 4 mm actively grown culture disc of fungal isolates (7-day old culture). The flasks were incubated at 25°C in the incubator in triplicates. The mycelium dry weight was recorded after 14-days using formula given by (Arey, 2010).

Weight of mycelium = (Weight of filter paper + Weight of Mycelium) – (Weight of filter paper)

3.8.2. Effect of hydrogen ion concentration (pH) on mycelial dry weight of fungi isolates

By using a sterile cork borer, mycelial discs measuring 4 mm taken from the margin of 7 days old pure culture on PDA were cut and used for inoculation onto 250 ml flasks containing 100 ml potato dextrose broth (PDB). The pH of PDB was varied in 0.5 units from 4 to 8.0 using 1N of HCl and 1N NaOH following the procedure of Tesfaye Alemu *et al*. (2014). Inoculated media were incubated at 25°C, with each treatment replicated three times. After 14 days, the mycelial mats were separated and harvested from the filtrate using Whatman No.1 filter paper and washed thoroughly with sterilized distilled water. Mycelial mats were oven dried at 65°C for 48 hours. The actual weight of dry fungal mycelium was then calculated using the formula given by Arey, (2010).

3.8.3. Effect of temperature on mycelial dry weight of different fungal isolates

Mycelial disks of each isolate (4-mm in diameter), was taken from the edge of 7-days old colony grown on PDA and inoculated into 250 ml flasks containing 100 ml Potato dextrose broth (PDB). The plates were incubated at six different temperatures values (15, 20, 25, 30, 35 and 40°C) for 14-days. Each treatment was replicated three times. Mycelium dry weights of each isolates were measured as described in section 3.8.2.

3.9. Evaluation of *Striga* seeds germination inhibition using fungi isolates in the presence of host crop

The effects of fungal isolates on *Striga* seeds germination inhibition were evaluated in the presence of sorghum root exudates under laboratory condition according to (Yonli *et al.,* 2010). *Striga* seeds were surface sterilized as before and washed five times with distilled water to remove the NaOCl. About fifty seeds were transferred to filter paper discs (2.5-cm diameter) laid down on Petri dishas over layered by filter paper. They were inoculated with fungal spore suspension (10^6 spores' ml^{-1}), sealed using parafilm. The petri dishes inoculated with only distilled sterile water without fungal spores were used as a negative control. All the treatments were done in five replicates incubated in the dark at 30^oC for preconditioning for 14-days.

In order to see the tripartite relationship (*Striga*, fungi and sorghum), seeds of susceptible sorghum *Teshale* variety were surface sterilized as before and rinsed more than four times with distilled water and allowed to germinate at 28^oC for 3-days. Single germinated sorghum seed was then transferred into new petri dishes filled with agar media together with fifty; 14-days preconditioned *Striga* seeds inoculated with the fungal spores and incubated at 25^oC for three days. The experiment was repeated at one week interval for 30 days by taking preconditioned and fungal inoculated *Striga* seeds from paper discs. The number of germinated and non-germinated *Striga* seeds were counted using stereomicroscope and computed in percentages as before.

3.10. Evaluation of fungi isolates on *Striga* seed mortality using tetrazolium test

Surface sterilized *Striga* seeds were dressed with fungal spore suspension (10^6 spores per ml) in Petri dish over layered by double whatsman filter paper No.1 on three discs and incubated for a month. This trial was replicated five times by completely randomized design. A single disc contained about fifty *Striga* seeds was picked and transferred to new Petri dishfor tetrazolium staining after two, three and four weeks of incubation according to Yonli *et al.,* (2010). *Striga* seeds viability and mortality was determined by adding 5 ml of 3000 ppm of triphenyl

tetrazolium chloride and incubated at 30^oC for eight days. After this, the mixtures were poured into a funnel lined with a 9 cm filter paper and was allowed to drain.

The filter papers were placed in a clean Petri dishand washed with 1% of commercial bleach solution for 3 minutes to barely cover the seed to lighten the seed coat to allow the red stained endosperm beneath to be seen and were examined under stereomicroscope at magnification power of (40X), looking for the red-stained endosperm indicative of viable seed. Then, the brick red seeds were counted as viable seeds, as non viable and mortal seeds remain light brown (Berner *et al.,* 1997). Moreover, slightly stained seeds were considered non-viable. Hence, the the *Striga* seeds mortality rate was determined from this laboratory experiments according to the formula stated by Van-Mourik *et al.* (2016).

Pathogenecity test

The isolates which inhibited *Striga* seeds germination in Petri-dish assays were tested for their pathogenicity to sorghum crop in green house. The sorghum seeds were sterilized as before and germinated on Petri dish having sterilized filter paper. Three days after emergence, seedlings were immersed in the inoculums at the concentration of 310^6 spores per milliliter for 10 minutes. Then the seedlings were transplanted in plastic plastic pots (18-cm diameter, 17.5-cm depth) each filled with 4- kg of agricultural soil collected from pawe 1 that was steam-sterilized for 4 hours and observed weekly for a month for the development of symptoms on the leaf and finally the sorghum root were uprooted, thoroughly washed with distilled water and tested on Potato dextrose agar for the growth of fungi to confirm the pathogenicity to sorghum plant following a little modification of Mohammad, (2010).

4. DATA ANALYSIS

All quantitative raw data collected were subjected to analysis of variance (ANOVA) using the statistical analysis system (SAS) version 9.2 software (Gomez and Gomez, 1984). The tukey test was used to separate the treatment means at 5% level of significance.

5. RESULTS AND DISCUSSION

5.1. Soil physicochemical properties of major *Striga* seed decaying soil

Some selected soil chemical properties of major *Striga* seed decaying soil samples were analyzed and their summaries are presented (Table 3). Soil pH of the different sites were different values ranged from 6.92 to 8.39 that qualify for neutral to saline alkaline (6.7-7.3 neutral and >8.0 strongly alkaline) according to the classification ranges suggested by Tekalign Mamo and Haque, (1991). The highest rate of organic carbon was obtained from E30, whereas the lowest soil organic carbon was obtained by E23, and the ranged between 0.01-0.12 also known as lower organic carbon, 0.12-0.25 classified to medium soil organic carbon and >0.25% classified to higher organic carbon content according to Berhanu Debele, (1980) classification. Soil samples collected from E30 had very high (262 mg kg^{-1}) available phosphorous as the range >59 classified under very high whereas, the lowest available phosphorous was recorded from E25 (with the range of 0-7 classified under very low) according to the ratings for some tropical soils (Bray, 1945) when extracted by the Bray II method.

Table 3: Soil physicochemical properties of the different soil samples collected from different parts of Northern Ethiopia

Sample code	Soil physicochemical properties							
	P.av (mg kg^{-1})	%TN	pH	OC (%)	CEC meq/100gm soil	% clay	%silt	%sand
E03	11.8	0.07	6.97	1.91	28.82	35	25	40
E11	5.17	1.48	7.49	0.86	32.24	16.25	27.5	56.25
E14	11.20	0.1	7.78	0.94	27.62	11.25	32.5	56.25
E16	14.4	0.04	7.33	0.57	27.78	17.5	52.5	30
E19	4.74	0.11	7.93	1.75	22.16	57.5	27.5	15
E22	5.171	0.07	7.11	2.26	10.48	18.75	15	66.25
E23	9.59	0.06	7.52	0.55	27.98	22.5	30	47.5
E25	4.35	0.06	8.39	2.03	33.76	56.25	27.5	16.25
E30	262	0.07	6.92	4.13	69.28	32.5	40	27.5
E38	7.15	0.11	7.44	1.75	23.70	55	22.5	22.5

Note: P.av-avaliable phosphorous, TN- total nitrogen, OC-organic carbon,

5.2. Assessment of *Striga* seedbank depleting soils from teabag experiment

Seeds mortality of *Striga hermonthica* was evaluated under laboratory using seed bag burial technique with three tests on exhumed seeds (Table 4). The teabag experiment showed that soil collected from Tigray region around Tahtay Adiabo (E22), showed the highest (19.7%) *Striga* seeds decaying ability. There was no significant difference ($p>0.05$) with results (15.0, 13.7 and 13.0%) of *Striga* seed depletion obtained from Amhara region around North Shewa: Kewot (E19), Dessie: Wuchale (E16) and north Wolo; Qobo (E23) , respectively. In contrast, the soil samples from North Wollo and Raya Kobo (E46) were low depletion percentage (3.6%). The exhumed *Striga* seed buried in sterilized river sand did not show any seed decay. This indicated that the *Striga* seed decay might be due to microbial activity. The value difference on *Striga* seed decay among the soil samples in the present study might be the existence of various microbes in the sites in terms of their potential, population and diversities.

The seed bag burial method at different incubation period, showed mortality rates of *S. hermonthica* seeds between 80 and 100% in the duration of one rainy season in field soils (Pieterse *et al.,* 1996). These authors noted that microbial activity caused most seed mortality. Van Mourik *et al.* (2003) have also stated that microbial activity is the major cause of *Striga* seed mortality resulted in 86% seed mortality rate of exhumed seeds within a period of 120-days under field condition using seed bag burial technique in Mali.

Table 4: Striga seeds decaying soils from teabag experiment in (%) and their respective soil collection site

Soil Sample	**Region**	**Zone**	**District :Village**	**Altitude (masl)**	**Germination (%)**	**Decayed (%)**	**Viable (%)**
E03	Amhara	Oromia	Jile-Tumuga: Karakore	1664	61.0^{bc}	10.0^{bcdefg}	29.0^{abcde}
E11	Tigray	Tembein	Abergele: Taytay-Hagum	1478	62.0^{bc}	10.7^{bcdef}	27.3^{abcdef}
E14	Tigray	West Tigray	Raya Azebo: Alamata	1468	67.7^{bc}	12.0^{bcde}	20.3^{cdef}
E16	Amhara	Dessie	Wuchale: Chisabalima	1617	67.0^{bc}	13.7^{abc}	19.3^{def}
E19	Amhara	North Shoa	Kewot: Abayatir	1373	64.3^{bc}	15.0^{ab}	20.7^{cdef}
E22	Tigray	West Tigray	Tahtay-Adiabo: Gemhalo	1040	58.0^{bc}	19.7^{a}	22.3^{bcdef}
E23	Amhara	North Wolo	Kobo	1503	59.3^{bc}	14.7^{ab}	26.0^{abcdef}
E25	Tigray	West Tigray	Tahtay-Adiabo: Simret	1079	64.6^{bc}	8.7^{bcdefg}	26.7^{abcdef}
E30	Amhara	Oromia	Artuma-Fursi: Hulatukuye	1460	62.7^{bc}	$13.^{abcd}$	24.3^{bcdef}
E38	Amhara	Oromia	Jile-Tumuga: Merewa	1421	61.3^{bc}	10.0^{bcdefg}	28.7^{abcde}
E26	Tigray	West Tigray	Haftay Humera:	619	63.3^{bc}	4.0^{fgh}	32.7^{abcde}
E35	Tigray	West Tigray	Haftay-Humera:Maykedira	642	59.7^{bc}	4.0^{fgh}	36.3^{abc}
E34	Tigray	West Tigray	Haftay-Humera:Maykedira	621	62.7^{bc}	3.7^{gh}	33.6^{abcde}
E46	Amhara	North Wolo	Raya-Azebo:Kebele 07	1425	54.7^{bc}	3.6^{gh}	41.7^{a}
Cont'l					88.7^{a}	0.0^{h}	11.3^{f}
X					68.3	5.5	26.2
CV					15.50	45.77	27.75
$LSD_{(p<0.05)}$					61.0	6.77	16.54

Groups that share the same superscript are not statistically significant (p>0.05) within the column, G- *Striga* seed germinated, D- *Striga* seed decayed, V- *Striga* seed viable, X-overall mean, CV- coeffecient of variation, LSD- least significant difference.

5.3. Isolation and characterization of fungal isolates decaying *Striga* seeds

From 46 soil samples 10 soil samples (E03, E11, E14, E16, E19, E22, E23, E25, E30, and E38) were selected based on the percentage of *Striga* seed decay and used for isolation of fungi suppressing *Striga* seed germination (Table 4). Hence, a total of 43 fungal isolates were characterized based on their cultural and microscopic characteristics (Table 5).

The cultural characteristics of the fungal isolates showed variation amongst one another (Table 5). Thus, the fungal isolates have different obverse and reverse colony colors and microscopically have different conidia shape (Figure 3). For instance, fungi isolate F-143 exhibit cottony pink (obverse) and pale pink (reverse) of colony color on Potato dextrose agar where as on Malt extract agar it was showed white fussy (obverse) and yellow color from the reverse colony (Table 5). Moreover, in this isolate, the colony color was whitish brown and pale yellow of the reverse and obverse on Czapex dox agar, respectively.

In general, almost all fungal isolates have different obverse and reverse colony colour on different media and have different morphological characteristics (Table 5). This finding is in line with the report of Gupta et al., (2010) who have found that that, single *Fusarium* species have different obverse and reverse colony colors on different media; i.e., *Fusarium oxysporum* F10 exhibit orange –cottony white of obverse colony color while the reverse were produce light brown on PDA. This isolate produces different color (cottony white of obverse and no color the reverse) on CDA. Finally they concluded that the variable colony character was designated as ideal colony character and metabolite colour ranged from yellowish to no colour for both *F. oxysporum* f. sp. *psidii* and *F. solani*.

An ideal colony growth pattern (*i.e.* cottony white) of *F. oxysporum* f. sp. *psidii* isolates was recorded when they were grown on CDA while. *F. solani* isolates when grown on CRBA media showed a cottony pinkish type growth. Both *F. oxysporum* f. sp. *psidii* and *F. solani* isolates produces no metabolite colour when grown on CMA media.

The works of Singh ., *et al* (2017) have also showed that the colour of the fungal mycelium of *Fusarium solani* EGY1 varied from cottony white on Czapek's dox agar, pale yellow on Richard's agar and creamy white on potato dextrose agar.

Table 5: Cultural and microscopic characteristics of the *Striga* decaying fungi on different media

Isolates code	MEA		CDA		PDA				Genus
	Colony color		Colony color		Colony color		Microscopic structures		
	Obverse	Reverse	Obverse	Reverse	Obverse	Reverse	Conidia	Hyphae structure	
F-032	Whitish/cottony	Pale yellow	Whitish	Yellow	Whitish pale violet	Pink	Falcate	Aseptate	Fusarium
F-040	Pale white	Pale yellow	Wooly	Pale yellow	Whitish pink	Dark pink	Falcate	Septate	Fusarium
F-143	Whitish fuzzy	Yellow	Whitish brown	Pale yellow	Cottony pink	Pale pink	Oval	Septate	Fusarium
F-191	Pale purple	Dark grey	Whitish pink	Pale yellow	White to pink	Pale-orange	Falcate	Aseptate	Fusarium
F-224	Whitish purple	Pale dark	Floccose	Pale yellow	Whitish to pink	Pale black	Falcate	Aseptate	Fusarium
F-252	Whitish purple	Pale purple	Whitish pink	whitish Pale yellow	Whitish pink	Dark pink	Oval	Septate	Fusarium
F-254	Pale purple	Dark brown	Whitish pale pink	Pale white	Whitish to pale violet	Pale yellow	Falcate	Aseptate	Fusarium
F-301	Pale purple	Dark pink	Pale purple	Whitish grey	Pale pink	Dark pink	Oval	Aseptate	Fusarium
F-304	Wooly pale pink	Dark purple	Whitish to pale pink	Pale Purple	Wooly pale pink	Dark purple	Fusiform	Septate	Fusarium
F-381	Powdery black	Yellow	Powdery black	Pale yellow	Powdery black	Pale yellow	Ampulliform	Septate	Aspergillus

MEA-malt extract agar, CDA- czapex dox agar, PDA-potato dextrose agar

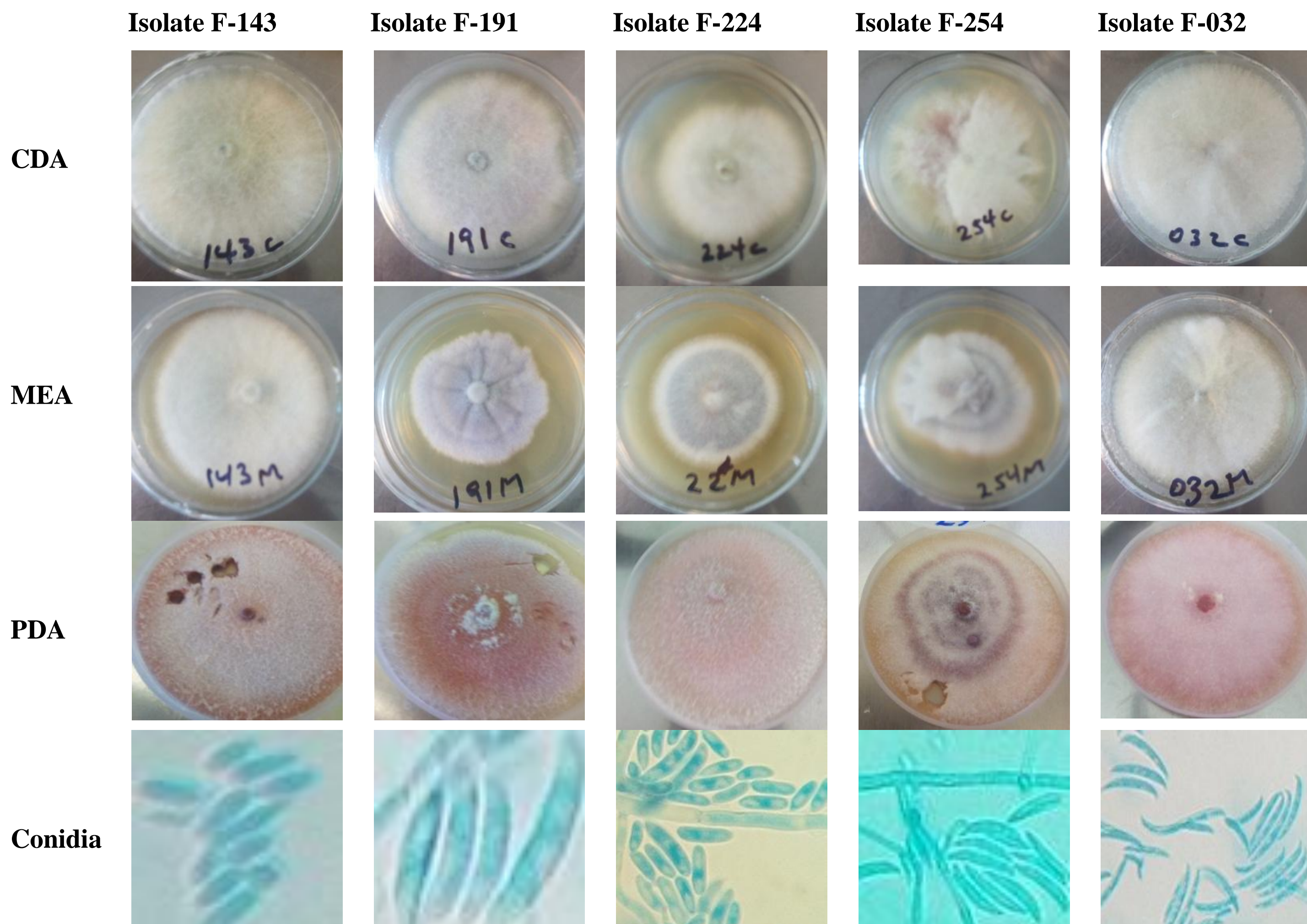


Figure 3: Different fungal isolates on different media and conidia structures

The current findings were also well supported by Nath., (2011) who have stated that *Fusarium oxysporum* f. sp. *ciceri of* different isolates exhibited variations in colony colors were purplish white, whitish orange, creamy white, cottony white on PDA meda. The growth and secondary metabolites of the fungal strains (*Aspergillus* fungi and *Penicillium* species) were greatly affected by the growth medium. The colour of the culture and secondary metabolites were noticeably altered and changed according to the growth medium used (Zain et al., 2009).

Based upon their cultural characteristics, the fungi were identified to the genus level and represented by four genera; 26 (60.5%) isolates belonged to the genus *Fusarium,* 11 (32.6%) belonged to *Aspergillus*, 5 (11.6%) isolates were identified as the genus *Penicillium* and one isolate (2.3%) assigned to genus *Alternaria* (Table 6).

Table 6: Different isolates with respective collection site and at genus identification

S.N	Isolate code	Taxa	Site of collection	S.N	Isolate code	Taxa	Site of collection
1	F-031	*Fusarium* sp.	Kemissie: Jile Tumuga	23	F-258	*Fusarium* sp.	West Tigray :Tahtay Adiabo
2	F-032	*Fusarium* sp.	Kemissie: Jile Tumuga	24	F-301	*Fusarium* sp.	Arxumafulsi : Hula Tukuye
3	F-033	*Fusarium* sp.	West Tigray :Tahtay Adiabo	25	F-302	*Fusarium* sp.	Arxumafulsi : Hula Tukuye
4	F-035	*Fusarium* sp.	West Tigray : Tahtay Adiabo	26	F-304	*Fusarium* sp.	Arxumafulsi : Hula Tukuye
5	F-036	*Fusarium* sp.	West Tigray : Tahtay Adiabo	27	F-037	*Aspergillus* sp.	Kemissie:Jile Tumuga
6	F-038	*Fusarium* sp.	Arxumafulsi : Hula Tukuye	28	F-041	*Aspergillus* sp.	Kemissie: Jile Tumuga
7	F-039	*Fusarium* sp.	Arxumafulsi : Hula Tukuye	29	F-112	*Aspergillus* sp.	Tembein : Abergele
8	F-040	*Fusarium* sp.	Arxumafulsi : Hula Tukuye	30	F-113	*Aspergillus* sp.	Tembein : Abergele
9	F-111	*Fusarium* sp.	Tembein : Abergelle	31	F-163	*Aspergillus* sp.	Dessie : Wuchale
10	F-143	*Fusarium* sp.	Raya Azebo : Alamaxa	32	F-165	*Aspergillus* sp.	Dessie : Wuchale
11	F-144	*Fusarium* sp.	Raya Azebo : Alamaxa	33	F-168	*Aspergillus* sp.	Dessie : Wuchale
12	F-162	*Fusarium* sp.	Dessie : Wuchale	34	F-194	*Aspergillus* sp.	North Shoa: Kewot
13	F-164	*Fusarium* sp.	Dessie : Wuchale	35	F-253	*Aspergillus* sp.	West Tigray :Tahtay Adiabo
14	F-166	*Fusarium* sp.	Dessie : Wuchale	36	F-259	*Aspergillus* sp	West Tigray :Tahtay Adiabo
15	F-167	*Fusarium* sp.	Dessie : Wuchale	37	F-381	*Aspergillus* sp.	Kemissie:JileTumuga/merwa
16	F-191	*Fusarium* sp.	North Shoa: Kewot	38	F-141	*Penicillium* sp.	Raya Azebo :Alamaxa
17	F-193	*Fusarium* sp.	North Shoa: Kewot	39	F-142	*Penicillium* sp.	Raya Azebo :Alamaxa
18	F-222	*Fusarium* sp.	WestTigray : Tahtay Adiabo	40	F-195	*Penicillium* sp.	North Shoa: Kewot
19	F-224	*Fusarium* sp.	WestTigray : Tahtay Adiabo	41	F-196	*Penicillium* sp.	North Shoa: Kewot
20	F-231	*Fusarium* sp.	North Wolo : Qobo	42	F-223	*Penicillium* sp.	West Tigray : Tahtay Adiabo
21	F-252	*Fusarium* sp.	West Tigray :Tahtay Adiabo	43	F-221	*Alternaria* sp.	West Tigray : Tahtay Adiabo
22	F-254	*Fusarium* sp.	West Tigray :Tahtay Adiabo	23	F-258	*Fusarium* sp.	West Tigray :Tahtay Adiabo

According to the finding of Abbasher *et al.* (1995), *Fusarium* species were isolated as *Striga* pathogens over 90% of the infected samples in northern Ghana. This spp. are long-lived soil inhabitants that can survive extended periods in the absence of their host by colonizing crop debris and producing chlamydospores, dormant resting propagules (Nelson, 1981). Other researcher also stated that *Fusarium* spp. are the dominant fungi that are able to control *Striga* in soil, indicating their ability to compete with other soil borne pathogens (Abbasher and Sauerborn, 1992). Next to *Fusarium* spp., *Aspergillus* is the second most dominant genus from the screened soil samples. Thus, Nekouam and Marley, (2003) have stated that *Aspergillus* species were the dominant pathogen attacking *Striga* seed and they are the most prevalent fungal genus in the rhizosphere of *Striga* and sorghum.

The abundance of fungal species also varied among the sampling sites (Table 7). Thus, Oromia: Artuma Fursi (E30) site showed the maximum colonization frequency of fungal species is (18.6%) per total fungal isolates followed by (16.3%) recorded from West Tigray around Taytay Adiabo (E22). But, the lowest abundance of (2.32%) was obtained from two sites; north Wolo around Kobo (E213) and Oromia around Jile Tumuga (E38) (Table 7). This variation in spatial distribution might be due to abiotic and biotic environmental variation (Deng *et al.,* 2016). Similarly, Leblanc *et al.* (2017) have reported that soil physicochemical characteristics influence the composition and richness of *Fusarium* communities in the soil. In the same manner, Tancic *et al.* (2017) have also stated that soil texture, organic matter content, pH and cation exchange capacity (CEC) can all significantly affect the diversity and abundance of soil fungi.

Fungi species diversity is dependent on the nature of substrate and temporal region that favors the colonization, growth and substrate possession of the fungi (Rani *et al.* 2010). The composition of the micro-biome in *Striga* field is dynamic and controlled by multiple factors. In the case of the Striga rhizosphere, temperature, pH, and the presence of chemical signals from fungi, plants, and nematodes all shape the environment and influence which organisms will flourish (Lakshmanan *et al.* 2014). Abiotic factors, such as temperature, soil pH, seasonal variation, and the presence of rhizospheric deposits, act as chemical signals for microbes and influence the microbiome community structure and function (Berg and Smalla, 2009). Total soil organic carbons, total nitrogen, are the main contributors to the variation of microbial community composition (Liu *et al.* 2019).

Table 7: Diversity of Striga decaying fungi isolated from different sampling sites of Ethiopia

S.C	Sampling collection site	Different Fungi genera				*Total isolate*	*CF (%)*
		Fusarium	*Aspergillus*	*Penicillium*	*Alternaria*		
E03	Kemissie: Jile Timuga	2	2	0	0	4	9.3
E11	Tembein: Abergelle	1	2	0	0	3	6.97
E14	Tigray:Raya Azebo	2	0	2	0	4	9.3
E16	Dessie:Wuchale	4	1	0	0	5	11.6
E19	North Shoa; Kewot	2	1	2	0	5	11.6
E22	Tigray :Taytay Adiabo	5	0	1	1	7	16.3
E23	North Wolo: Kobo	1	0	0	0	1	2.3
E25	Taytay Adiabo:Simret	3	2	0	0	5	11.6
E30	Oromia :Artuma Fursi	6	2	0	0	8	18.6
E38	Oromia:Jile Timuga	0	1	0	0	1	2.32
Tot.		26	11	5	1	43	
FD (%)		60.5	25.6	11.6	2.3		

FD- Frequency dominance, CF- Colonization frequency

5.4. Screening of *Striga* seedbank depleting fungi in absence of host plant

A total of 43 fungal isolates were screened for their ability to inhibit *S.hermonthica* seed germination (Table 8; Annex 7).The result showed that the fungal isolates had different seed germination inhibition ability ranging from 6.8 percent to 91.84 percent of which 20 isolates decayed *Striga* seeds by more than 50%. The highest *Striga* seed germination inhibition was recorded from isolate F-254 (91.84%) followed by 89.92% from isolate (F-224). The lowest seed germination inhibition was 6.8% recorded from the treatment with isolate (F-195).

The germination inhibition percentage of *Striga* seed difference among fungal isolates tested might be due to the genetic potential of individual isolates. Similar observation indicated that *Fusarium* isolates differed in pathogenecity to *Striga* seeds and the highest *Striga* seed germination inhibition (91%) was observed with *Fusarium* isolate 12-Sh-Mai collected in Mali (Abbasher *et al.,* 1998), whereas isolate 9-1-BH from Burkina Faso produced the highest (71%)

Striga germination inhibition rate (Abbasher *et al.,* 1998). Sauerborn *et al.,* (1996b) have reported that the mechanism of inhibition in the laboratory with the soil-borne pathogenic fungus *Fusarium nyagamai* using electron microscopic analysis showed that the mycelium penetrated the seed coat of intact seeds and destroyed the endosperm resulting in germination inhibition.

Table 8: Screening of fungal isolates in the presence of synthetic stimulant GR-24 for their ability to inhibit Striga seeds germination using paper disc method

Isolate code	Taxonomic group	Average number of *Striga* seed germination inhibition/50seed Disc 1	Disc 2	Disc 3	Disc 4	Disc 5	Total Seed	Average number of Striga seed germination inhibited	Striga Seed germination inhibited (%)
F254	*Fusarium* sp.	46.6	47.6	43.4	46.4	45.6	250	229.6	91.8^A^
F224	*Fusarium* sp.	45.8	44.6	44	45	45.4	250	224.8	89.9^AB^
F304	*Fusarium* sp.	45.8	44.2	44.2	45	44.2	250	223.4	89.3^AB^
F191	*Fusarium* sp.	43.8	42.8	42.6	43.8	43.4	250	216.4	86.5^BC^
F381	*Aspergillus* sp.	42.2	44.2	41.8	44.4	42.8	250	215.4	86.1^BCD^
F032	*Fusarium* sp.	44.2	42.8	42.2	41.8	42.6	250	213.6	85.4^BCDE^
F301	*Fusarium* sp.	42.4	41.4	41.4	41	41	250	207.2	82.8^CDEF^
F143	*Fusarium* sp.	42.8	40.4	40.6	40.6	40	250	204.4	81.7^DEFG^
F040	*Fusarium* sp.	38.8	41.6	40.8	40	42.8	250	204	81.6^EFG^
F252	*Fusarium* sp.	41	40.4	39.4	39.6	38.8	250	199.2	79.6^FGH^
F193	*Fusarium* sp.	11.4	10.6	9.6	10.6	10.2	250	52.4	20.^9de^
F162	*Fusarium* sp.	6.8	9.2	8.2	9.6	11	250	44.8	17.9^e^
F195	*Penicillium* sp.	2.8	2	3	3.6	5.6	250	17	6.8^f^
CV									3.6
LSD									4.5

Groups that share the same superscript are not statistically significant at P>0.05. CV: coefficient of variation, LSD: Least significant differences

5.5. Physiological and biochemical studies

5.5.1. Effect of different liquid media on mycelium dry weight of different fungi isolates

In the present investigation, three different types of culture media affected the mycelial growth rate of different fungi isolates (Table 9). The results showed that there was significant different ($p<0.05$) among fungi isolates. All fungal isolates showed different growth on different media. The maximum mycelium dry weight of (689.7±17.47 mg) and (669.7±17.61 mg) was attained by isolate F-191 which was significantly ($p<0.05$) superior to all isolates using MEB and PDB medium, respectively. However, the least mycelium dry weight (322.0±15.27 g/100ml liquid medium) was recorded by isolate F-252 from PDB and 442.3±19.73 of mycelium dry weight on MEB from isolate F-224. Among the isolate tested on CDB, the maximum mycelium dry weight (573.3±41.04 mg) was recorded by isolate F-304, which were no significant ($p>0.05$) difference with isolate (F-381), (F-032) and (F-040). In contrast, the least mycelium dry weight 411.0±23.81 was recorded from CDB with isolate F-224. Overall, isolate F-191 was showed maximum gowth on PDB and MEB, when compared to other isolates, whereas, isolate F-304 was showed best growth on CDB.

The present finding is in agreement with the result of Kumawat *et al.,* (2016) who have reported that, among the various liquid medium, MEB (1007 mg) was the most suitable liquid culture medium incubated for 14-days for the growth of *Chrysosporium queenslandicum,* and moderate mycelial growth was observed in the CDB. The higher preference for one nutrient over other nutrient for each isolates might be due to preferable microelement contents which are important for good growth of fungi species. This possibly indicates that the pathogen has preference for certain nutritional components, which could be related to specificity for host. Similarly, (Chaudhary *et al.,* 2018) have showed the maximum mycelium dry weight (348.3mg) of *Fusarium udum* on Potato dextrose broth was higher when compared to Czapex dox broth medium for 21-days of incubation.

Table 9: Effects of different liquid media on mycellium dry weight of different fungal isolates

		Mycellium dry weight/100ml of Liquid media		
Isolates	**Taxonomic group**	**PDB**	**MEB**	**CDB**
F-032	*Fusarium* sp.	632.0^{bc}±19.51	557.0^{bc}±30.19	528.0^{a}±48.75
F-040	*Fusarium* sp.	643.0^{ab}±19.51	559.7^{bc}±15.14	534.7^{a}±22.94
F-143	*Fusarium* sp.	604.0^{cd}±17.52	571.7^{bc}±12.22	425.0^{c}±27.22
F-191	*Fusarium* sp.	669.7^{a}±17.61	689.7^{a}±17.47	474.0^{b}±13.74
F-224	*Fusarium* sp.	554.3^{e}±21.12	442.3^{e}±19.73	411.0^{c}±23.81
F-252	*Fusarium* sp.	372.0^{f}±17.52	450.3^{e}±22.74	412.7^{c}±16.25
F-254	*Fusarium* sp.	610.7^{cd}±11.06	522.3^{cd}±18.03	405.7^{c}±13.86
F-301	*Fusarium* sp.	403.3^{f}±18.61	606.3^{b}±64.78	418.7^{c}±35.00
F-304	*Fusarium* sp.	322.7^{g}±15.27	489.0^{de}±53.81	573.3^{a}±41.04
F-381	*Aspergillus* sp.	597.0^{d}±24.02	541.7^{cd}±25.16	533.0^{a}±16.52
X ± SD		**540.86±18.47**	**543±32.48**	**471.6±28.35**
CV		**3.4**	**5.9**	**6.0**
$LSD_{(p=0.05)}$		**31.5**	**55.3**	**48.29**

Groups that share the same superscript are not statistically significant (p>0.05) within the column. X- Total mean, CV-coefficient of variance, LSD- least significant difference, PDB-potato dextrose broth, MEB- malt extract broth, CDB- czapex dox broth.

5.5.2. Effect of hydrogen ion concentration (pH) on the mycelial growth of fungi isolates

The results of the present investigation showed that maximum mean mycelial dry weight of all fungi isolates (623.6±11.0 mg) was recorded at pH 6.0 which was significantly (P<0.05) superior to growth at other pH levels (P<0.05) (Table 10). Mycelial growth of all the isolates increased consistently from pH 4.0 to pH 6.0 and then started declining from pH 7.0 to pH 8.0; the least mean mycelium dry weight was observed at pH 8.0 (115.3±10.6 mg).

The maximum mycelial dry weight at pH 6.0 was recorded by isolate (F-252) with (774.7±7.09 mg) of mycelium dry weight which was significantly (P<0.05) different from each other, whereas the least mycelium dry weight (506.0±18.52 mg) was obtained from isolate (F-224). In case of pH 6.5, the maximum and minimum mycelium dry weight was obtained by fungi isolate of (F-032) and (F-252) with (709.7±15.01) and (444.7±12.66 mg) respectively. Overall, isolates

(F-254) and (F-224) showed the maximum and minimum mycelial dry weight respectively. Therefore, pH 6.0 and 6.5 is favorable for fungi isolates obtained in this study.

Singh and Chauhan, (2013) studied the effect of pH on the growth of *Aspergillus flavus* and *Penicillium chrysogenum* in vitro and the maximum growth of *A. flavus* was observed at pH 6.0 with (506 mg) whereas pH 7.0 with mycelium dry weight (356 mg) was most suitable for the growth of *P. chrysogenum*. Likewise, Abubakar et al., (2013) reported the highest mycelial dried weight (355.67mg) was at pH 4.0 followed by 353.3mg at pH 7.0 of *Aspergillus parasiticus*.The variation among the findings might be due to the difference in the sensitivity of the different isolates to hydrogen concentration.

Table 10: Effect of hydrogen ion concentration (pH) on the mycelial growth of Fungi isolates on Potato destrose broth medium

Isolates	Mycelium dry weight in (mg)/100ml of PDB media								
	pH4	pH 4.5	pH 5	pH 5.5	pH 6	pH 6.5	pH 7	pH 7.5	pH 8
F-032	227.0^{bcd}±2.64	332.7^{a}±48.41	429.3^{abc}±16.65	479.7^{d}±14.04	582.3^{e}±14.29	709.7^{a}±15.01	246.7^{b}±16.44	149.0^{ab}±12.53	118.0^{ab}±7.55
F-040	240.7^{bc}±8.62	298.7^{abc}±15.01	410.7^{cd}±6.11	483.7^{d}±8.32	617.3^{d}±10.59	682.3^{b}±10.26	298.7^{a}±10.97	156.3^{ab}±12.22	129.3^{a}±15.01
F-143	247.7^{b}±17.00	271.3^{bc}±17.50	371.7^{de}±54.50	603.7^{a}±17.24	743.3^{b}±10.69	523.3^{f}±11.06	197.3^{d}±9.60	144.0^{abc}±8.18	124.3^{a}±6.02
F-191	244.3^{b}±13.86	268.7^{bc}±15.63	442.7^{abc}±22.74	539.0^{c}±16.09	612.7^{d}±6.02	510.3^{f}±6.02	219.7^{c}±5.03	135.0^{bcd}±11	125.7^{a}±16.26
F-224	256.0^{b}±9.16	322.0^{ab}±20.51	417.0^{bc}±9.53	440.3^{e}±14.29	506.0^{g}±18.52	583.0^{d}±11.14	198.3^{d}±15.5	123.7^{cd}±13.01	111.3^{abc}±4.04
F-252	209.7^{d}±14.01	247.3^{c}±10.06	314.7^{f}±10.06	583.0^{ab}±14.10	774.7^{a}±7.09	444.7^{g}±12.66	150.0^{e}±7.55	124.7^{cd}±10.69	100.7^{bc}±3.21
F-254	300.0^{a}±46.36	320.0^{ab}±75.02	349.3^{ef}±20.13	558.3^{bc}±23.86	687.0^{c}±6.24	423.3^{h}±12.86	216.0^{cd}±13.08	162.3^{a}±11.5	121.3^{a}±10.21
F-301	245.0^{b}±10.58	339.0^{a}±21.07	426.7^{abc}±20.52	447.7^{e}±12.22	583.3^{e}±10.59	624.3^{c}±6.50	288.0^{a}±9	148.7^{ab}±26.16	123.0^{a}±9.84
F-304	213.3^{cd}±7.50	292.0^{abc}±11.53	452.7^{ab}±17.24	487.3^{d}±12.42	553.3^{f}±7.76	635.3^{c}±11.5	292.7^{a}±13.2	121.7^{d}±4.16	102.3^{bc}±9.07
F-381	240.3^{bcd}±9.71	310.0^{ab}±10.14	456.3^{a}±14.29	566.0^{b}±11.35	575.7^{e}±12.01	545.7^{e}±16.04	206.3^{cd}±11.02	126.0^{cd}±7	96.7^{c}±15.57
X ± SD	242.4±18.0	300.2±31.5	407.1±23.1	518.9±14.9	623.6±11.0	568.2±11.7	231.4±11.6	139.1±12.9	115.3±10.6
CV	7.4	10.5	5.6	2.8	2.0	2.1	5.0	9.2	9.2
$LSD_{(p=0.05)}$	30.7	53.7	39.3	25.4	18.7	19.9	19.8	21.9	18.1

Groups that share the same superscript are not statistically significant (p>0.05) within the column, X-overall means, SD- standard deviation, CV-coefficient of variation, LSD- least significance difference

5.5.3. Effect of varying temperatures on mycelial growth of fungi isolates

All the isolates showed different responses to the different incubation temperatures (Table 11). There was increase in mycelial growth from 15- 25°C but decreased from 25 to 40°C in all fungi isolates (Table 11). The variation in mycelial dry weight among the isolates of the test fungal isolates at different temperatures was found to be different (Table 11).

Isolate F-252, showed maximum mycelium dry weight (836.0±16.7 mg) at 25°C, which was significantly ($p<0.05$) superior to all other treatments. The isolate (F-254) showed maximum mycelium dry weight (427±11 mg) at 30°C and (212.6±5.50 mg) at 35°C which was also significantly ($p<0.05$) superior over all other treatments except for isolate F-252 recorded statistically almost similar mycelium dry weight (408±11 mg) at 30°C. Although the maximum mycelial dry weight (96.3±8.74 mg) was obtained by isolate F-252 at 40°C, there were no significant ($p>0.05$) corrected different with most isolates tested. However, there were significant ($p<0.05$) difference with two isolates (F-032) and (F-301). Overall, it was depicted that the maximum mycelium dry weight (mean) was when the temperature was at 25°C (625.4±18.42 mg) for all isolates followed by a temperature at 30°C (375.5±11.25) (Table11). However, all most all fungal isolates were showed minimum mycelium dry weight at 40°C.

Singh and Chauhan, (2013) also studied the effect of temperature on the growth of *Aspergillus flavus* and reported that the suitable temperature for the growth of *A. flavus* was 25°C (228 mg). Somesh *et al*. (2019) also reported that optimum temperature range for growth of *Fusarium oxysporum* f.sp. *lini* was 25°C to 30°C and the minimum growth was recorded at 45° C and 10°C on PDA.

The variations among the present finding and the previous research works results for different mycelium dry weight in the different temperature levels of individual isolate seem to reflect inherent biochemical and physiological differences among fungi isolates inhibiting *Striga* seed germination and their sensibility to different temperature. Moreover, it might be the growth of fungi is retarded due to low metabolic rate of the fungus at low temperature whereas at high temperatures the metabolism increases which results in exhaustion of nutrients. The high temperature could also lead to fungal cell rupture, loss of membrane or damage to the intra-cytoplasmic compounds result for minimum mycelium dry weight.

Table 11: Effect of varying temperatures on mycelial growth of fungi isolates on the PDB medium

	Mycelium dry weight in (mg)/100ml of PDB at different temperature levels					
Isolates	**15°C**	**20°C**	**25°C**	**30°C**	**35°C**	**40°C**
F-032	82.0^{b}±6.55	285.7^{a}±8.62	573.0^{e}±7.55	356.0^{cd}±10.81	131.7^{cde}±6.80	66.0^{c}±6.24
F-040	87.3^{ab}±10.01	230.3^{b}±6.11	651.7^{d}±14.29	353.3^{d}±12.66	121.3^{def}±3.05	86.7^{ab}±9.07
F-143	95.3^{ab}±12.53	285.7^{a}±8.50	795.0^{b}±24.63	380.6^{b}±5.50	194.0^{b}±8.88	85.66^{ab}±8.14
F-191	91.0^{ab}±6.55	277.0^{a}±5.56	646.7^{d}±30.66	385.3^{b}±9.07	142.7^{c}±8.02	87.0^{ab}±4.58
F-224	96.7^{ab}±9.07	273.7^{a}±5.03	420.0^{h}±7.21	383.0^{b}±15.09	133.3^{cde}±10.97	84.0^{ab}±70
F-252	84.7^{b}±10.01	285.3^{a}±10.50	836.0^{a}±16.7	408.0^{a}±11	120.7^{ef}±14.98	96.3^{a}±6.66
F-254	86.7^{ab}±6.02	284.7^{a}±9.01	746.7^{c}±19.6	427.0^{a}±11	212.6^{a}±5.50	85.0^{ab}±10.8
F-301	83.7^{b}±10.69	278.3^{a}±3.05	579.7^{e}±12.01	374.7^{bc}±8.50	110.7^{f}±3.51	80.7^{b}±8.74
F-304	87.7^{ab}±9.07	274.7^{a}±8.62	521.3^{f}±16.86	329.0^{e}±8.88	111.0^{f}±8.54	84.3^{ab}±6.11
F-381	101.7^{a}±5.13	235.7^{b}±9.50	483.7^{g}±21.03	358.7^{cd}±15.94	136.3^{cd}±11.24	90.6^{ab}±8.74
X± SD	**89.7±8.86**	**271.1±7.78**	**625.4±18.42**	**375.5±11.25**	**141.4±8.86**	**84.6±7.8**
CV	**9.8**	**2.8**	**2.9**	**3.0**	**6.2**	**9.2**
$LSD_{(p=0.05)}$	**15.1**	**13.26**	**31.38**	**19.16**	**15.09**	13.29

Groups that share the same superscript are not statistically significant. (p>0.05); X-overall mean, SD-standard deviation, CV- coefficient of variation, LSD- least significance difference

5.6. Evaluation of fungal isolates on *Striga* seed germination inhibition in the presence of host crop

The result showed significant (p<0.05) differences in *Striga* seed germination inhibition among the fungal isolates ranged from 23.6±3.84% to 74.4±3.57% upon 15-days of incubation (Table 12). Hence, *Striga* seed germination inhibition at 15-DOI were the highst (74.4±3.57) by isolate F-254 when compared with all fungal isolates. This result was highly significant (p<0.05) difference when compared to all isolates except for isolate F-304 which was almost similar result (68.4±2.60) recorded. However, the lowest striga seed germination (23.6±3.84) was recorded by isolate F-191 at 15 DOI. Moreover, three isolates (F-032, F-040 and F-252) have no significant (p>0.05) difference on *Striga* seed germination inhibition at 15DO.

Upon 21-DOI, the maximum (82.8±3.03) percentage of *Striga* seed germination inhibition was obtained by isolate F-254 which was stistically no significant (p>0.05) difference with isolate

F-304 with 79.2±3.03 of *Striga* seed germination inhibition. But, the lowest (34±4.14%) *Striga* seed germination inhibition was recorded by isolate F-191.

Although there was no significant ($p>0.05$) difference between the two isolates *Fusarium* (F-254 and F-304) at 30-DOI, numerically the highest (92.8±2.28) *Striga* seed germination inhibition was recorded by isolate F-254 and have highly significant ($p<0.05$) different when compared to all fungal isolates. However, the lowest (46.4±5.17) *Striga* seed germination inhibition was also obtained when *Striga* seeds were inoculated with isolate F-191. The variation of *Striga* seed germination inhibition among all the isolates indicates that there might be a difference in their natural potential in terms of pathogenesity to *Striga* seeds.

The mean *Striga* seeed germination inhibition by isolate F-254 (74.4±3.57) at 15-days of incubation was less than the report of Abbasher *et al.,* (1995) who found that two *F. oxysporum* isolates inhibited the *Striga* germination by 88 and 98 percent, respectively in Ghana. Likewise, Abbasher and Sauerborn, (1995) have also reported that from twenty-eight fungi isolates evaluated in Sudan, several of the isolates showed similar pattern of inhibition with the isolates of the present study. The deviation occurred between this study and others might be due to variation in fungal isolates.

Marley *et al.* (1999) have also stated that, among *Fusarium* species isolated from Nigerian Savannah, only *Fusarium oxysporum* PSM-197 was identified as potential candidate for *Striga* control that completely inhibited *Striga* seed germination when grown on sorghum grain. Sauerborn *et al.* (1996a) reported that, fungal parasite *Fusarium nygamai* was able to reduce the germination of *Striga hermonthica* seeds by more than 70 percent.

Idris *et al.,* (2003) reported that, toxic metabolites (fusaric acid, 9, 10-acid dehydrofusaric, 2-ester-methyl) produced in-vitro by *Fusarium nygamai* completely inhibited *Striga* germination. Thomas *et al.* (1996) have that fusaric acid has shown a very effective inhibitor of *Striga* germination even at very low concentrations. Other studies also showed that the virulence factors could be enzymes that degrade cell walls, facilitating the entry of the microorganism and the phytotoxic components and phytotoxins or secondary metabolites that can interfere in the metabolism of the *Striga* seeds (Ghorbani *et al.,* 2005; Harding *et al.,* 2015; Junior *et al.,* 2019).

Table 12: Effects of fungal spores on Striga seed germination inhibition at different incubation periods

Isolate codes	Taxa	*Striga* seed germination inhibition (%) ±SD		
		15 DOI	**21 DOI**	**30 DOI**
F-032	*Fusarium* sp.	46.4^{c}±7.12	66^{cd}±5.09	80.4^{cd}±5.54
F-040	*Fusarium* sp.	42.4^{cd}±6.06	64^{cd}±6.32	77.6^{cd}±3.84
F-143	*Fusarium* sp.	43.2^{cd}±5.93	59.2^{de}±6.41	71.6^{d}±5.72
F-191	*Fusarium* sp.	23.6^{e}±3.84	34.8^{f}±4.14	46.4^{f}±5.17
F-224	*Fusarium* sp.	33.2d^{e}±4.60	50.8^{e}±5.40	61.2^{e}±5.21
F-252	*Fusarium* sp.	46.8^{c}±4.60	70^{bc}±5.09	82.8^{bc}±1.78
F-254	*Fusarium* sp.	74.4 ^{a}±3.57	82.8^{a}±3.03	92.8^{a}±2.28
F-301	*Fusarium* sp.	52.8^{bc}±4.81	70.8^{bc}±4.81	82.8^{bc}±4.81
F-304	*Fusarium* sp.	68.4^{a}±2.60	79.2^{ab}±3.03	90.8^{ab}±3.63
F-381	*Aspergillus* sp.	57.6^{b}±6.69	72^{bc}±4.69	82.8^{bc}±3.03
Control	$_{d}H_{2}0$+*Striga*	0.0^{f}±0	0.0^{g}±0	0.0^{g}±0
Mean ± SD		**44.43±4.93**	**59.05±4.7**	**69.9±4.11**
CV		**11.1**	**7.96**	**5.89**
LSD$_{(P=0.05)}$		**10.6**	**10.1**	**8.8**

Keys: DOI: days of incubation, CV: coefficient of variation, LSD: least significant difference, Groups that share the same superscript are not statistically significant (p >0.05) within column

5.7. Effects of fungal isolates on *Striga* seed mortality

This study showed fungal isolates had considerable variations on the viability of *Striga* seed (Table 13). The average of *Striga* seeds mortality was significantly (p<0.05) affected by different incubation periods and the *Striga* seed mortality was between 16.4±3.84% to 70 ±5.47% in 15 days of incubation whereas; in 30 days it was between 39.6±8.98% to 92±3.16%. However, the highest *Striga* seed mortality (70%±5.47, 86 ±3.16% and 92±3.16%) was obtained when the *Striga* seeds were treated with fungal isolate isolate (F-254) at incubation periods of 15, 21 and 30 days of incubation respectively. Isolate (F-254) showed the highest *Striga* seed mortality in all incubation period, followed by *isolate* (F-381) causing 57.2±8.64%, 78±3.28% and 89.1±2.96% *Striga* seed mortality at days of 15, 21, and 30 days of incubation respectively.

This suggests that there was no statistically significant ($p>0.05$) difference between the two isolates isolate (F-254) and isolate (F-381) on *Striga* seed mortality. All taken together, the highest *Striga* seed mortality was recorded from *Striga* seeds treated with *Fusarium* isolate (F-254), followed by isolate (F-381). A study in Ghana showed that fungal species that belong to *Fusarium* spp. reduced the emergence of *S. hermonthica* by 76 percent, and consequently improved sorghum yield by 26 percent compared to the control without *Striga* (Abbasher *et al.,* 1995).

In Burkina Faso, native *Fusarium* species were investigated to use them to use them as bio-control agents to *S. hermonthica* under controlled environmental conditions. Yonli *et al.,* (2010) have stated that percentage of *Striga* mortality ranged from 17-37% between 14 and 28 days after inoculation with spores of *F. oxysporum* 34-Fo and *F. equiseti* 5-Kou. The variation on seed mortality might be the potential of each isolates difference. On the other hand, the biological inhibitions of *Striga* seed viability were probably due to negative impact of fungal species on the *Striga* seed physiological activity through production of different enzymes.

Table 13: Effects of fungi isolates on Striga seeds mortality at different incubation period in percentage

Isolate code	Tentative identification	*Striga* seed mortality (%) 14-DOI	21-DOI	30-DOI
F-032	*Fusarium* sp.	25.2^{e} ±5.40	40.4^{de} ±8.87	56.4^{cd} ±5.54
F-040	*Fusarium* sp.	20^{e} ±3.16	33.2^{ef} ±6.09	39.6^{f} ±8.98
F-143	*Fusarium* sp.	26^{e} ±3.16	50.4^{d} ±3.84	66.8^{c} ±3.34
F-191	*Fusarium* sp.	18.8^{e} ±4.81	34.4^{ef} ±4.77	55.6^{de} ±7.12
F-224	*Fusarium* sp.	50.8^{bc} ±8.67	72^{bc} ±5.09	88.8^{ab} ±3.63
F-252	*Fusarium* sp.	$28d^{e}$ ±4.47	38.8^{ef} ±5.40	59.6^{cd} ±2.60
F-254	*Fusarium* sp.	70^{a} ±5.47	86^{a} ±3.16	92^{a} ±3.16
F-301	*Fusarium* sp.	16.4^{e} ±3.84	29.6^{f} ±4.33	45.6^{ef} ±6.22
F-304	*Fusarium* sp.	40^{cd} ±10	65.2^{c} ±2.28	80^{b} ±3.16
F-381	*Aspergillus* sp.	57.6^{ab} ±8.64	78.4^{ab} ±3.28	90.4^{ab} ±2.96
Control	$_{d}H_{2}0$+*Striga*	0.0^{f} ±0	0.0^{g} ±0	0.0^{g} ±0
X ± SD		**32.07±5.9**	**48.03 ±4.8**	**61.34 ±4.87**
CV		**18.65**	**10**	**7.9**
LSD		**12.7**	**10.3**	**10.4**

Keys: DOI: days of incubation, Groups that share the same superscript are not statistically significant at (p >0.05) within each column CV: coefficient of variation, LSD: least significant difference, SD: standard deviation, X: overall mean

Pathogenecity test

Interestingly, there was no any disease symptoms observed in all pot experiments. This indicates that the evaluated fungal isolates are host specific to *Striga* and was not attack the sorghum plant.

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

Soil sample collected from Tigray region around Tahtay Adiabo (E22), Abayatir (North Shewa: Kewot) (E19), Kobo (North Wolo) (E23), Chisabalima (Dessie: Wuchale) (E16) showed the highest *Striga* seeds decaying ability. A total of 43 fungi isolates associated with *Striga* seed decay were obtained and belonged four genera (*Fusarium*, *Aspergillus*, *Penicillium* and *Alternaria*) and the isolates from the genus *Fusarium* were the dominant of all the isolates. The physiological and biochemical characteristics showed that the suitable temperature for the cultural growth of the isolates was between 25°C and 30°C, while most grew best at the temperature of 25°C. All the isolates of *Striga* seed pathogenic fungi required pH 6.0 for maximum mycelial dry weight and showed high adaptability and growth range of pH value from 5.5 to 6.5. Based on *Striga* seed germination inhibition and *Striga* seed mortality analysis, it can be also concluded that there were different fungi species which can reduce *Striga* seedbanks and their decaying ability was different from isolates to isolates. Three isolates (F-254, F-304 and F-381) were more effective on *Striga* seed germination inhibition and seed mortality. This implies that the three fungal isolates have a potential as biocontrol for *Striga* weed and soil seedbank depletion.

6.2. Recommendations

Based on the results of this study, the following recommendations are made for future consideration.

- From the total fungi isolated and evaluated fungi, three isolates were best biocontrol of *Striga* and studied under in-vitro condition. Therefore, it is recommended that future work will be expected to test under greenhouse and field condition for authentication and use as *Striga* biocontrol.
- Morphological identification of fungi at species levels difficult. Therefore, phylogenetic analyses are particularly helpful to assign isolates to their respective species.
- This study was limited to specific area where *Striga* weeds were infested and hence need attention to cover large areas for further studies

7. REFERENCES

Abubakar, A., Suberu, H.A., Bello, I.M., Abdulkadir, R., Daudu, O.A. & Lateef, A.A. (2013). Effect of pH on mycelial growth and sporulation of *Aspergillusparasiticus, J. Plant Sc*, **1** (9):64-67.

Abbasher, A. A. & Sauerborn, J. (1995). Pathogens attacking *Striga hermonthica* and their potential as biological control agents. In Proceedings of the Eight International Symposium on Biological Control of Weeds. Lincoln University, Melbourne, Australi (pp. 527-533).

Abbasher, A. A., Hess, D. E. & Sauerborn, J. (1998). Fungal pathogens for biological control of *Striga hermonthica* on sorghum and pearl millet in West Africa. *Afr. Crop Sci.* ***6***(2): 179-188.

Abbasher, A. A. & Sauerborn, J. (1992). *Fusarium nygamai,* a potential bioherbicide for *Striga hermontica* control in sorghum. *Biol. Control*. **2**: 291-296.

Abbasher, A. A. (1994). Microorganisms associated with *Striga hermonthica* and possibilities for their utilization as biological control agents. ***12***(1): (pp 144)

Adugna Asfaw (2012). Population genetics and ecological studies in wild sorghum [*Sorghum bicolor* (L.)] in Ethiopia: implications for germplasm conservation. *Addis Ababa, Ethiopia: Addis Ababa University. OpenURL*. Alive

Aflakpui, G. K. S., Bolfrey-Arku, G. E. K., Anchirinah, V. M., Manu-Aduening, J. A. & Adu-Tutu, K. O. (2008). Incidence and severity of *Striga* spp. in the coastal savanna zone of Ghana. Results and implications of a formal study. Outlook on Agriculture, **37**(3): 219-224.

Ahonsi, M. O., D. K. Berner, A. M., Emechebe. & S. T. Lagoke. (2002). Selection of rhizobacterial strains for suppression of germination of *Striga hermonthica* (Del.) Benth. seeds. *Biol. Control.* **24** (2): 143–152.

Amusan, I. O., Rich, P. J., Menkir, A., Housley, T. & Ejeta, G. (2008). Resistance to *Striga hermonthica* in a maize inbred line derived from Zea diploperennis. *New Phytol.* ***178***(1): 157-166.

Amhara BoANRD (Amhara Bureau of Agriculture and National Resources Development) (2016). Plan for ISC Project Activities in Amhara Region for 2016 (prepared in Amharic). Amhara BoANRD, Bahr Dar, Ethiopia.

Arey, N.C. (2010). Manual of Environmental Analysis. Ane Books Pvt Ltd, New Delhi, India. 424.

Ayana Mirkana (2017). ISC Project annual progress report for 2016 in Oromia region. A paper presented during the six annual reviews and planning workshop, EIAR, Addis Ababa, Ethiopia.

Babiker, A. G. T., Ejeta, G., Butler, L. G. & Woodson, W. R. (1993). Ethylene biosynthesis and strigol induced germination of *Striga asiatica. Physiol. Plant.* ***88***(2): 359-365.

Benishangul Gumuz BoANRD (Benishangul Gumuz Bureau of Agriculture and National Resources Development) (2016). Infestation of *Striga* in Benishangul Gumuz Region (written in Amharic). Benishangul Gumuz BoANRD, Assosa, Ethiopia.

Berg, G. & Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *Microbial. Ecol*, **68**(1): 1-13.

Berhane Sibhatu (2016). Review on *Striga* weed management. *Int. J. Life. Sci. Scienti. Res.* **2**(2): 110-120.

Berhanu Debele (1980). Survey of studies conducted about soil resources appraisal and evaluation for rural development in Ethiopia.

Berner, D. K., Winslow, M. D., Awad, A. E., Cardwell, K. F., Raj, D. M. & Kim, S. K. (1997). *Striga* research methods. *Manual, The Pan-African Striga Control Network (PASCON) and the International Institute of Tropical Agriculture (IITA)*, ***64***(22): 13-20.

Berner, D. K., Kling, J. G. & Singh, B. B. (1995). *Striga* research and control. A perspective from Africa. *Plant Dis.* ***79***(7): 652-660.

Berner, A. (2007). *Striga*: The spreading scourge in Africa. *Regulation of Plant Growth & Development*. ***42***(1): 74-87.

Black, C.A. (1965). Methods of Soil Analysis. Part I, American Society of Agronomy. Madison, Wisconsin, USA, 1572 p.

Bray R.H. & L.T. Kurtz. (1945). Determination of Total, Organic and Available Phosphorus in Soils. *Soil Sci. J*. **59**(1): 39-45.

Bouwmeester, H. J., Matusova, R., Zhongkui, S. & Beale, M. H. (2003). Secondary metabolite signalling in host–parasitic plant interactions. *Current opinion in Plant Biology*. ***6***(4): 358-364.

Bouyoucos, G.J. (1962). Hydrometer method improvement for making particle size analysis of soils. *Agron. J*. **54**: 179-186.

Chang, M. and Lynn, D.G. (1986). The haustorium and the chemistry of host recognition in parasitic angiosperms. *J. Chem. Ecol.***12**: 561-579.

Cardoso, C., Ruyter-Spira, C. & Bouwmeester, H. J. (2011). Strigolactones and root infestation by plant-parasitic *Striga*, Orobanche and Phelipanche spp. *Plant Sci*. ***180***(3): 414-420.

Charudattan, R. & Dinoor, A. (2000). Biological control of weeds using plant pathogens: accomplishments and limitations. *Crop Prot. **19***(8-10): 691-695.

Charudattan, R. (2001). Biological control of weeds by means of plant pathogens: significance for integrated weed management in modern agro-ecology. *BioControl*. **46**(2): 229-260.

Chaudhary, B., Sanjeev, K., Ratan L. S. & Shish Ram Jakhar. (2018). Effect of Different Media, pH and Temperature on Growth and Sporulation of *Fusarium udum* Causing Wilt of Pigeonpea. *Int.J.Curr.Microbiol.App.Sci.* **6**: 2005-2011

Ciotola, M., Watson, A. K. & Hallett, S. G. (1995). Discovery of an isolate of *Fusarium oxysporum* with potential to control *Striga hermonthica* in Africa. *Weed Res. **35***(4): 303-309.

Ciotola, M.A., DiTommaso, A., Watson, A.K. (2000). Chlamydospores production, inoculation methods and pathogenicity of *F. oxysporum* M12-4A, a biocontrol for *Striga hermonthica*. Biocontrol Sci. Technol. **10**: 129-145.

Cochrane, V. & Press, M. C. (1997). Geographical distribution and aspects of the ecology of the hemiparasitic angiosperm *Striga asiatica* (L.) Kuntze: a herbarium study. *J. Trop. Ecol.* ***13***(3): 371-380.

Compant, S., Brader, G., Muzammil, S., Sessitsch, A., Lebrihi, A. & Mathieu, F. (2013). Use of beneficial bacteria and their secondary metabolites to control grapevine pathogen diseases. *Biol. Control*. ***58***(4): 435-455.

CSA (Central Statistics Agency). (2015). Report on Area and Production of Crops of Central Statistics Agency. Central Statistics Agency, Addis Ababa, Ethiopia.

CSA (Central Statistics Agency). (2019). Report on Area and Production of Crops of Central Statistics Agency. Central Statistics Agency, Addis Ababa, Ethiopia.

Deng, Tu, Q., Y., Yan, Q., Shen, L., Lin, L., He, Z., & Enquist, B. J. (2016). Biogeographic patterns of soil diazotrophic communities across six forests in the North America. *Mol. Ecol.* ***25***(12): 2937-2948.

Dorr, I. (1997). How *Striga* parasitizes its Host: a TEM and SEM study. Annals of Botany, **79:** 463-472.

EIAR (Ethiopian institute of agricultural Research) (2014). Ethiopian strategy for sorghum 2014-2024.EIAR, Addis Ababa, Ethiopia.

Elzein, A. & Kroschel, J. (2004). *Fusarium oxysporum* Foxy 2 shows potential to control both *Striga hermonthica* and *S. asiatica. Weed Res.* ***44***(6): 433-438.

Fasil Reda & Verkleij, J. A. C. (2007). Cultural and cropping systems approach for *Striga* management a low cost alternative option in subsistence farming. In *Integrating New Technologies for Striga Control: Towards Ending the Witch-Hunt* (pp. 229-239).

Frost, D. L., Gurney, A. L., Press, M. C. & Scholes, J. D. (1997). *Striga hermonthica* reduces photosynthesis in sorghum: the importance of stomatal limitations and a potential role for ABA? *Plant Cell & Environ.* ***20***(4): 483-492.

Gebisa Ejeta & Butler, L. G. (1993). Host-parasite interactions throughout the *Striga* life cycle, and their contributions to *Striga* resistance. *Afr. Crop Sci. J.* ***1*** (2): 75-80.

Gebisa Ejeta, Babiker, A.G.T. & Butler, L. (2002). New approaches to the control of *Striga,* a training workshop on *Striga* resistance. Melkassa, May 14-17 (2002). Nazareth, Ethiopia. pp. 4 - 8.

Gebisa Ejeta (2006) .International Symposium on Integrating New Technologies for *Striga* Control. Purdue University, USA. www.agry.purdueedu/ *Striga* Conference/pdf/1-Ejeta/ 7/11/2006

Gebisa Ejeta (2007). Breeding for *Striga* resistance in sorghum: exploitation of intricate host–parasite biology. ***Crop*** *Sci*. ***47***(3): S-216.

Gebremedhin Woldewahid., Goudriaan, J. & Naber, H. (2000). Morphological, phenological and water-use dynamics of sorghum varieties (Sorghum bicolor) under *Striga hermonthica* infestation. *Crop Prot.* ***19***(1): 61-68.

Ghorbani, R., Leifert, C. & Seel, W. (2005). Biological control of weeds with antagonistic plant pathogens. *Adv. Agron.* ***86****(3)*: 191-225.

Girmay Shinun (2017). ISC Project annual progress report for 2016 in Tigray region. A paper presented during the six annual reviews and planning workshop, EIAR, Addis Ababa, Ethiopia.

Gobena Daniel, Shimels, M., Rich, P. J., Ruyter-Spira, C., Bouwmeester, H., Kanuganti, S., & Gebisa Ejeta, G. (2017). Mutation in sorghum Low Germination Stimulant 1 alters strigolactones and causes *Striga* resistance. *Proc Natl Acad Sci.* ***114***(17): 4471-4476.

Gomez Gomez, K. A. & Gomez, A. A. (1984). *Statistical procedures for agricultural research*. 2nd Edn., John Wiley and Sons Inc., New York, USA., Pages: 680.

Gupta, V., Misra, A. & Gaur, R. (2010). Growth characteristics of *Fusarium* spp. causing wilt disease in Psidium guajava L. in India. *J. Plant Prot. Res.* ***50***(4): 452-462.

Gurney, A. L., Grimanelli, D., Kanampiu, F., Hoisington, D., Scholes, J. D., & Press, M. C. (2003). Novel sources of resistance to *Striga hermonthica* in Tripsacum dactyloides, a wild relative of maize. *New Phytol.* ***160***(3): 557-568.

Harding, D. P. & Raizada, M. N. (2015). Controlling weeds with fungi, bacteria and viruses: a review. *Front. Plant Sci.* ***6****(2)*: 659.

Hayelom Berhe (2014). Advance research on *Striga* control: A review. *J. Plant Prot. Res.* ***8***(11): 492-506.

Hearne, S. J. (2009). Control the *Striga* conundrum. *Pest Management Science: formerly Pestic. Sci.* ***65***(5): 603-614.

Hess, D. E., Kroschel, J., Traoré, D., Elzein, A. E. M., Marley, P. S., Abbasher, A. A. & Diarra, C. (2000). *Striga*: Biological control strategies for a new millennium. *Sorghum and Millet Diseases*, 165-170.

Heydari, A. & Pessarakli, M. (2010). A review on biological control of fungal plant pathogens using microbial antagonists. *J. Biol. Sci.* ***10***(4): 273-290.

Idris, A. E., Abouzeid, M. A., Boari, A., Vurro, M. & Evidente, A. (2003). Identification of phytotoxic metabolites of a new *Fusarium* sp. inhibiting germination of *Striga hermonthica* seeds. *Phytopathol. Mediterr*. ***42***(1): 65-70.

Júnior, F. W. R., Scariot, M. A., Forte, C. T., P, Olfi, L., Dil, J. M., Weirich, S. & Galon, L. (2019). New perspectives for weeds control using autochthonous fungi with selective bioherbicide potential. *Heliyon*, ***5***(5): 01676.

Keyes, W. J., O'malley, R. C., Kim, D. & Lynn, D. G. (2000). Signaling organogenesis in parasitic angiosperms: xenognosin generation, perception, and response. *J. Plant Growth Regul.* ***19***(2): 217-231.

Khan, Z. R., Midega, C. A., Pittchar, J. O., Murage, A. W., Birkett, M. A., Bruce, T. J. & Pickett, J. A. (2014). Achieving food security for one million sub-Saharan African poor through push–pull innovation by 2020. *Philosophical Transactions of the Royal Society B: Biol. Sci.* ***369***(1639): 20120284.

Kumawat, T. K., Sharma, A. & Bhadauria, S. (2016). Effect of culture media and environmental conditions on mycelium growth and sporulation of *Chrysosporium queenslandicum*. *Int J ChemTech Res*, ***9***(11): 271-277.

Kroschel, J., Hundt, A., Abbasher, A. A. & Sauerborm, J. (1996). Pathogenicity of fungi collected in northern Ghana to *Striga hermonthica*. *Weed Res.* ***36***(6): 515-520.

Labrada, R. (2006). Recommendations for improved weed management. FAO of the united nations, Rome, Italy

Lakshmanan, V., Selvaraj, G. & Bais, H. P. (2014). Functional soil microbiome: belowground solutions to an aboveground problem. *Plant physiology*, ***166***(2): 689-700.

Leblanc, N., Kinkel, L. & Kistler, H. C. (2017). Plant diversity and plant identity influence *Fusarium* communities in soil. *Mycologia*, ***109***(1): 128-139.

Leck Astrid, (1999). Preparation of Lactophenol cotton blue slide mounts, *community eye health*. **12**(30):24

Lendzemo, V. W. & Kuyper, T. W. (2001). Effects of arbuscular mycorrhizal fungi on damage by *Striga hermonthica* on two contrasting cultivars of sorghum, Sorghum bicolor. *Agr Ecosyst Environ.* ***87***(1): 29-35.

Liu, M., Sui, X., Hu, Y. & Feng, F. (2019). Microbial community structure and the relationship with soil carbon and nitrogen in an original Korean pine forest of Changbai Mountain, China. *BMC microbiology*, **19**(1): 218.

Marley, P. S., Ahmed, S. M., Shebayan, J. A. Y. & Lagoke, S. T. O. (1999). Isolation of *Fusarium oxysporum* with potential for biocontrol of the witchweed (*Striga hermonthica*) in the Nigerian savanna. *Biocontrol Sci. Techn.* ***9***(2): 159-163.

Marley, P.S. & Shebayan, J.A.Y. (2005). Field assessment of *Fusarium oxysporum* based mycoherbicide for control of *Striga hermonthica* in Nigeria. *BioControl* **50**, 389-399.

Mesfin Abate (2016). Assessment of *Striga* infestation and evaluation of sorghum landraces for resistance/tolerance to *Striga hermonthica* (del.) benth in North Western Ethiopia. PhD thesis, Haramaya University, Haramaya.

Mbuyi, D. A., Masiga, C. W., Kuria, E. K., Masanga, J., Wamalwa, M., Mohamed, A. & Runo, S. M. (2017). Novel sources of witchweed (*Striga*) resistance from wild sorghum accessions. *Front. Plant Sci.* ***8(4)***: 116.

MOA (Ministry of Agriculture) (2014): Sorghum Sector Development Strategy (Working Document 2015-2020) MOA, Addis Ababa, Ethiopia.

Mohamed, E.A.A. (2002). Evaluation of *Fusarium* sp. Abu Haraz as a biocontrol for *Striga hermonthica* (Del). Benth. on Sorghum bicolor (L.) M. Sc. Thesis, University of Gezira, Sudan.

Mohammad Reza Safari Motlagh, (2010). Isolation and characterization of some important fungi from *Echinochloa* spp. the potential agents to control rice weeds, *I.J.Crop. Sci.* **4**(6):457-460

Mrema, E., Shimelis Hussien., Laing, M. & Bucheyeki, T. (2017). Screening of sorghum genotypes for resistance to *Striga hermonthica* and S. asiatica and compatibility with *Fusarium oxysporum* f. sp. *Striga*e. *Acta Agriculturae Scandinavica, Section B—Soil & Plant Sci.* ***67***(5): 395-404.

Muller-Stover. D., Nybroe, O., Baraibar, B., Loddo, D., Eizenberg, H., French, K. & Christensen, S. (2016). Contribution of the seed microbiome to weed management. *Weed Res.* **56**(5): 335-339.

Musselman, L. J. (1980). The biology of *Striga*, Orobanche, and other root-parasitic weeds. *Annual review of phytopathology*, ***18***(1): 463-489.

Musyoki, M. K., Cadisch, G., Enowashu, E., Zimmermann, J., Muema, E., Beed, F. & Rasche, F. (2015). Promoting effect of *Fusarium oxysporum* [f.sp.] on abundance of nitrifying prokaryotes in a maize rhizosphere across soil types. *Biol. Control*. ***83****(11)*: 37-45.

Nath, N. (2011). Morphological and physiological variation of *Fusarium oxysporum* f. sp. ciceri isolates causing wilt disease in chickpea (Doctoral dissertation, Department Of Plant Pathology, Sher-E-Bangla Agricultural University).

Nekouam, N. & Marley, P. S. (2003). Micro-organisms associated with *Striga hermonthica* and rhizosphere soil in the Nigerian savannah: isolation and evaluation for possibilities in biological control of the witchweed.

Nelson, Paul E. (1981). "Life cycle and epidemiology of *Fusarium* oxysporum." *Fungal wilt diseases of plants*. 51-80.

Neondo, J. O. (2017). Exploring biological control and transgenic weed management approaches against infestation by Striga hermonthica in maize (Doctoral dissertation, JKUAT).

Nzioki, H. S., Oyosi, F., Morris, C. E., Kaya, E., Pilgeram, A. L., Baker, C. S. & Sands, D. C. (2016). *Striga* biocontrol on a toothpick: a readily deployable and inexpensive method for smallholder farmers. *Front. Plant Sci.* ***7****(3)*: 1121.

Pageau, K., Simier, P., Le Bizec, B., Robins, R. J. & Fer, A. (2003). Characterization of nitrogen relationships between Sorghum bicolor and the root hemiparasitic angiosperm *Striga hermonthica* (Del.) Benth. using K15NO3 as isotopic tracer. *J.Exp. Bot.* ***54***(383): 789-799.

Pal, K. K. & Gardener, B. M. (2006). Biological control of plant pathogens. Plant Heal Instr. 1-25

Parker, C. & Riches, C. R. (1993). *Parasitic weeds of the world: biology and control*. CAB International Press, Wallingford, Oxon, Ox 108 DE, UK,332p.

Pieterse, A. H., Verkleij, J. A. C., Den Hollander, N. G., Odhiambo, G. D. & Ransom, J. K. (1996). Germination and viability of *Striga hermonthica* seeds in Western Kenya in the

course of the long rainy season. In *Congresos y Jornadas-Junta de Andalucia (Espana)*. JA, DGIA.

Rani, C. & Panneerselvam, A. (2010). Fungal diversity in the sediments of Point Calimere, east coast of India. *J. Pure Appl. Microbiol*, **4**(1):199-206.

Ransom, J. K. (2000). Long-term approaches for the control of *Strata* in cereals: field management options. *Crop Prot.* ***19***(8-10): 759-763.

Rebeka Gebrestadik (2013) Integrating Sorghum [*Sorghum bicolor* (L.) Moench] Breeding and Biological Control Using *Fusarium oxysporum* Against *Striga hermonthica* in Ethiopia (Doctoral Dissertation)

Rodenburg, J., Cissoko, M., Kayongo, N., Dieng, I., Bisikwa, J., Irakiza, R. & Scholes, J. D. (2017). Genetic variation and host–parasite specificity of *Striga* resistance and tolerance in rice: the need for predictive breeding. *New Phytol.* ***214***(3): 1267-1280.

Rhodes. D.J. (1993). Formulation of biological control agents. In: Jones, D.G. (Ed.), Exploitation of Microorganisms. Chapman and Hall, London, pp. 411-439.

Rousk, J., Bååth, E., Brookes, P. C., Lauber, C. L., Lozupone, C., Caporaso, J. G. & Fierer, N. (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil. *The ISME journal*, ***4***(10): 1340-1351.

Rubiales, D. & Fernández-Aparicio, M. (2012). Innovations in parasitic weeds management in legume crops. A review. *Agronomy for Sustainable Development*, ***32***(2):433-449.

Sato, D., Awad, A. A., Takeuchi, Y. & Yoneyama, K. (2005). Confirmation and quantification of strigolactones, germination stimulants for root parasitic plants *Striga* and Orobanche, produced by cotton. *Biosci Biotech Bioch.* ***69***(1): 98-102.

Satish, K., Gutema, Z., Grenier, C., Rich, P. J. & Gbebisa Ejeta. (2012). Molecular tagging and validation of microsatellite markers linked to the low germination stimulant gene (lgs) for *Striga* resistance in sorghum [Sorghum bicolor (L.) Moench]. *Theor Appl Genet.* ***124***(6): 989-1003.

Sauerborn, J., Abbasher, A. A., Kroschel, J., Cornes, D. W., Zoschke, A. & Hine, K. T. (1996a). *Striga hermonthica* control with *Fusarium nygamai* in maize. In *Proceedings of the 9th International Symposium on Biological Control of Weeds* (pp. 461-466).

Sauerborn, J., Dörr, I., Abbasher, A., Thomas, H. & Kroschel, J. (1996b). Electron Microscopic Analysis of the Penetration Process of *Fusarium nygamai*, a Hyperparasite of *Striga hermonthica*. *Biol. Control*. ***7***(1): 53-59.

Sauerborn, J., Müller-Stöver, D. & Hershenhorn, J. (2007). The role of biological control in managing parasitic weeds. *Crop Prot.* ***26***(3): 246-254.

Sicuia, O. A., Grosu, I., Constantinescu, F., Voaides, C. & Cornea, C. P. (2015). Enzymatic and genetic variability in Bacillus spp. strains with plant beneficial qualities. *Agro.Life Scient. J.* ***4****(3)*: 124-131.

Sisay Lemawork (2017). ISC Project annual progress report for 2016 in SNNP region. A paper presented during the six annual reviews and planning workshop, EIAR, Addis Ababa, Ethiopia.

Singh, K. (1991). *An illustrated manual on identification of some seed-borne Aspergilli, Fusaria, Penicillia and their mycotoxins*. Danish Government Institute of Seed Pathology for Developing Countries.

Singh, P. & Chauhan, M. (2013). Influence of environmental factors on the growth of building deteriorating fungi: *Aspergillus flavus* and *Penicillium chrysogenum*. *Int. J. Pharm. Sci. Res.* ***4***(1): 425-429.

Smith, M. C. & Webb, M. (1996). Estimation of the seedbank of *Striga* spp. (Scrophulariaceae) in Malian fields and the implications for a model of biocontrol of *Striga hermonthica*. *Weed Res*. **36:** 85-92.

Somesh, N. S., Behera, L., Bais, R. K., Tiwari, A. & Kumar, S. (2019). Effect of temperature and pH on growth and sporulation of *Fusarium oxysporum* f. sp. lini (Bolley) Synder and Hensan causing linseed wilt under environmental condition. *Journal of Pharmacognosy and Phytochemistry*, ***8***(2): 1427-1430.

Tancic Zivanov, S., Nesic,L., Jevtic,R., Belic, M., Ciric, V., Lalosevic, M. & Veselic, J. (2017). Fungal diversity as influenced by soil characteristics. *Zemdirbyste-Agriculture*, ***104***(4).

Taye Tessema & Gebisa Ejeta (2019). Sorghum production in transition through *Striga* management. Ethiopian Institute of Agricultural Research. Addis Ababa, Ethiopia. pg (5-11)

Tekalegn Mamo & Haque, I. (1991). Phosphorus status of some Ethiopian soils, II. Forms and distribution of inorganic phosphates and their relation to available phosphorus. *J. Trop. Agric.* ***68***(1): 2-8.

Tesfaye Alemu, Getachew Gashaw & Kassahun Tesfaye (2014). Morphological, physiological and biochemical studies on Pyricularia grisea isolates causing blast disease on finger millet in Ethiopia, *J. Appl. Biosci.* **74**: 6059– 6071

Tesso Tesfaye., Zenbaba Gutema Aberra & Gebisa Ejeta, 2007. An integrated *Striga* management option offers effective control of *Striga* in Ethiopia. P199-212.

Thomas, H. (1996). Phytotoxic metabolites produced by *Fusarium nygamai* from *Striga hermonthica*. Proc. of the IX Int. Symposium on Biological Control of Weeds.

Van Mourik, T. A., Stomph, T. J. & Westerman, P. R. (2003). Estimating *Striga hermonthica* seed mortality under field conditions. *Aspects of applied biology*, ***69***: 187-194.

Van Reeuwijk. (1992). Procedures for Soil Analysis, 3rd edition. International Soil Reference and Information Center (ISRIC), Wageningen, the Netherlands, 34p.

Voisard, C., Keel, C., Haas, D. & Dèfago, G. (1989). Cyanide production by Pseudomonas fluorescens helps suppress black root rot of tobacco under gnotobiotic conditions. *The EMBO Journal*, ***8***(2): 351-358.

Walkley & I.A. Black, (1934). An Examination of the Degtjareff Method for Determining Soil Organic Matter and a Proposed Modification of the Chromic Acid Titration Method. *Soil Sci.*, **37**: 29-38.

Westwood, J. H., Yoder, J. I., Timko, M. P. & dePamphilis, C. W. (2010). The evolution of parasitism in plants. *Trends in Plant Sci.* ***15***(4): 227-235.

Wondimu Bayu, Binor Solomon & Admassu Legesse. (2001). Tolerance of sorghum landraces and varieties to *Striga* (*Striga hermonthica*) infestation in Ethiopia. Acta Agron. Hung., **49**(2): 343 -349.

Xie, X., Yoneyama, K., Kisugi, T., Uchida, K., Ito, S., Akiyama, K. & Yoneyama, K. (2013). Confirming stereochemical structures of strigolactones produced by rice and tobacco. *Mol. Plant*. ***6***(1): 153-163.

Xie, X., Yoneyama, K. & Yoneyama, K. (2010). The strigolactone story. *Annual review of phytopathology*, ***48***(number):93-117.

Yoneyama, K., Xie, X., Kisugi, T., Nomura, T., Sekimoto, H., Yokota, T. & Yoneyama, K. (2011). Characterization of strigolactones exuded by Asteraceae plants. *J. Plant Growth Regul.* ***65***(3): 495-504.

Yonli, D., Traoré, H., Sérémé, P., Hess, D. E. & Sankara, P. (2010). Pathogenicity of *Fusarium* isolates to *Striga hermonthica* in Burkina Faso. *Pak J Biol Sci. PJBS*, ***13***(5): 201-208.

Yonli, D., Traoré, H., Hess, D.E, Sankara, P. & Sérémé, P. (2006). Effect of growth media, *Striga* seed burial distance and depth on efficacy of *Fusarium* isolates to control *Striga hermonthica* in Burkina Faso. Weed Res. **46**, 73-81.

Zain, M. E., Razak, A. A., El-Sheikh, H. H., Soliman, H. G. & Khalil, A. M. (2009). Influence of growth medium on diagnostic characters of *Aspergillus* and *Penicillium* species. *Afr. J.Microbiol. Res.* **3**(5): 280-286.

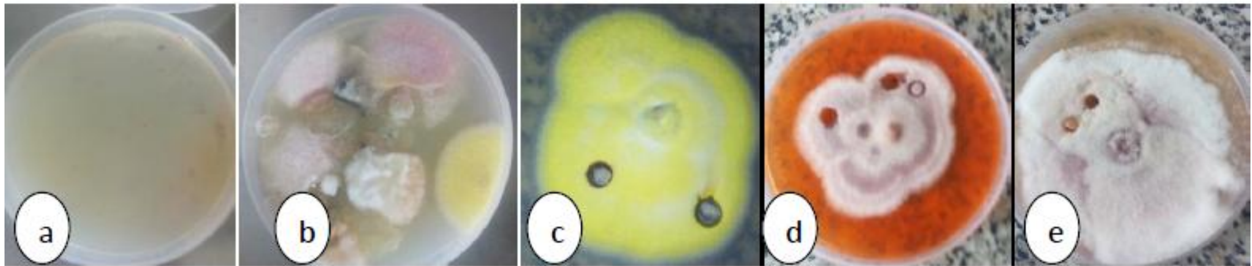
8. APPENDIXES

Annex 1: Assessment of Striga seed decaying soil teabag experiment result

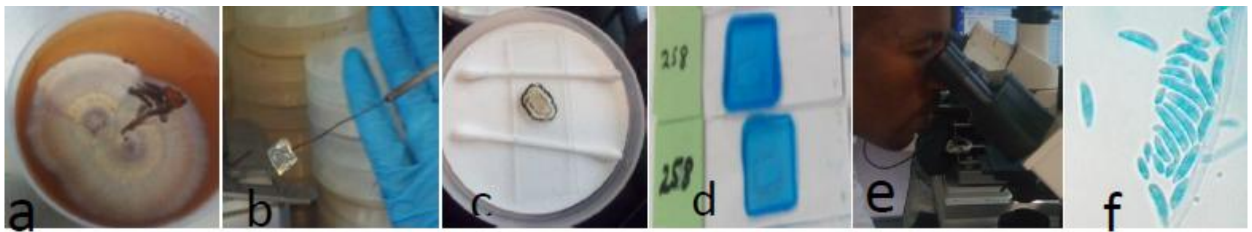
Sample	SSG (%)	SSD (%)	SSV (%)	Sample	SSG (%)	SSD (%)	SSV (%)
Control	88.7^{a}	0.0^{h}	11.3^{f}	**E25**	64.7^{bc}	8.7^{bcdefg}	26.7^{abcdef}
E01	74.7^{ab}	7.0^{cdefg}	18.3^{ef}	**E26**	63.3^{bc}	4.0^{fgh}	32.7^{abcde}
E02	67.3^{bc}	6.3^{defgh}	26.3^{abcdef}	**E27**	63.0^{bc}	7.0^{cdefg}	30.0^{abcde}
E03	61.0^{bc}	10.0^{bcdefg}	29.0^{abcde}	**E28**	61.0^{bc}	4.7^{fgh}	34.3^{abcde}
E04	64.0^{bc}	6.7^{defgh}	29.3^{abcde}	**E29**	56.0^{bc}	6.7^{defgh}	37.3^{ab}
E05	66.3^{bc}	5.7^{efgh}	28.0^{abcde}	**E30**	62.7^{bc}	13.^{abcd}	24.3^{bcdef}
E06	67.0^{bc}	7.3^{cdefg}	25.7^{abcdef}	**E31**	62.3^{bc}	7.0^{cdefg}	30.7^{abcde}
E07	64.3^{bc}	7.0^{cdefg}	28.7^{abcde}	**E32**	60.7^{bc}	7.7^{cdefg}	31.7^{abcde}
E08	64.3^{bc}	6.3^{defgh}	29.3^{abcde}	**E33**	61.0^{bc}	4.7^{fgh}	34.3^{abcde}
E09	61.7^{bc}	7.3^{cdefg}	31.0^{abcde}	**E34**	62.7^{bc}	3.7^{gh}	33.7^{abcde}
E10	61.7^{bc}	5.7^{efgh}	32.7^{abcde}	**E35**	59.7^{bc}	4.0^{fgh}	36.3^{abc}
E11	62.0^{bc}	10.7^{bcdef}	27.3^{abcdef}	**E36**	63.3^{bc}	6.3^{defgh}	30.3^{abcde}
E12	65.0^{bc}	6.3^{defgh}	28.7^{abcde}	**E37**	60.7^{bc}	5.3^{efgh}	34.0^{abcde}
E13	59.0^{bc}	6.7^{defgh}	34.3^{abcde}	**E38**	61.3^{bc}	10.0^{bcdefg}	28.7^{abcde}
E14	67.7^{bc}	12.0^{bcde}	20.3^{cdef}	**E39**	58.7^{bc}	7.0^{cdefg}	34.3^{abcde}
E16	67.0^{bc}	13.7^{abc}	19.3^{def}	**E40**	59.7^{bc}	4.7^{fgh}	35.^{7abcd}
E17	65.7^{bc}	7.0^{cdefg}	27.3^{abcdef}	**E41**	62.0^{bc}	4.7^{fgh}	33.3^{a}
E18	65.0^{bc}	7.7^{cdefg}	27.3^{abcdef}	**E43**	63.3^{bc}	7.7^{cdefg}	29.0^{abcde}
E19	64.3^{bc}	15.0^{ab}	20.7^{cdef}	**E45**	62.7^{bc}	5.0^{fgh}	32.3^{abcde}
E20	63.0^{bc}	6.3^{defgh}	30.7^{abcde}	**E46**	54.7^{c}	3.7^{gh}	41.7^{a}
E21	60.3^{bc}	7.0^{cdefg}	32.7^{abcde}	**E47**	62.0^{bc}	5.3^{efgh}	32.7^{abcde}
E22	58.0^{bc}	19.7^{a}	22.3^{bcdef}	**S1**	57.0^{bc}	7.3^{cdefg}	35.7^{abcd}
E23	59.3^{bc}	14.7^{ab}	26.0^{abcdef}	**S2**	60.7^{bc}	6.3^{defgh}	33^{abcde}
E24	61.7^{bc}	5.3^{efgh}	33.0^{abcde}	**Mean**	**63**	**7.4**	**29.6**

SSG (%) - *Striga* seed germinated, SSD (%) - *Striga* seed decayed, SSV (%) - *Striga* seed viable

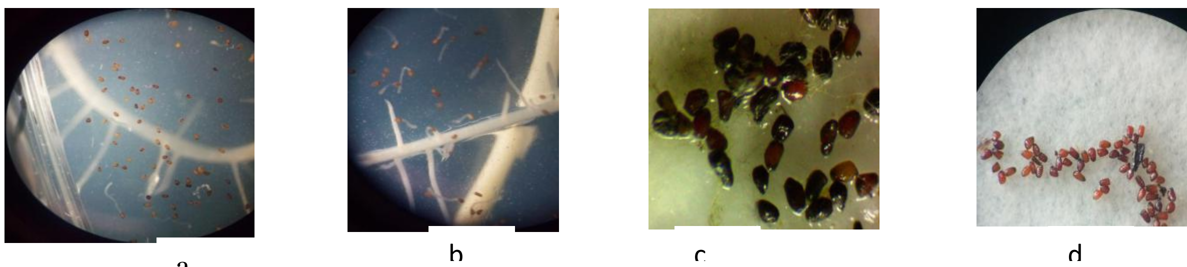
Annex 2: Tea bag experiment trial photo during soil sample evaluation in the laboratory



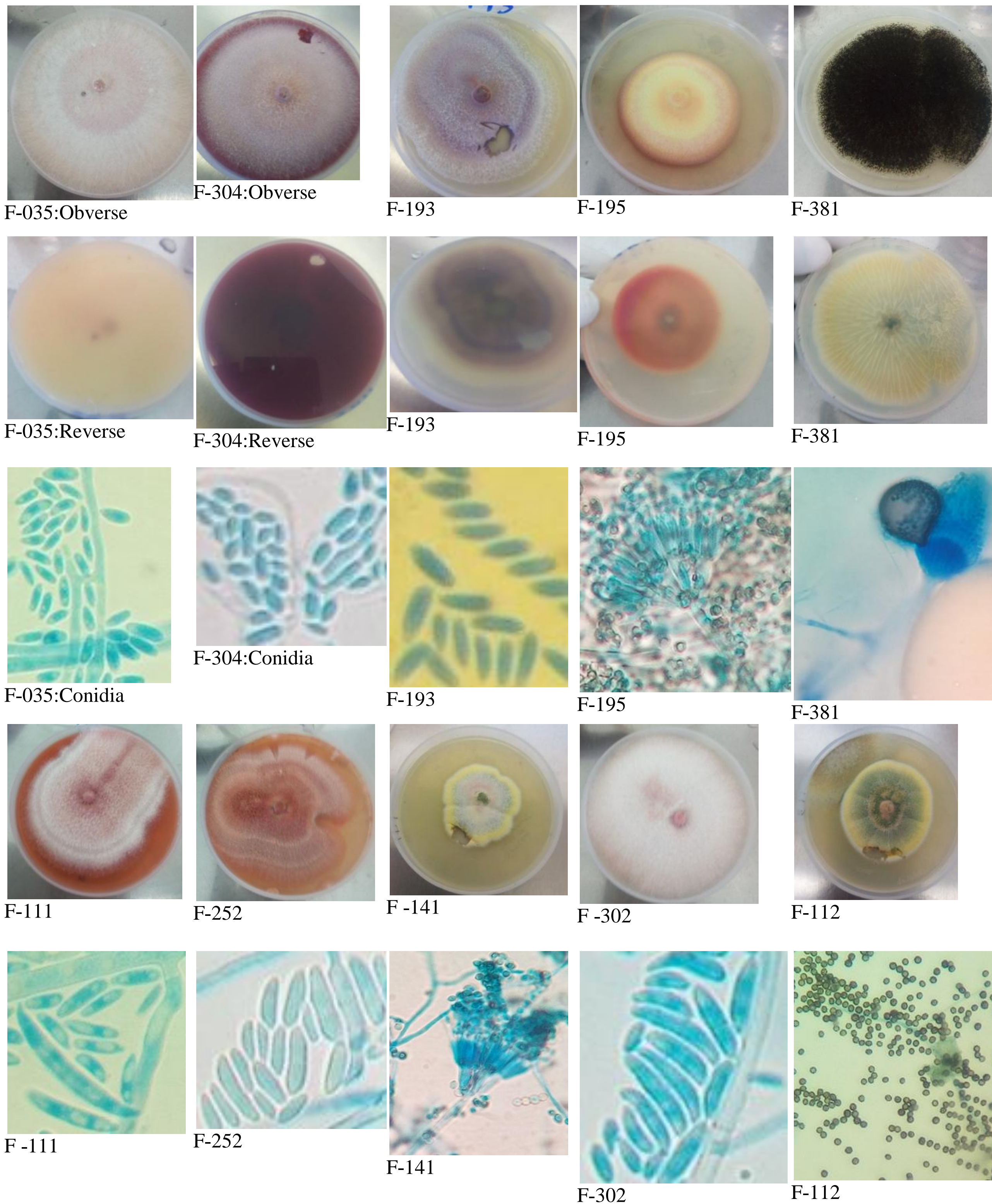
Annex 3: Seed trap methods for purposive/targetic fungi isolation from soil sample: *Striga* Seed on PDA b) different fungal genera c, d, and e) pure fungal colony after 7-days



Annex 4: Slide culture technique procedure for microscopic fungal identifications: a) Pure iolate b) Agar block c) Culture incubated on agar block for 7 days d) Lactophenol cotton blue staining e) Discovering under microscope f) Conidia



Annex 5: Striga seed germination inhibition test (a and b), using agar media and seed mortality (viability test c and d) using Tetrazolium test



Annex 6: Different fungi isolate colony morphology and conidia on PDA

Annex 7: Screening of Striga seedbank decaying fungi isolates

Isolate code	Disc 1	Disc 2	Disc 3	Disc 4	Disc 5	TSS	SSD	SSD (%)	Remark
F-111	13.4	15	16.2	15.2	18.6	250	78.4	31.36	
F-112	14	10.4	17.2	14.8	16	250	72.4	28.96	
F-113	20.4	16.2	23.8	22.8	21.8	250	105	42	
F-141	36	34.6	35.2	36	36	250	177.8	71.12	
F-142	28	29.4	25.8	27.6	27	250	137.8	55.12	
F-143	42.8	40.4	40.6	40.6	40	250	204.4	81.76	Selected
F-144	19.8	18.4	18	17.4	18.2	250	91.8	36.72	
F-162	6.8	9.2	8.2	9.6	11	250	44.8	17.92	
F-163	20.8	17.8	24	22.8	22.8	250	108.2	43.28	
F-164	35	34.2	33.4	34	34	250	170.6	68.24	
F-165	37.8	37.6	38.6	38	37.4	250	189.4	75.76	
F-166	20.4	18.2	17.8	18.8	18.8	250	94	37.6	
F-167	9.2	8.2	14.4	13.4	12.8	250	58	23.2	
F-168	14.2	12.2	19.8	14.6	15.8	250	76.6	30.64	
F-191	43.8	42.8	42.6	43.8	43.4	250	216.4	86.56	Selected
F-193	11.4	10.6	9.6	10.6	10.2	250	52.4	20.96	
F-194	37.2	34.6	34.8	34.8	36.4	250	177.8	71.12	
F-195	2.8	2	3	3.6	5.6	250	17	6.8	
F-196	25.2	23.8	23.2	23.4	22.8	250	118.4	47.36	
F-221	29.6	28.6	28.4	28.2	29.2	250	144	57.6	
F-222	40.2	39.8	39.2	39.8	39	250	198	79.2	
F-224	45.8	44.6	44	45	45.4	250	224.8	89.92	Selected
F-223	15	14.8	18.4	15.8	18.8	250	82.8	33.12	
F-231	32	30	30	29	30.4	250	151.4	60.56	
F-252	41	40.4	39.4	39.6	38.8	250	199.2	79.68	Selected
F-253	14	11.8	17	16.8	15.4	250	75	30	
F-254	46.6	47.6	43.4	46.4	45.6	250	229.6	91.84	Selected
F-258	17.4	15.4	19.4	20.6	20.6	250	93.4	37.36	
F-259	23.2	22	23.4	21.8	26.4	250	116.8	46.72	
F-301	42.4	41.4	41.4	41	41	250	207.2	82.88	Selected
F-302	38.4	38.8	37.8	37.8	37.2	250	190	76	
F-304	45.8	44.2	44.2	45	44.2	250	223.4	89.36	Selected
F-031	40.6	39	37.6	37.8	38.8	250	193.8	77.52	
F-032	44.2	42.8	42.2	41.8	42.6	250	213.6	85.44	Selected
F-033	16.5	14.2	21.2	17.9	17.8	250	87.6	35.04	
F-035	16	15.2	18.4	16.8	18.6	250	85	34	

F-036	9.4	6.4	13	10.8	11.8	250	51.4	20.56	
F-037	21.2	19.8	24.6	23.6	23.6	250	112.8	45.12	
F-038	12.6	9.8	12.2	11.2	14.8	250	60.6	24.24	
F-381	42.2	44.2	41.8	44.4	42.8	250	215.4	86.16	Selected
F-039	16.2	14.4	21.4	19.6	18.4	250	90	36	
F-040	38.8	41.6	40.8	40	42.8	250	204	81.6	Selected
F-041	20.6	20.2	22.4	21	24.2	250	108.4	43.36	
Mean	26.7	25.6	27.2	26.8	27.4	250.0	133.7	53.5	

TSS – Total *Striga* seed, SSD – *Striga* seed decayed