College of Natural and Computational Sciences School of Post Graduate Studies

Department of Microbial, Cellular and Molecular Biology

Characterization of *Striga* sp. Seed Bank Depleting Bacteria from *Striga* Infested sorghum (*Sorghum bicolor* (L.) Moench) Growing Areas of Northern and Eastern Ethiopia

A Thesis Submitted to the School of Post Graduate Studies of Addis Ababa University in Partial Fulfillment of the requirement for the Degree of Masters of Sciences (M.Sc) in Applied Microbiology

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Addis Ababa Ethiopia

DECLARATION

I, Getachew Yilma, hereby declare that "Isolation and Characterization of *Striga hermonthica* Seed Bank Depleting Bacteria from *Striga* Infested Sorghum Growing Areas of Northern and Eastern Ethiopia" is my own work and it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University except where due acknowledgement has been made in the text. It is submitted for the degree of Master of Science in Applied Microbiology to Addis Ababa University College of Natural and Computational Sciences School of Post Graduate Studies.

Candidate	**signature**	**date**
Getachew Yilma	----------	----------

ADVISORS APPROVAL SHEET

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Applied Microbiology Stream

As thesis research Advisor, we here certify that the thesis prepared by Getachew Yilma which were under our guidance throughout the research work, entitled "**Isolation and Characterization of *Striga hermonthica* Seed Bank Depleting Bacteria from *Striga* Infested Sorghum Growing Areas of Northern and Eastern Ethiopia**" and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Applied Microbiology fulfills with the regulations of the University and meets the accepted standards with respect to originality and quality.

Advisors name	**signature**	**date**
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DEDICATION

I want to dedicate this MSc thesis to my father Yilma Abebe, who was deceased when I started my education in grade 1. We were both unfortunate to celebrate the occasion together.

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ACRONYMS

AAU	Addis Ababa University
EIAR	Ethiopian Institute of Agricultural Research
GR-24	Growth Regulator (synthetic hormone)
LSD	List Significant Different
NIOO-KNAW	Netherlands Institute of Ecology, Royal Dutch Academy of Arts & Sciences
PROMISE	Promoting Root Microbes for Integrated *Striga* Eradication
SAS	Statistical Analysis System
SDH_2O	Sterile Distilled water
Spp. /sp.	Species
SXSg	Sorghum Xenognosin

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ABSTRACT

Striga hermonthica is a very devastative parasitic weed of (*Sorghum bicolor* (L.) Moench) which is the third important crop in Ethiopia. It is hemi-parasite living with the host plant by attaching small sucker root system to host plant. A study was undertaken to evaluate the effect of bacterial isolates obtained from soil samples collected from *Striga* infested sorghum growing fields in Amhara, Tigray and Oromia Regions to inhibit (deplete) the *Striga* seed bank. Surface sterilized and conditioned *Striga* seeds were buried in the soil by using teabag to determine germinated, viable and decayed seeds at different times after burial. A total of 44 isolates were isolated from germinated and decayed striga seeds and tested for their effect on germination and seed decay of *Striga* seeds. Six isolates (GS29, GS32, GS34, GS39, GS42 and GS45) were stimulated 10.33, 9.0, 9.67, 8.33, 5.33 and 8.33 *Striga* seeds germination from the total of 35 seeds respectively in the absence of host plant synthetic stimulant. On the other hand four isolates (SD3, SD9, SD36 and SD46) significantly decayed *Striga* seeds at $P<0.05$ (21, 20, 21.33 and 21 seeds respectively) in order to reduce *Striga* attack. Selected isolates were characterized by using biochemical tests, and three isolates were classified under the genus *Pseudomonas* while the other three isolates were grouped under the genus *Klebsiella*. The four isolates that showed seed decay were classified under the genus *Bacillus*.

KEY WORDS; *Germination, stimulant, seed decay, viability, GR-24*

1. INTRODUCTION

Sorghum bicolor (L.) Moench is an important crop for 500 million of people in Sub-Saharan Africa which is mainly cultivated in drier areas, especially on shallow and heavy clay soils (Wortmann et al., 2009). In Africa, the area under sorghum production is about 23.14 million ha and total production and average yield being 23.35 million metric tons and 1.01 ton/ha, respectively (FAO, 2016). It is the third largest cereal crop in Ethiopia and is produced in most parts of the country, especially in drought prone areas (Demeke and Di Marcantonio, 2013 and CSA, 2019). It is known for its versatility and diversity and is produced over a wide range of agro-ecological zones. However, the production and productivity of sorghum is significantly affected due to several abiotic and biotic factors such as drought, *Striga* weed, diseases, insect pests, and birds.

Striga hermonthica (Del.) is hemi-parasite weed lives with host plant attached to small sucker root system of host plant. It is a parasitic weed that is being a major constraint to cereal production including sorghum in Sub-Saharan Africa where it makes one of the gravest threats to food security in the region. *Striga* weed causes blotching, scorching, wilting, loss of vigor and finally death of the plant. It also causes a reduction in the ear size, plant height, stem diameter and weight of the whole plant. It also imparts severe damage on roots and causes stem lodging (Mahmoud et al., 2013). Hence, *Striga* infestation is one of the most yield limiting constraints of sorghum causes up to 100% yield loss (Schneider, et al., 2010).

So far, cultural and chemical methods as well as breeding for host resistance have been practiced to control *Striga* (Berhane, 2016). But *Striga* control becomes difficult due to tiny seeds that are dust-like and produced in large quantities (50,000 to 500,000 seeds per plant) and retain their germination up to 20 years (Berner et al., 1995). *Striga* infested farm can cause 65-100% yield loss. *Striga* control using hand weeding is not effective, because it emerges after damaging the host crop.

It is clearly showed that different bacteria have the ability to suppress *Striga* seed germination by different mechanisms (Babalola et al., 2007, Bouillant et al., 1997 and Ahonsi et al., 2002). Bacteria can suppress *Striga* infestation by ethylene production in the absence of sorghum, and

hence reduce the *Striga* seed bank. This is by synthesizing and releasing ethylene gas to the soil which is a *Striga* seed germination stimulant. The bacterium *Pseudomonas syringae* pathovar glycinea synthesizes relatively large amounts of ethylene and stimulates *Striga* seed germination more than ethylene gas (Berner et al., 1999 and Babalola et al., 2007)

There are also seed decaying activities by antibiosis and enzymatic properties of potential bio-control isolates of some *Bacillus* spp. which recorded high antibiosis, enzymatic and seed decay values (Neondo et al., 2017). Some saprophytic bacteria also have an important role in decaying of *Striga* seed because of their nutritional versatility, fast growth rate, and high specificity of their inhibitory activity (Elliot and Lynch, 1984; Kennedy et al., 1991) cited in (Subbapurmath, 2012).

Some bacteria are capable of promoting plant growth by different mechanisms and help the plant to overcome abiotic and biotic stress. Other bacterial species are capable of scavenging the root exudate released by the host that stimulates *Striga* seed germination and hence suppress it. Hiba, et al., (2013) also showed that addition of bacteria suspensions (*Pseudomonas*) to the root exudates of the host plant (sorghum) significantly reduced (100% germination) the ability of the exudates to induce germination of *Striga* seeds under in vitro conditions.

In Ethiopia even if some research works have been undertaken on different *Striga* management mechanisms, there is a limited information on the isolation and testing of soil bacteria that are capable of depleting *Striga* seed bank in the soil either by stimulating germination in the absence of host or decaying and killing the *Striga* seed (Berhane, 2016).
Therefore, the current study was initiated in order to determine the suitability of soil bacteria to control *Striga* infestation.

1.1. General Objective

The general objective of this study is to isolate and characterize different *Striga* seed bank depleting bacterial isolates from the *Striga* infected *Sorghum* growing areas of Northern and Eastern parts of Ethiopia.

1.1.1. The specific objectives of this study are:

- To isolate *Striga* seed bank depleting bacteria from soils collected from *Striga* infested Sorghum growing areas
- To characterize *Striga* seed bank depleting bacterial isolates under *in-vitro* and *in-vivo* conditions.

1.2. Hypothesis

- Bacterial isolates that are found in *Striga* infested soils are capable of depleting *Striga* seed bank by seed decaying and inhibiting germination.
- Some bacterial isolates from *Striga* infested soil are able to stimulate *Striga* seed germination in the absence of the host crop.

2. LITERATURE REVIEW

2.1. Sorghum and production constraints

Sorghum (*Sorghum bicolor*) is an important staple crop which is indigenous to Africa and grown in arid and moisture stressed areas (Plessis, 2008). It belongs to the grass family, *Poaceae*, genus *Sorghum* and classified as *S. bicolor* (L.) Moench (Berenji et al., 2011). Sorghum is the fifth most important cereal globally and the dietary staple of around 500 million people (Wortmann et al., 2009). Sorghum is crucially important to food security in Africa as it is uniquely drought resistant among cereals and can withstand periods of high temperature. It is an important food crop in many parts of eastern and southern Africa as stated by Awika (2011).

In Ethiopia it is the third most important cereal crop both in area coverage and production following teff and maize. It can grow in a wide range of agro-climatic conditions from a mean monthly rain fall of 228 ml to 43 ml and in a temperature range from 18 ^{0}C- 27^{0}C. Its altitude also ranges from 680-2100 masl (Wortmann et al., 2009). It is the single most important staple crop in drought prone areas (Demeke and Di Marcantonio, 2013). Sorghum can be consumed in different types of non-fermented and fermented foods. Non-fermented food is whole grain either boiled or roasted, bread from Sorghum flour prepared from non-fermented dough, porridge, while the fermented foods include fermented bread, *enjera* and traditional beverages (Taylor, 2003).

Sorghum though very important crop in Africa especially for poor farmers, there are many production constraints. Among the constraints of production drought, low soil fertility, *Striga* infestation, storage pests, damage by birds, lack of improved varieties, lack of production inputs (fertilizers, insecticides, herbicides, fungicides, and improved seeds) and among these constraints *Striga* ranks first (Mrema et al., 2017).

2.2. *Striga hermonthica*

The word '*Striga'* is the Latin word which means 'witch'. Witch weed (*akenchira* in Amharic) is a common name for *Striga* because plants diseased by *Striga* display stunted growth and an overall drought-like phenotype long before *Striga* plants appear. The genus *Striga*, erected by the

Italian botanist, Loureiro, in 1790, is characterized by opposite leaves, irregular flowers with a corolla divided in to a tube and spreading lobes, herbaceous habitat, and parasitism. The flowers are pink, red, white, purple or yellow, the seeds are tiny, some 0.3 mm long and 0.15 mm wide (Babiker, 2007).

Approximately 30 *Striga* species (family: Orobanchaceae) have been described in the genus *Striga* and most parasitize grass species (Poaceae) except *Striga gesnerioides* (Willd.) which parasitize legumes (Spallek et al., 2013). A single *Striga* plant can release 50,000 up to 500,000 seeds and it can be stay dormant in the soil until the conditions are favorable for germination or the host plant release root exudate (Berner et al., 1995). *Striga* spp. are serious weeds of important food crops including Sorghum, maize, millet and rice, in sub-Saharan Africa, the Middle East and Asia. Their effects are severe, and complete loss of harvest is common in heavily infested areas. *Striga hermonthica* is one of the devastating parasitic weeds of this group and mainly attacks cereal crops (Sorghum, *Sorghum bicolor* (L. Moench), maize *Zea mays* L.), pearl millet (*Pennisetum glaucum* (L.) (Leeke), upland rice (*Oryza sativa* (L).) *S. hermonthica* plant beside its well-known devastating impacts on the most important food cereal crops in Africa and is deemed to be one of the main factors that threatens the food security in this continent (Faisal, 2011).

2.3. Status of *S. hermonthica* in Ethiopia

As different research reports showed Ethiopia is one of *Striga hermonthica's* habitations and it is assumed that the origin of *Striga* is around the border of Sudan and Ethiopia (Obilana, 1992) and (Faisal, 2011). It is a major biotic constraint in Sorghum growing parts of Ethiopia (Atsbha et al., 2016). Another research shows that genetic diversity was assessed in 12 *S. hermonthica* populations from different locations in Ethiopia. Of these, seven populations were parasitic on Sorghum, two each on tef and maize, and one on finger millet. Genetic differentiation between populations was relatively high, and all populations were significantly different from each other. The genetic differentiation value of the population (FST values) ranged from 0.032 to 0.293 and averaged 0.146 (Welsh et al., 2011). Under drought-prone agro-ecologies of Ethiopia according to farmer's perception next to moisture stress, *Striga hermonthica* is one of the major limiting factors of Sorghum production (Amelework et al., 2016).

2.4. Effect of *S. hermonthica* in Sorghum production

S. hermonthica is an economically very important parasitic weed of sorghum. The infestation affects pre and post flowering stages of the crop growth phases; about 50% reduction in seedling vigor and 9% delayed days to 50% flowering for pre-flowering stress, while post flowering traits under *Striga* stress resulted in 37% reduction in panicle weight and 45% reduction in grain yield (Francis, 2006). It has pink flowers and can invade the whole farm (figure 1). Growth reduction and the consequent loss of Sorghum yield as a result of infestation and parasitic activity of *Striga* was observed. It was thus concluded that *Striga* had a significant negative effect on growth of *Sorghum bicolor.* (Akomolafe, 2018).

Figure 1. *Striga* infested farm at western Hararghe photo taken during sample collection (author)

2.4.1. The mechanism of attachment of *Striga* to sorghum root

The seeds of parasitic plants of the genus *Striga* will only germinate if there is an induction by a chemical signal exuded from the roots of their host. *Striga* have very small seeds that can survive for only a few days after germination before forming an association with a host. The limited carbohydrate reserves in *Striga* seeds restrict seedling root elongation before host attachment.

Thus, arranging for germination to coincide with proximity of an appropriate host root is critical to *Striga* seedling survival. To ensure that germination occurs near host roots, *Striga* seeds germinate only in the presence of sustained (10–12 h) high concentrations of germination inducers exuded into the soil by host roots (Figure 2).

Germination inducers vary between different *Striga* hosts. To date, the only plant-produced *Striga* germination inducer that has been identified and characterized is Sorghum xenognosin (SXSg). SXSg is highly unstable in aqueous solution, a useful trait for a *Striga* germination inducer because it is unlikely to persist in the soil and falsely indicate the presence of a host. However, SXSg is so unstable that it initially seemed difficult to explain how SXSg persisted and traveled in the soil in quantities sufficient to affect nearby *Striga* seeds (Weir et al., 2010). For *Striga* spp. several germination stimulants have been identified in the root exudates of host and non-host plants which are collectively described as the strigolactones (Matusova et al., 2005).

Phenols and quinones are common in the rhizosphere where they are known to function as signal molecules acting between plant roots and other organisms, including roots of other plants. The biological activity of these molecules is often associated with their redox state, and in some cases, bioactivity is a function of the oxidoreduction cycle itself. Plants and other organisms have evolved detoxification systems that limit the cytotoxicity of radical molecules generated during redox cycling, and these mechanisms function in parasitic plants as well. However, parasitic plants have further evolved to use the redox-active molecules as signals to initiate haustorium development. In this way parasitic plants recruit biologically active and generally toxic molecules to signal the transition to a heterotrophic lifestyle (Bandaranayake et al., 2013).

Haustorium is a unique multicellular organ that formed upon detection of haustorium-inducing factors derived from the host plant. This multicellular organ penetrates the host root and connects to its vasculature which allows exchange of materials such as water, nutrients, proteins, nucleotides, pathogens, and retrotransposons between the host and the parasite (Yoshida et al., 2016).

Xylem connections between parasites and hosts (*Striga* and sorghum) involve very specific, clustered intrusions into the host's water conducting elements, mainly into the large vessel

elements. One haustorial cell can penetrate a host vessel element with more than one intrusion. Then all intrusions become covered by an additional electron-opaque wall layer. During subsequent differentiation, dissolution of specific wall parts of the cell intrusions occurs so that open, cup- or trunk-like structures result. The vessel-like host contact can comprise up to five openings within a single intrusion. Concomitantly, the intrusions and the haustorial cells to which they belong lose their protoplasts and transform into elements which absorb water. Walls of the haustorial cells and both wall parts of their appendages come to be strongly lignified. The water and nutrient absorbing structures inserted into the host vessel are named 'oscula' (Dörr, 1997).

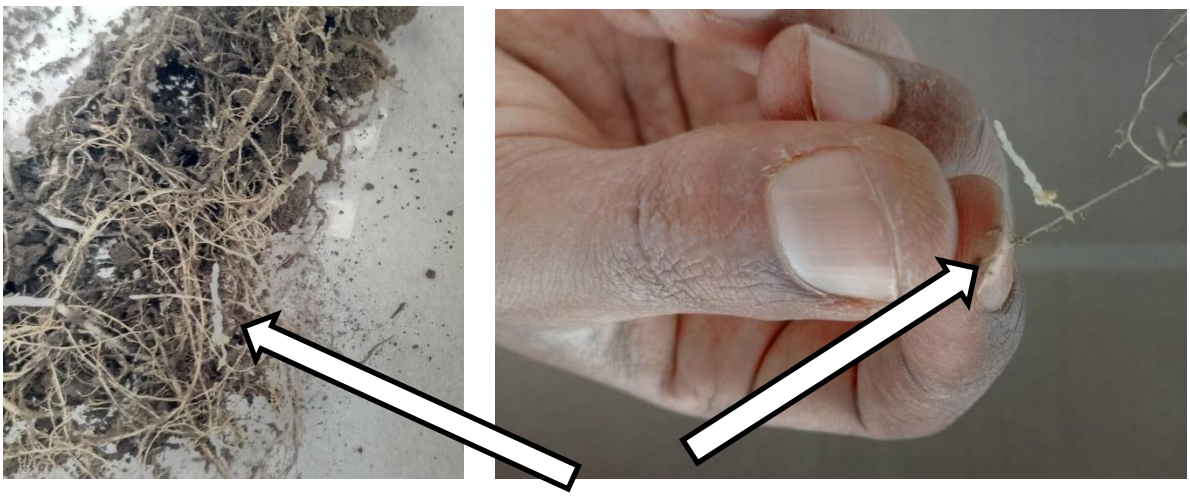


Figure 2 *Striga* attachment to sorghum root a photo taken during sample collection (author)

2.5. *Striga* control methods

Many researches were conducted on the control mechanisms of *Striga* weed since the first half of 20th century. *Striga* weed management is described under three principles. They are reducing number of *Striga* seeds in soil, preventing production of new seeds and preventing spread from infested to non-infested soils (Obilana et al., 1992 and Runo et al., 2018). Host –plant resistance, host seed treatment, transplanting, and biological control reduce the amount of *Striga* seeds in the soil, stop *Striga* reproduction and reduce crop loss (Berner et al., 1995. These control methods can be generalized as cultural, chemical, and biological.

2.5.1. Cultural control

Cultural *Striga* management includes hand weeding, crop-rotation, inter-cropping, trap and catch croping and improving soil fertility. As many scholars agreed hand weeding is not effective management of *Striga* weed (Babiker, 2007). Still these practices are not adopted by farmers. Because they are perceived by poor farmers as unaffordable or uneconomical, labor intensive, impractical, or not congruent with their other farm practices (Berhane, 2016).

2.5.2. Chemical

Various chemicals including herbicides, fumigants (e.g, methyl bromide) and germination stimulants (e.g., ethylene) have been reported as means of control of *Striga* Herbicides like Imazapyr and pyrithiobac applied as seed dressing to maize were reported to give efficient control of the parasite. The excellent control capacity of the herbicides is most likely due to their relatively long persistence in the rhizosphere. Furthermore, multi-location testing showed that this herbicide provided excellent early season control of both *S. asiatica* and *S. hermonthica* and could increase yield 3 to 4-fold in heavy infested fields (Kanampiu et al., 2003)

2.5.3. Biological

The biological control of *Striga* weed includes herbivorous insects, microorganisms and smothering plants (Berhane, 2016). Insects can attack *Striga* in different sites and can be classified according to their site of damage. They are defoliators such as *Junonia* spp., gall forming such as *Smicronyx* spp., shoot borers as *Apanteles* spp., miners as *Ophiomyia Strigalis*, inflorescence feeders as *Stenoptilodestaprobanes* and fruit feeders as *Eulocastra* spp. (Kroschel, 1999). Twenty eight fungi and two bacteria were found to be associated with *Striga*hermonthica in Sudan. Among the fungi, only Fusarium nygamai and Fusarium semitectum var. majus showed potential to be used as bio-agents for the control of *Striga* (Zahran, 2008)

But in general, it was reviewed that no single management option has been found effective across locations and time. Hence, an integrated *Striga* management approach, currently, offers the best possibility for reducing impact at the farm level (Berhane, 2016). It was also reported that adoption of an integrated approach encompassing high yielding *Striga* resistant and/or tolerant crop cultivars and bacterial inoculation may provide a novel, cheap and easy to apply

method for *Striga* control under substance low-input farming systems (Hassan et al., 2009). Especially in Africa the control methods should be simple, easy to apply, inexpensive and sustainable (Babiker, 2007).

2.6. The role of microorganisms in *Striga* management

Currently the use of microorganisms for the control of *Striga* is getting a quite attention. As different research reports show different fungi and bacterial isolates have very important roles in *Striga* weed management in different mechanism. According to *Striga hermonthica* seed mortality under field conditions with non-sterile soil was 86% greater than sterile soil and due to this it was suggested that microbial activity is the major cause of seed mortality during the rainy season (Van Mourik et al., 2003). There are soil microbes with potential of being used as bio control agents produce several extra-cellular lytic enzymes and antibiotic compounds (Neondo et al., 2017). Subbapurmath also reported that some soils have microorganisms which naturally suppress the *Striga* germination and it is possible to isolate, test and use these microorganisms in managing *Striga* menace.

Some microbial isolates are not only controlling *Striga* attachment, but also found to have a significant role in improving plant growth. Total nitrogen, P concentration, chlorophyll content, plant height and plant biomass were significantly increased by the inoculation of *Striga* suppressive bacteria, fungi and actinomycete isolates (Subbapurmath, 2012). Babiker also suggested that the microorganisms involved in soil suppressiveness need to be identified and crop management practices which enhance their proliferation and effectiveness need to be ascertained (Babiker, 2007). It was also reported that good control of parasitic *Striga* could be achieved by manipulating host-rhizosphere microorganisms in combination with *Striga* tolerant Sorghum cultivars (Hassan et al., 2009).

2.7. Bacteria effect on *Striga* control

It was reported that some of the bacterial strains and isolates reduced and delayed *Striga* emergence on sorghum, others reduced *Striga* infestation and growth, while some had enhancing effects. Some bacterial strains and isolates increased sorghum growth in comparison to the *Striga* infested un-treated control and bacteria strains and isolates were more suppressive to *Striga* emergence on resistant and tolerant sorghum cultivars than on the susceptible (Hassan et al.,

2009). Bacterial isolates are found to deplete *Striga* seed bank by different mechanisms. These mechanisms are by stimulation of germination for suicidal germination, decaying *Striga* seed or inhibiting seed germination (Babalola et al., 2007, Bouillant et al., 1997 and Ahonsi et al., 2002). Therefore, bacteria can be considered as an important biological control of *Striga hermonthica*.

2.7.1. *Striga* seed germination stimulant bacteria

Different bacterial isolates were reported to germinate *Striga* seeds in the absence of the host crop. *Pseudomonas* sp. was reported to germinate *Striga* seeds significantly (Babalola et al., 2007). *Klebsiella*, which is known to be an ethylene producer, elicited considerable germination of the parasite. Microbes – derived ethylene could be used to induce suicidal germination of the parasite and thus deplete the seed reserves in the soil (Hassan et al., 2010). It was also reported that ethylene-producing bacteria are highly effective in promoting seed germination in *Striga* spp. and also suggested as a practical means of biological control of *Striga* spp. (Berner et al., 1999).

The use of more than one bacterial strain for *Striga* management was also reported as the best way of *Striga* inhibition. Combinations of bacterial strains were often more suppressive to haustorium initiation than individual isolates and strains. A combination of the bacterial strains *Azospirillum brasilense* and *Pseudomonas putida; A. brasilense* and *Azomonas spp; Azotobacter vienlandi* and *Bradyrhizobium japonicum; A. brasilense* and *Azomonas spp.* inhibited germination by 18 to 34% in comparison to the corresponding control (Hassan et al., 2010).

2.7.2. *Striga* seed decaying bacteria

Neondo et al. (2017) reported that antibiosis and enzymatic properties of potential bio-control isolates of some *Bacillus* spp. correlated positively and recorded high antibiosis, enzymatic and seed decay values. Some saprophytic bacteria also have an important role in decaying of *Striga* seed because of their nutritional versatility, fast growth rate, and high specificity of their inhibitory activity (Elliot and Lynch, 1984; Kennedy et al., 1991) cited in (Subbapurmath, 2012).

2.7.3. Bacterial Inhibition of *Striga* seed germination

It was reported that out of four strains of *Azospirillum brasilense*, isolated from soil where Sorghum is grown, have been tested for their effect on germination of *Striga* hermonthica seeds

assayed two of them significantly inhibited germination of the parasite (Bouillant et al., 1997)**.** On the other hand, 15 *Pseudomonas fluorescens/P. putida* isolates were tested and all of them significantly inhibited germination of *S. hermonthica* seeds (Ahonsi et al., 2002). Similarly, Hiba et al., 2013) have reported that a bacterial isolate that belongs to the genus *Pseudomonas* was found to inhibit *Striga* germination up to 100% in greenhouse condition. This is because of growth of the bacteria with production of the active enzyme could result in germination stimulant being destroyed as fast as it is produced or it computes for strigol.

2.8. Formulation of bacterial inoculants in *Striga* management and its success under greenhouse and field condition

Now a day the use of microbial inoculants in agricultural crop production is become common practice. Because bacterial inoculants can enhance plant growth and minimize the effect of loss topsoil, soil infertility, poor plant growth, low yield index and insufficient diversity of indigenous microbes (Babalola and Glik, 2012). Microbial inoculants are preferred in agriculture not only for their yield improvement, but they are very natural with no effect to the environment and agricultural sustainability (Alori, & Babalola, 2018). Among the microbial application in agriculture is biocontrol of weed, pests and diseases. Since *Striga* is one of the most yields limiting weeds of sorghum formulation of bacterial inoculants for biocontrol very important. Based on the greenhouse experiment result Hassan et al., (2009) recommended that use of bacterial inoculant integrated with resistant variety was effective to mitigate *Striga* challenge. Babalola, et al., (2007) formulate bacterial isolates by pelleting with a mixture of bacterial cells from 24 h. old culture (5×10^7 CFU ml^{-1} that was suspended in 1% methylcellulose (mc))to inoculate *Striga* in greenhouse experiment. They also reported that MC is effective as a bacterial preservative and does not significantly influence seed germination.

3. MATERIALS AND METHODS

3.1. Sampling Sites and Sample Collection

Sampling sites cover some of the major Sorghum growing areas of Northern Ethiopia in Kemise Oromia Special Zone, North Shewa and Wollo in Amhara, Shiraro, Humera and Abergelle areas in Tigray and western in Oromia regions (Figure 3).

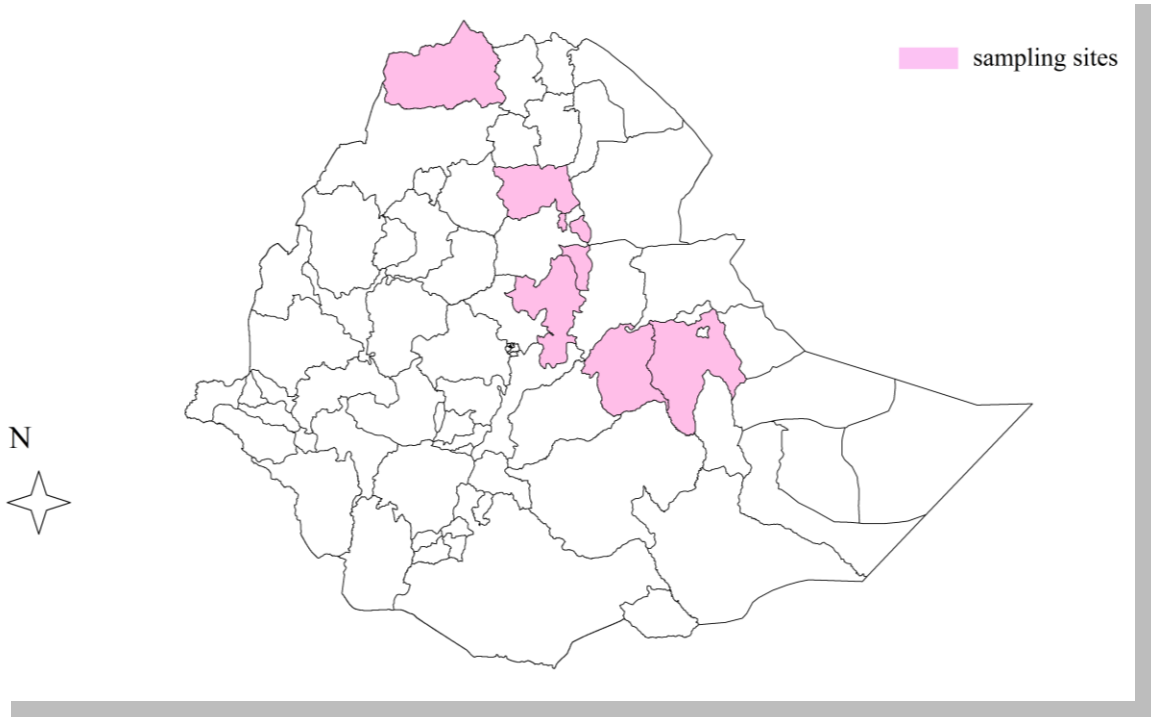


Figure 3. Soil sample collection sites. A map produced by Arc GIS (author).

Geo-referenced soil samples from the top layer (20 cm depth) were collected from four individual sites per each farm in a rectangular pattern and combined in to one composite sample. Soil sampling was based *Striga* infested sorghum growing agroecology and level of *Striga* infestation during 2018 crop seasons at milking stage of sorghum.

Five (5) Kg of bulk composite soil samples were taken from each location. Soils around and under the sorghum root for those current sorghum grown fields were considered. *Striga* infestation was recorded as presence/absence based on the current observation of *Striga* plant in the field during soil sampling (Appendix 1). A total of 44 isolates were collected. Each properly

labeled soil sample was transported to Holeta, National Agricultural Biotechnology Research Center and stored at cool area and further processed by drying and sieving. For the execution of and bioassay experiments, all the 44 composite soil samples were used. The *Striga* seed used for this study were collected from Abergelle area in Tigray region.

Physico-chemical analysis of each soil was analyzed in Holeta agricultural research center soil laboratory (Appendex 2).

3.2. Assessment of *Striga* seed bank depleting bacterial communities

3.2.1. *Striga* seed conditioning

Striga seeds were surface sterilized by using the procedure stated in Amusan et al., (2011). Seeds were surface sterilized by immersing and well shaking in 70% ethanol for 2 minutes. After removing the alcohol, disinfection by sterilizing di-aldehyde solution (Metricide [Metrex Research Corporation] diluted 10-fold) for 3 minutes was made. The seeds were then rinsed three times in sdH_2O. A 4.5 ml of sdH_2O and 1.5 mL of a 0.015% benomyl (Benlate 50% WP, Du Pont) solution was then added to each flask followed by 10 mL of sterile water. The purpose of benomyl solution is to control fugal growth during conditioning. The flasks were then placed into a 30°C incubator for conditioning for 14 days. Every 2- 3 days, benomyl was removed and changed by fresh benomyl solution similar to the previous steps and returned to the incubator (Mohamed, et al., 1998) This procedure was repeated in fourteen days. After 14 days the benomyl solution was removed and *Striga* seeds dried under aseptic condition in the luminal flow-hood and used for the required purpose. For every experiment *Striga* seed were conditioned by this method.

3.2.2. *Striga* seed germination and viability test

Nylon pouches (tea bag mesh size, 40 μm) with an area of 3 cm x 6 cm were filled by 3 mg viable and conditioned *Striga* seeds in aseptic condition. Then the tea bag containing *Striga* seeds were stapled and tied with the help of nylon thread for pulling out. Nine by nine cm area and 10 cm depth plastic pots were filled by the target soil samples that used for isolation and four tea bags each containing 3 mg viable seed were buried in single pot with three replications. The soil samples with buried *Striga* seed were moisten to field capacity by sterile distilled water and

incubated at 30^0C in incubator (DRY C/ DGG-9620A) under dark condition by switching of the fan.

After one, two, four and six weeks, one nylon thread was pulled up and some *Striga* seeds were taken and added to Petri dishes that were covered by filter paper in the lamina flow hood. After one, two, four and six week one pouch was removed from the soil and seeds in the teabag were added in to a 1.5 ml Eppendorf tube. 30-50 write 30 or 40gram *Striga* seeds were added to each petri dishes that were covered with a 9cm diameter filter paper. The added *Striga* seeds were observed under a stereo microscope with magnification of 100 X (SZ-ST1) to see whether the seeds were germinated or not. After observation under microscope, GR-24 was added to each plate. This was made to observe the effect of soil microbes on germination of *Striga* seeds. After two days of incubation at 30 ^{O}C *Striga* seed germination was also observed again under stereo microscope.

Then tetrazolium solution (3000 ppm) was added to each plate and incubated under 30 ^{O}C for 8 days to observe whether the seed is viable or not (Kroschel 2002). After 8 days of incubation *Striga* seeds were observed under stereo microscope and seeds that are non-germinated and not changed to red color were non-viable seeds. It was also repeated on each week of pulling up in the same manner and test for viability and decay. Based on the germination results from seed burying soils E04, E09, E18, E25, E29, E34, E39 were selected for isolation of bacteria that can stimulate *Striga* seed germination. From the tetrazolium test, soil samples E14, E16, E19, E20, E22, E23, and E30 were selected to isolate bacteria that might be capable of damaging the *Striga* seed viability in the soil.

3.3. Isolation of *Striga* Seed Bank Depleting Bacteria

To isolate bacteria from *Striga* seeds, nutrient agar medium was prepared and sterilized at 121^OC for 15 minutes (Vertical Pressure Steam Sterilizer LS-50HJ). The medium was poured on 9 cm diameter Petri-dishes and about 35 *Striga* seeds extracted from selected soils at week 4 were dispersed over in aseptic condition. After 24 hours incubation at 30^OC each colony from the plates were transferred to nutrient broth in sterile Eppendorf tube (Eppendorf® Safe-Lock micro centrifuge tubes). Continuous sub-culturing was made to extract each culture into individual pure colony. Finally, a total of 42 (26 isolates from germinated *Striga* seeds and 16 from non-viable

Striga seed) pure bacterial isolates were obtained and stored for further germination and decay tests. Next trials and tests were made by using these bacterial isolates.

3.4. Standardization of Bacterial suspension

To have a uniform number of bacterial cells for the same test standardization was made. Purified bacterial isolates suspensions in the nutrient broth medium were pooled and diluted to an Optical Density at wavelength of 600 nm (OD_{600}) of 0.5 (Nova spec II spectrophotometer; Pharmacia Biotech, United Kingdom) by adding distilled sterile water to have an approximately 10^9 CFU/ml (Biesta et al., 2010). The suspension was added to 100 ml nutrient broth medium and incubated overnight on the incubator shaker.

3.5. In-Vitro Evaluation of bacterial isolates for *Striga* Seed Germination Stimulation

A total of twenty eight bacterial isolates obtained from soils that showed maximum germination in seed burying test were tested for their capacity to stimulate germination of *Striga* seeds under in-vitro condition. Agar was prepared and filled up to half height on tissue culture thread plates (Corning Incorporated **costar**®) having 24 wells 15.22 mm diameter and a volume of 3ml. 6mm diameter glass fiber discs (Whatman™ GLASS MICROFIBER FILTERS GF/A) were placed on each agar containing wells by using forceps. 35 ±5 conditioned *Striga* seeds were added on each glass fibers by using a pasture pipette. Distilled sterile water was added to the conditioned *Striga* seeds to suck seeds using a pasture pipette. Microbial isolates from nutrient broth were added to each *Striga* seed containing glass fibers in each well by using a micro pipate. 25 µl nutrient broth media containing approximately $2x10^8$ cells/ml of pure bacterial isolate was added to each corresponding test wells. For positive control 25 µl GR-24 was add as germination stimulant while for negative control distilled sterile water is add with the same volume. It was replicated 3 times and after covering with aluminum foil it was incubated at 30 ^{O}C in the incubator (BIOAIR® EuroΘlone Division). After 4 days of incubation the data were recorded by observing *Striga* seeds under stereo microscope (SZ6745) with a magnification of 30X and Germinated *Striga* seeds were recorded (Kroschel, 2002).

3.6. *In Vitro* Evaluation of *Striga* Seed Decaying Bacterial Isolates

Eighteen isolates that were isolated from soils that were selected for this purpose were tested to evaluate their effect on *Striga* seed germination. Agar was prepared and filled up to half height on tissue culture thread plates (Corning Incorporated costar®) having 24 wells 15.22 mm diameter and a volume of 3 ml. A 6mm diameter glass fiber discs (Whatman™ GLASS MICROFIBER FILTERS GF/A) were placed on each agar containing wells by using forceps. A 25 ±5 conditioned *Striga* seeds were added on each glass fibers by using a pasture pipette. Distilled sterile water was added to the conditioned *Striga* seeds to suck seeds using a pasture pipette.

Microbial isolates from nutrient broth were added to each *Striga* seed containing glass fibers in each by using a micro pippette. A 25 µl nutrient broth medium containing approximately $2x10^8$ cell ml^{-1} of pure bacterial isolate was added to each corresponding test wells. For positive control 25 µl GR-24 was add as germination stimulant while for negative control distilled sterile water was added with the same volume. It was replicated 3 times and after covering with aluminum foil it was incubated at 30°C in the incubator (BIOAIR® EuroΘlone Division) for one week and 25 µl of GR-24 was added to each wells of the test plates to observe whether the isolates had effect on *Striga* seed germination or not. After 2 days of incubation at 30°C the data of *Striga* seed germination was recorded. Following the germination result 0.5 ml of 3000 ppm of triphenyl tetrazolium chloride was added to each wells of the Tissue culture plate and incubated for eight days. After eight days of incubation the color change of *Striga* seeds were recorded. Number of non-viable *Striga* seeds was recorded by subtracting both germinated and viable (red color) seeds from the total number of seeds.

3.7. Bio assay on the effect of bacterial isolates on *Striga* sorghum association

A total of eighteen bacterial isolates were tested for their inhibition effect on *Striga* seeds. Conditioned *Striga* seeds were placed and dispersed in 9cm diameter Petri dishes. Sterile agar was suspended on each plate. One conditioned and well germinated sorghum seedling was transferred to each petri-dish containing preconditioned *Striga* seeds and inoculated with 1 ml of each microbial isolate having approximately $2x10^8$ cells/ml of culture on the same date. Sorghum

variety used was *Teshale* which is *Striga* susceptible. The Petri dish that was not inoculated with microbial inoculation was used as a negative control while GR-24 was added as a positive control. Each treatment was replicated three times and randomly incubated at 28^0C. After three, five, and ten days of incubation, the effects on seed germination, radical elongation and *Striga* attachment on the roots of sorghum was recorded, respectively, under the stereo microscope (SZ-ST1) connected with camera. Data was taken by counting number of germinated seeds.

3.8. Morphological and Biochemical identification of selected isolates

Morphological and biochemical tests were made to characterize the selected isolates. Morphological tests were colony structure, gram staining and cell shape. Biochemical tests were catalase test, citrate test, motility test and urease tests. Motility test was made by inoculating fresh colony straight down in the Motility test semi-solid medium and observing bacterial growth after 24 hours incubation at 30 ^{O}C. Catalase test was done by using sterile loop a fresh colony was placed to the slide. By using a dropper 1 drop of 3% H_2O_2 was added onto the organism on the microscope slide. Bubble formation was observed to determine if the isolate was catalase positive or negative. For citrate test a citrate slant medium was prepared and bacterial isolates were streaked over the slant. After 24 hours of incubation the color change due to citrate positive isolates were recorded. Isolates that changed to blue color were citrate positive while those remain green are considered citrate negative. To do urease test Christensen's Urea Agar (4, 5) slant was prepared and fully streaked by fresh culture and incubated overnight. The color change to red was due to urease positive bacterial isolates (Benson 1990).

Finally, all targeted isolates were classified in to genera level by using Bergey's manual of bacteriology (Staley & Krieg, 1984))

3.9. Data Analysis

Data were analyzed by using the Statistical Analysis System (SAS) Procedure version 9.0 at 5% probability ($P<0.05$). Means separation was done by Tukey's multiple range tests for each numerical result and used as comparison.

4. RESULTS AND DISCUSSION

4.1. Germination of buried *Striga* seed with teabag in the soil without the presence of the host

Out of the 46 soil samples tested, only 7 soil samples (15%) showed seed germination in the absence of the host indicating that there were some microorganisms capable of inducing germination of the seeds (Table 1). *Striga* seed germination was observed from soils E04, E09, E18, E25, E29, E34, and E39. The highest amount of germinated *Striga* seed was 6 (11%) recorded from E25, whereas the lowest germination 1 *Striga* seed (2%) was recorded from soil E04, E09 and E29.

The reason for low percentage of germination in the soil may be due to high competition of other microbes and lower number of target bacteria that are capable of inducing germination. For soils that didn't show any germination the target microbes may not exist at all or in sufficient amount to stimulate *Striga* seed germination. With regard to the role of incubation time on seed germination, the highest *Striga* germination was observed at week four. Therefore, *Striga* seeds that are extracted from teabags that were buried in these soils at week four were selected for isolation of bacterial isolates and all the 28 isolates were isolated from *Striga* seeds that were buried from these 7 soils. Similarly, Babalola et al. (2007) have stated that some bacterial species that are capable of stimulating *Striga* seed germination buried in the soil in the absence of the host plant.

Table1. Mean percent *Striga* seed germination from seed burying in the soil samples in the absence of the host plant up on four weeks of incubation.

Soil Code.	Germinated *Striga* Seeds	Total Seed Counted.	Percentage germination
E04	1	50	2.0
E09	1	64	1.6
E18	2	51	3.9
E25	6	53	11.3
E29	1	44	2.3
E34	3	46	6.5
E39	2	58	3.4
Total	16	366	4.43

4.2. Non-viability result from buried *Striga* seed with teabag in the soil

In this study, the tetrazolium test showed that seven selected soils, (E14, E16, E19 E20, E22, E23 and E30) showed non-viable seeds that did not show any color change by the addition of tetrazolium solution after 8 days of incubation (Table 2). The highest number of non-viable seeds was 46 which was 80.7% of the total whereas the lowest was 11 or 26.2%. All soil samples that had more than 25% non-viable *Striga* seeds were used for isolation of bacteria. Similarly, Verma et al., (2013) have observed that non-viable *Striga* seeds were seeds which didn't show any color change whereas viable seeds were changed to red.

Non-viability of *Striga* seeds that were buried in the selected soils was assumed to be due to the action of microbes in the soil. Similarly, Neondo et al. (2017) have reported that antibiosis and enzymatic properties of potential bio-control isolates of some *Bacillus* spp. correlated positively with seed decay values. It is also reported that some saprophytic bacteria play a role in decaying of *Striga* seed because of their nutritional versatility, fast growth rate, and high specificity of their inhibitory activity (Elliot and Lynch, 1984; Kennedy et al., 1991) cited in Subbapurmath, 2012). Therefore, the non-viability of these *Striga* seeds may be due to the availability of such kind of bacteria. On greenhouse experiment it was also reported that some of the bacterial isolates reduced and delayed *Striga* emergence on sorghum, others reduced *Striga* infestation and growth, while some had enhancing effects. Some bacterial isolates increased sorghum growth in comparison to the *Striga* infested un-treated control and bacteria isolates were more suppressive

to *Striga* emergence on resistant and tolerant sorghum cultivars than on the susceptible (Hassan et al., 2009).

Table 2 Soil samples that showed non-viable *Striga* seeds using tetrazolium test after 8 days of incubation.

Soil Code.	**Non-viable *Striga* Seeds**	**Total seed Counted.**	**Percentage**
E14	46	57	80.7
E16	23	66	34.8
E19	29	50	58.0
E20	20	51	39.2
E22	11	42	26.2
E23	32	56	57.1
E30	27	52	51.9
Total	188	374	49.7

4.3. Isolation of bacteria from the soil samples showing seed germination and decay

A total of 28 bacterial isolates were collected from germinated *Striga* seeds that were buried in the seven soils whereas 18 bacterial isolates were collected from non-viable *Striga* seeds (Table 3). The twenty-eight isolates were tested for their capacity of stimulating *Striga* seeds in the absence of the host and without any germination stimulant whereas eighteen isolates were tested for the effect on *Striga* seed viability and decaying.

Similarly, Hassan et al., (2010) also isolated 211 bacterial isolates from 80 soil samples that are capable of inhibiting GR-24 stimulated *Striga* seed germination. On the other hand, Babalola, et al., (2007) were isolated 140 bacterial isolates in the rhizosphere of two sorghum varieties. The difference of the number of isolates might be due to the difference in sample size and the nature of soil samples used.

Table 3 bacterial isolates from selected soil samples for germination stimulant and seed decay

Soil samples	Isolates for *Striga* germination stimulant	Soil samples	Isolates for *Striga* decaying
E04	GS1, GS2,	E14	SD3,SD7, D8, D9, SD10
E09	GS4, GS5, GS6	E16	SD17, SD18
E18	GS11, GS12, GS13, GS14, GS15, GS16	E19	SD19, D20, SD21
E25	GS24, GS25, GS26, GS29, GS32, GS33	E20	SD22, SD23
E29	GS34, GS35	E22	SD27, SD28, SD30, SD31
E34	GS37, GS38, GS39, GS40, GS41, GS42	E23	SD36
E39	GS43, GS44, GS45	E30	SD46
Total	28		18

4.4. Bioassay of *Striga* seed germination

Twenty eight isolates were tested for their effect on *Striga* seed germination on agar field tissue culture plate in the absence of the host and without application of germination stimulant. Out of 28 bacterial isolates, 6 isolates (GS29, GS32, GS34, GS39, GS42 and GS45) stimulated more than five *Striga* seeds, and 16 isolates stimulated less than five *Striga* seeds (Table 4). However, 6 isolates did not show any germination of the *Striga* seeds at all. The six isolates that stimulated more than five *Striga* seeds were considered as promising candidate for biological control of *Striga hermonthica* to stimulate suicidal germination in the absence of the host, (sorghum). The six potential isolates were characterized based upon cultural, morphological and biochemical characteristics (Table 7).

Isolates 29 and 34 showed an average of 10 *Striga* seed germination which was exactly similar to the number of *Striga* seeds germinated by GR-24 synthetic stimulant (the positive control). Sixteen isolates showed low percentage (<14%) of *Striga* seed germination. six isolates GS1, GS4, GS5, GS12, GS13 and GS35 didn't show any germination of *Striga* seeds. The reason for no germination may be due to their non *Striga* germination stimulating bacteria that were attached to germinated *Striga* seed during seed burying and isolated in resemblance. There was

also no any germination of *Striga* seed on the negative control to which only distilled water was added (Table 4).

Gafar,et al. (2015) reported that among fourteen isolates tested for *Striga* seed germination two isolates were stimulate significantly high *Striga* seeds than the control which were 14% and 19%. On the other hand Babalola, et.al. (2007) also reported from tested forty isolates only three isolates were able to stimulate *Striga* seed germination. He also hypothesized that the lower percentage of germination by bacterial isolates might be due to the high diffusion rate of stimulant produced by the isolates.

Table 4; Mean separation result of germinated *Striga* seeds by bacterial isolates and by GR-24.

Isolate	Germination	Germination by GR	Isolate	Germination	Germination by GR	Total no. of seeds
GS1	0.00^{k*}	37.0^{abc}	**GS32**	**9.00^{bc}**	36.0^{abc}	35±5
GS2	0.33^{k}	38.7^{ab}	GS33	4.33^{e}	39.7^{ab}	35±5
GS4	0.00^{k}	35.7^{abc}	**GS34**	**9.67^{ab}**	37.3^{abc}	35±5
GS5	0.00^{k}	32.0^{abc}	GS35	0.00^{k}	32.3^{abc}	35±5
GS6	0.67^{k}	36.0^{abc}	GS37	2.67^{gh}	35.0^{abc}	35±5
GS11	2.33^{hi}	33.7^{abc}	GS38	3.33^{gf}	36.0^{abc}	35±5
GS12	0.00^{k}	35.3^{abc}	**GS39**	**8.33^{c}**	33.7^{abc}	35±5
GS13	0.00^{k}	36.7^{abc}	GS40	4.67^{ed}	34.3^{abc}	35±5
GS14	4.00^{ef}	32.0^{abc}	GS41	2.33^{hi}	36.7^{abc}	35±5
GS15	0.33^{k}	35.7^{abc}	**GS42**	**5.33^{d}**	38.7^{ab}	35±5
GS16	0.33^{k}	38.0^{ab}	GS43	0.33^{k}	29.3^{bc}	35±5
GS24	3.33^{gf}	35.3^{abc}	GS44	1.00^{jk}	36.0^{abc}	35±5
GS25	1.67^{hi}	41.33^{a}	**GS45**	**8.33^{c}**	26.0^{c}	35±5
GS26	4.67^{ed}	37.7^{abc}	GR-24	**10.33^{a}**	31.3^{abc}	35±5
GS29	**10.33^{a}**	33.0^{abc}	(- ve) control	0.00^{k}	36.0^{abc}	35±5
LSD**	0.88	9.65		0.88	9.65	
CV***	16.5	16.7		16.5	16.7	

* Means with the same letters within the same column are statistically similar at P<0.05; **LSD: The list significant different; ***CV: Coefficient of variance.

4.5. Germination of *Striga* seeds after addition of GR-24

Growth regulator GR-24 was added to each well of tissue culture plate containing *Striga* seed inoculated by corresponding bacterial isolate to evaluate the effect of bacteria on *Striga* seed viability. *Striga* seed germination after addition of GR-24 on each testing plate was totally changed from the previous bioassay result (Table 4). In almost all plates, more than 75% of *Striga* seeds were germinated. There was no any significant difference among treatments, except isolate 43 and 45, even negative (only distilled water) and positive (with GR-24) controls are the same in germination after addition of GR-24.

The result indicates that some *Striga* seeds were not stimulated by bacterial isolates even though they were stimulated by the synthetic stimulant GR-24. This is because all bacterial isolates didn't affect *Striga* seed viability or have fewer negative effects on *Striga* seed viability and GR-24 is the best stimulant of *Striga* seed. Other researches also showed that GR-24 can stimulate 65% of the total tested *Striga* seeds (Babalola, et al., 2007). Toh, et al., (2014) also reported that GR-24 was the best parasitic plant germination stimulant chemical and used for soil seedbank depleting.

4.6. Seed viability test result

Even if all the isolates did show non-viable *Striga* seeds, significantly the highest number of non-viable *Striga* seeds at $P<0.05$ was recorded from *Striga* seeds inoculated with isolates SD3, SD9, SD36, and SD46 which were 21,20, 21.3 and 21 respectively (Table 5). Isolates SD3 and SD9 were collected from soil sample E14, whereas isolates SD36 and SD46 were collected from soil of sample E30.

Striga seeds inoculated with other isolates also showed non-viable *Striga* seeds which were significantly different at $P<0.05$ from the negative control. But the number of non-viable *Striga* seeds was less than 60%. So, it indicates that the ability of these isolates to decay *Striga* seed is less and they may not be effective under natural environment where many kinds of microbes are there. For this reason, these isolates were not selected for in-vitro evaluation.

This result indicates that there are some saprophytic bacteria that can decay *Striga* seed. Neondo et al. (2017) reported that antibiosis and enzymatic properties of some potential bio-control

isolates of some *Bacillus* spp. recorded high antibiosis, enzymatic and seed decay values. Subbapurmath, (2012) also reported that some saprophytic bacteria also have an important role in decaying of *Striga* seed because of their nutritional versatility, fast growth rate, and high specificity of their inhibitory activity.

Table 5. Mean number of non-viable *Striga* seeds in tissue culture plate by the inoculation of bacterial isolates after 4 days of incubation.

Isolate	Nonviable seeds	Isolate	Nonviable seeds
SD3	**21.0^{a}**	SD22	16.33^{def}
SD7	12.67^{hij}	SD23	17.0^{cde}
SD8	16.33e^{df}	SD27	15.67d^{efg}
SD9	**20.0^{ab}**	SD28	13.33^{ghi}
SD10	14.33^{fghi}	SD30	18.0^{bcd}
SD17	12.33^{ij}	SD31	17.33^{cde}
SD18	10.67^{j}	SD36	**21.33^{a}**
SD19	18.0^{bcd}	SD46	**21.0^{a}**
SD20	15.0^{efgh}	-ve control	0.33^{k}
21	13.33^{ghi}		
LSD		2.6	
CV		5.4	

*Means with the same letters with in the same column are statistically similar at P<0.05; **LSD: The list significant different; ***CV: Coefficient of variance.

4.7. In-vitro Bioassay test on *Striga* seed decay

The inoculation of isolates SD3, SD9, SD36 and SD46 significantly reduced germination of *Striga* seeds at P<0.05 with respective germination rate of 1, 0.33, 1 and 0.33, respectively (table 6). The low amount of *Striga* germination was due to the effect of bacterial isolates during seed viability and germination. These isolates also significantly affected viability of *Striga* seed on *Striga* seed viability test. Therefore, these isolates had the potential to serve as candidate isolates for biological control of *Striga* weed by affecting germination and seed viability.

Similarly, Ahonsi et al., (2002) have found that some *Pseudomonas* species were capable of significantly affecting *Striga* seeds germination under in-vitro condition and they recommended as a biological control of *Striga hermonthica* and for plant growth promoting effect.

The highest number of *Striga* seed germination was recorded from negative and positive controls (14 and 10 seeds, respectively) which were not inoculated with bacterial isolates (Table 6). Isolates SD10 and SD22 also suppress *Striga* germination in a lower amount relative to other isolates (6.33 and 4.33 *Striga* germination). This shows that the sorghum *Teshale* variety was highly susceptible and stimulated *Striga* seed germination.

Table 6. Mean number of *Striga* seed decay obtained from in-vitro bioassay test due to isolates in the presence of sorghum

Isolate	Mean *Striga* seed germinated*	Isolate	Mean *Striga* seed germinated
SD3	1.0fg	SD22	2.33ef
SD7	3.0de	SD23	2.0efg
SD8	2.67def	SD27	2.33ef
SD9	0.33g	SD28	2.67def
SD10	6.33c	SD30	2.33ef
SD17	2.33ef	SD31	2.0efg
SD18	2.33ef	SD36	1.0fg
SD19	3.67ed	SD46	0.33g
SD20	2.67def	-ve control	10.0b
21	4.33d	GR-24	14.0a
LSD**		1.9	
CV***		18.2	

*Means with the same letters with in the column are statistically similar at P<0.05; **LSD: The list significant different; ***CV: Coefficient of variance.

4.8. Characterization of selected isolates

Gram staining, cell shape, catalase test and sugar utilization test results are summarized in Table 7. According to colony shape, cell morphology, gram stain test and biochemical tests of the isolates were classified to the genus level. Accordingly, the isolates were classified under the genera *Bacillus, Klebsiella* and *Pseudomonas* using Bergey's Manual Systematic Bacteriology (Staley & Krieg, 1984).

Thus, isolates SD3, SD9, SD36 and SD46 were classified under the genus *Bacillus.* These isolates were rod, gram positive, motile, citrate positive, urease negative and catalase positive with round smooth colony (Table 7).

Bioactive bacterial isolates belonged to *Bacillus; Streptomyces* and *Rhizobium* genera were identified with antibiotic and enzymatic properties of potential bio-control *Striga* seed decaying (Neondo et al., 2017). In this research other bacterial isolates didn't obtain except *Bacillus*. The difference may be the absence of other bacterial species in the selected soils.

Isolates GS19, GS29, GS32 showed rod shape gram negative cells with motility, catalase, citrate and urease positive results and classified as genus *Pseudomonas.* These isolates showed a significant inhibition of *Striga* seed germination at $P<0.05$. *Pseudomonas fluorescens/P. putida* isolates that significantly inhibited germination of *S. hermonthica* seeds (Ahonsi et al., 2002). From tested three bacterial isolates only *Pseudomonas* sp. 4MKS8 gave significant stimulation of *S. hermonthica* seed at $P<0.05$ as compared to the control (Babalola et al., 2007).

The rest of the isolates GS34, GS39 and GS45 were classified as genus *Klebsiella.* They were gram negative non-motile and short rod. They also showed positive result for catalase and citrate tests while the urease production test result was negative. *Klebsiella* spp. are known to produce large amount of ethylene and show efficacy in germinating seeds of *S. hermonthica.* Incubation of conditioned *S. hermonthica* seeds with *Klebsiella* sp. resulted in considerable germination that ranged from 34-49% (Hassan et al., 2010).

Isolates GS42, GS29, GS32, GS34, GS39 and GS45 significantly stimulate *Striga* seed germination and were isolated from soils E20, E25, E25, E29, E34 and E39, respectively. The rest isolates, i.e., isolates SD3, SD9, SD36 and SD46 that were selected for the decay of *Striga* seeds were obtained from soil samples E04, E14, E29 and E39, respectively. Out of ten soil samples used to isolate the target bacterial isolates, eight of them were from soils samples that were collected from Tigray region while soil samples E04 and E30 from which isolate SD3 and GS45 were isolated, respectively, were collected from Amhara region.

Table 7 morphological and biochemical test results of selected bacterial isolates.

Isolate	Colony shape, margin and Surface characteristics respectively	Cell shape	Gram stain	Motility	Catalase	Citrate	Urease	Genera
SD3	Round, smooth, smooth	Rod	+	+	+	+	-	*Bacillus*
SD9	Round, smooth, smooth	Rod	+	+	+	+	-	*Bacillus*
SD36	Round, smooth, smooth	Rod	+	+	+	+	-	*Bacillus*
SD46	Round, smooth, smooth	Rod	+	+	+	+	-	*Bacillus*
GS29	Round, smooth, smooth	Rod	-	+	+	+	+	*Pseudomonas*
GS32	Round, smooth, smooth	Rod	-	+	+	+	+	*Pseudomonas*
GS42	Round, smooth, smooth	Rod	-	+	+	+	+	*Pseudomonas*
GS34	Irregular, smooth, smooth	Rod	-	-	+	+	-	*Klebsiella*
GS39	Irregular, smooth, smooth	Rod	-	-	+	+	-	*Klebsiella*
GS45	Irregular, smooth, smooth	Rod	-	-	+	+	-	*Klebsiella*

Table 8 Detail information of soils from which selected bacterial isolates were obtained.

Isolate	Soil Code	Collection sites				Geographic location			Average *Striga* count/m^2
		Region	Zone	Woreda	Kebele	Altitude(m)	Longitude	Latitude	
SD3	E14	Amhara	Oromiya	Jiletumuga	Merewa	1416	10.17.43.2	033.58.08.6	199.25
SD9	E14	Tigray	Rayaazebo	Alamata	Alamata	1468	12.29.49.7	039.37.30.3	124.25
GS29	E25	Tigray	West Tigray	TahtayAdiabo	Simret	1079	14.24.22.8	037.52.11.6	46.75
GS32	E25	Tigray	West Tigray	TahtayAdiabo	Simret	1079	14.24.22.8	037.52.11.6	46.75
GS34	E29	Tigray	West Tigray	Haftayhumera	Maykedira	635	14.10.27.4	036.36.11.3	2.5
SD36	E30	Tigray	West Tigray	Haftayhumera	Maykedira	635	14.10.27.4	036.36.11.3	2.5
GS39	E34	Tigray	West Tigray	Haftayhumera	Maykedira	621	14.0457.6	036.34.23.2	0
GS42	E34	Tigray	West Tigray	Haftayhumera	Maykedira	621	14.0457.6	036.34.23.2	0
GA45	E39	Tigray	West Tigray	Haftayhumera		628	14.14.14.6	036.40.52.5	64.25
SD46	E30	Amhara	Oromiya	Artumafulsi	Hulatukuye	1460	10.33.46.8	039.55.50.5	0

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The present research result showed that some isolates had the capacity of stimulating *Striga* seed germination in the absence of the host plant. The number of germinated *Striga* seeds was statistically similar to the number of *Striga* seeds that were germinated by the synthetic germination stimulant GR-24. Bacterial isolates have the effect of *Striga* seeds decaying or affecting the viability of *Striga* seeds. *Pseudomonas* was the best isolate that stimulate the same amount of *Striga* seeds that were stimulated by the synthetic stimulant GR-24.

5.2. Recommendation

Based on results obtained from this study, the author recommends the following points.

- Green house and on farm tests of these isolates should be conducted to verify their efficiency of depleting *Striga* seed banks in the soil and their effect on Sorghum crop.
- Detail molecular classification of the selected isolates is important to confirm the biochemical based classification.
- Further isolation of bacterial isolates to find more strains with the capacity of depleting *Striga* seed bank in the soil.
- Evaluation and characterization *Striga* under in-vitro and in-vivo tests in the lab, greenhouse and at field condition may greatly assist the *Striga* control in order to improve sorghum productivity.

6. REFERENCES

Ahonsi, M. O., Berner, D. K., Emechebe, A. M., & Lagoke, S. T. (2002). Selection of Rhizobacterial Strains for suppression of Germination of *Striga hermonthica* (Del.) Benth. Seeds. *Biological control*: 24(2): 143-152.

Akomolafe, G. F., Terna, T. P., & Okogbaa, J. I. (2018). Occurrence and growth inhibitory activity of *Striga hermonthica* (Delile) Bent on *Sorghum bicolor* (L.) Moench in Lafia, Nigeria. Nigerian *Journal of Basic and Applied Science* 26(1): 65-69.

Alemayehu Seyoum Taffesse, Dorosh P. and Sinafikeh Asrat (2011). Crop Production in Ethiopia: Regional Patterns and Trends. *Food and agriculture in Ethiopia: Progress and policy challenges*, 74(2): 53.

Ali, H. A., Elamin, H. B., & Dirar, H. A. (2013). Biological control of *Striga hermonthica* Del. Bent: Screening for Bacteria Scavenging Strigol. *American Journal of Biochemistry:* 3(4): 89-92.

Amelework Beyene, Hussein Shimelis, Pangirayi T., Fentahun Mengistu, Mark D L. & Dawit, Getnet (2016). Sorghum production systems and constraints, and coping strategies under drought-prone agro-ecologies of Ethiopia. *South African Journal of Plant and Soil*, 33(3): 207–217

Amusan, I. O., Patrick, J. R. Housley, T. & Gebisa Ejeta, 2011. An *in vitro* method for identifying post-attachment *Striga* resistance in maize and sorghum. *Agronomy Journal*, 103: 1472-1478.

Atsbha Gebreslasie, Taye Tessema, Ibrahim Hamza and Demeke Nigussie (2016). Abundance and distribution of *Striga* (*Striga hermonthica* (del.) Bent.) Infestation in selected sorghum (*sorghum bicolour* l. Moench) growing areas of Tigray region, Ethiopia. *African Journal of Agricultural Research*: 11(45): 4674-4682.

Awika, J. M. (2011). Major cereal grains production and use around the world. In *Advances in cereal science: implications to food processing and health promotion* (pp. 1-13). American Chemical Society.

Babalola, O. O., Berner, D. K., & Amusa, N. A. (2007). Evaluation of Some Bacterial Isolates as Germination Stimulants of *Striga Hermonthica*. *African Journal of Agricultural Research*, 2(1):27-30.

Babiker, AGT. (2007). *Striga*: the spreading scourge in africa. *Regulation of Plant Growth & Development*, 42(1): 74-87.

Bandaranayake, P. C., & Yoder, J. I. (2013). Haustorium initiation and early development. In *Parasitic Orobanchaceae* (pp. 61-74). Springer, Berlin, Heidelberg.

Berenji, J., Dahlberg, J., Sikora, V., & Latkovi, D. (2011). Origin, history, morphology, production, improvement, and utilization of broomcorn [*Sorghum bicolor* (L.) Moench] in Serbia. *Economic Botany*, 65(2): 190-208.

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

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

Berner, D. K., Schaad, N. W., & Völksch, B. (1999). Use of ethylene-producing bacteria for stimulation of *Striga* spp. Seed germination. *Biological Control*, 15(3): 274-282.

Biesta-Peters, E. G., Reij, M. W., Joosten, H., Gorris, L. G., & Zwietering, M. H. (2010). Comparison of two optical-density-based methods and a plate count method for estimation of growth parameters of *Bacillus cereus*. *Applied Environmental Microbiology*, *76*(5): 1399-1405.

Bouillant, M. L., Miché, L., Ouedraogo, O., Alexandre, G., Jacoud, C., Sallé, G., & Bally, R. (1997). Inhibition of *Striga* seed germination associated with sorghum growth promotion by

soil bacteria. Comptes Rendus de l'Académie des *Sciences-Series III-Sciences de la Vie*, 320(2): 159-162.

CSA (Central Statistics Agency). Report on Area and Crop Production Forecast for Major Crops. Statistical Bulletin V.1. Addis Ababa, Ethiopia April 2019.

Demeke Bayable, Di Marcantonio F., 2013. Analysis of incentives and disincentives for Sorghum in Ethiopia. *Technical notes series*, MAFAP, FAO, Rome.

Dörr, I. (1997). How *Striga* parasitizes its host: a TEM and SEM study. *Annals of Botany*, 79(5): 463-472.

Faisal, H. M. (2011). *Striga hermonthica* (Del.) Bent: phytochemistry and pharmacological properties outline. *Journal of Applied Pharmaceutical Science*, 1(07): 01-05.

FAOSTAT. 2016. http://Faostat.fao.org (accessed on February 16, 2019)

Fassil Reda (2002). *Striga hermonthica* in Tigray (northern Ethiopia): prospects for control and improvement of crop productivity through mixed cropping. PhD. Thesis work Institute of Ecological Science, Vrije Universiteit Amsterdam.

Francis, A. (2006). Effect of *Striga hermonthica* on yield and yield components of Sorghum in Northern Guinea Savanna of Nigeria. *Journal of Plant Sciences*, 1(1): 67-71.

Gafar NY, Hassan MM, Rugheim AME, Osman AG, Mohamed IS, Abdelgani ME & Babiker AGT. (2015). Evaluation of endophytic bacterial isolates on germination and haustorium initiation of *Striga* hermonthica (del.) Benth. *International Journal of Farming and Allied Sciences* 4 (4): 302-308

Hassan, M. M., & Babiker, A. G. T. (2011). Effects of bacterial strains and isolates on in situ germination, subsequent developmental stage of *Striga* hermonthica onto sorghum roots. *Advances in Environmental Biology*, 5(10): 3263-3270.

Hassan, M. M., Abdelgani, M. E., Babiker, A. E., & Osman, M. G. (2010). Effect of Klebsiella spp. and different ethylene inhibitors on *Striga hermonthica* Bent. (Del.) seeds germination. *Asian Journal of Agricultural Sciences*, 2(3): 94-98.

Hassan, M. M., Gani, M. E. S. A., & Babiker, A. G. E. T. (2009). Management of *Striga hermonthica* in Sorghum using soil rhizosphere bacteria and host plant resistance. *Int. J. Agric. Biol*, 11: 367-373.

Hiba A. Ali, Hassan B. Elamin, Hamid A. Dirar, Abdel Moneim E. Sulieman, (2013), Biological Control of *Striga hermonthica* Del. Bendth: Screening for Bacteria Scavenging Strigol *American Journal of Biochemistry* 3(4): 89-92

Kanampiu FK, Kabamble V, Massawe C, Jasia L, Friesen D, Ransom JK and Gressel J. (2003). Multi-site, multi-season field tests demonstrate that herbicide seed-coating, herbicide-resistance maize controls *Striga* spp. and increases yield in several African countries. *Crop Prot*. 22: 697 - 706.

Kroschel, J. (1999). Analysis of the *Striga* problem, the first step towards future joint action. *Advances in parasitic weed control at on farm level*, *1*, 3-26.

Kroschel, J. (Ed.). (2002). *A technical manual for parasitic weed research and extension*. Springer Science & Business Medium.

Matusova, R., Rani, K., Verstappen, F. W., Franssen, M. C., Beale, M. H., & Bouwmeester, H. J. (2005). The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanche* spp. are derived from the carotenoid pathway. *Plant physiology*, 139(2): 920-934.

Mohamed, A. H., Gebisa Ejeta, Butler, L. G., & Housley, T. L. (1998). Moisture content and dormancy in *Striga* asiatica seeds. *Weed research (Print)*, *38*(4), 257-265.

Mohamed, A. H., Gebisa, Ejeta & Housley, T. L. (2013). *Striga asiatica* seed conditioning and 1-aminocyclopropane-1-carboxylate oxidase activity. *Weed research*, 41(2): 165-176.

Mrema, E., Shimelis, H., Laing, M., & Bucheyeki, T. (2017). Farmers' perceptions of Sorghum production constraints and *Striga* control practices in semi-arid areas of Tanzania. *International Journal of Pest Management*, 63(2): 146-156.

Neondo, J. O., Alakonya, A. E., & Kasili, R. W. (2017). Screening for potential *Striga hermonthica* fungal and bacterial biocontrol agents from suppressive soils in Western Kenya. *Biological Control*, 62(5): 705-717.

Obilana, A.T., & Ramaiah, K.V. (1992). *Striga* (witchweeds) in Sorghum and millet: nowledge and future research needs. *International Crops Research Institute for the Semi-Arid Tropics* 1 (CP 741): 87-201

Plessis, J. D. (2008). Sorghum production. *Department of Agriculture. South Africa, Pretoria*.

Runo S, Kuria EK (2018). Habits of a highly successful cereal killer, *Striga*. *PLoS Pathog*, 14 (1)

Schneider, K., & Anderson, L. (2010). Yield gap and productivity potential in Ethiopian agriculture: Staple grains & pulses. *EPAR brief*, *98*.

Spallek, T., Mutuku, M., & Shirasu, K. (2013). The genus *Striga*: a witch profile. *Molecular plant pathology*, 14(9): 861-869.

Staley & Krieg, (1984). *Bergey's Manual of Systematic Bacteriology*

Subbapurmath, P. S. (2012). Development of Microbial Strains for Control of *Striga* spp., A Parasitic Weed (Doctoral dissertation, UAS, Dharwad). NP

Taylor, J. R. N. (2003). Overview: Importance of Sorghum in Africa. In Afripro: Workshop on the Proteins of Sorghum and Millets: Enhancing Nutritional and Functional Properties for Africa, Pretoria 2 (4).

Toh, S., Holbrook-Smith, D., Stokes, M. E., Tsuchiya, Y, & McCourt, P (2014). Detection of parasitic plant suicide germination compounds using a high-throughput Arabidopsis HTL/KAI2 Strigolactone Perception System. *Chemistry & Biology* 21(8), 988–998

Van Mourik, T. A. (2007). *Striga hermonthica* seed bank dynamics: process quantification and modelling. NP

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.

Verma, P., & Majee, M. (2013). Seed germination and viability test in tetrazolium (TZ) assay. *Bio-protocol*, *3*, 884.

Weir, T. L., Perry, L. G., Gilroy, S., & Vivanco, J. M. (2010). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology*, 57(2):233-266

Welsh, A. B., & Kemal, I. Mohamed (2011). Genetic diversity of *Striga hermonthica* populations in Ethiopia: Evaluating the role of geography and host specificity in shaping population structure. *International Journal of Plant Sciences*, 172(6): 773-782.

Wortmann, C. S., Mamo, M., Mburu, C., Letayo, E., Abebe, G., Kayuki, K. C., ... & Ndacyayisenga, T. (2009). Atlas of Sorghum (Sorghum bicolor (L.) Moench): Production in Eastern and Southern Africa. *Intsormil Scientific Publications*. 2.

Yoneyama, K., Awad, A. A., Xie, X., Yoneyama, K., & Takeuchi, Y. (2010). Strigolactones as germination stimulants for root parasitic plants. *Plant and Cell Physiology*, 51(7): 1095-1103.

Yoshida, S., Cui, S., Ichihashi, Y., & Shirasu, K. (2016). The haustorium, a specialized invasive organ in parasitic plants. *Annual Review of Plant Biology*, 67(1): 643-667.

Zahran, E. (2008). Biological control of *Striga* hermonthica (Del.) Benth. using formulated mycoherbicides under Sudan field conditions. PhD. Thesis, University of Hohenheim

7. APPENDIX

Appendix 1. Detailed information of total soils collected for screening and isolation.

Sample code	Collection site				GPS coordinate			Average *Striga* count/m^2
	Region	Zone	Woreda	Kebele	Altitude(m)	Longitude	Latitude	
E01	Amhara	Oromiya	Kewet	Abayatir	1394	09.54.49.3	04.00.53.4	0
E02	Amhara	North shoa	Kewet	Abayatir	1386	09.55.25.3	040.00.45.4	0
E03	Amhara	Oromiya	Jile-Timuga	Kara-Kore	1664	10.27.33.3	039.56.12.4	102.5
E04	Amhara	Oromiya	Jile-Timuga	Merewa	1416	10.17.43.2	033.58.08.6	199.25
E05	Tigray	West Tigray	Haftay-Humera		668	14.11.35.9	036.46.35.2	3.5
E06	Tigray	Tembein	Abergelle	Yechila	1629	13.13.58.3	038.59.33.2	12.5
E07	Amhara	Oromiya	Artuma-Fursi	Chefa-Dire	1457	10.31.17.9	039.55.36.4	1.25
E08	Amhara	Oromiya	Artuma-Fursi	Jara-Kichini	1456	10.30.54.0	039.56.51.3	16.5
E09	Tigray	Tembein	Abergelle	Yechila	1587	13.15.26.2	038.59.36.5	3.5
E10	Tigray	West Tigray	Haftay-Humera	Maykedira	668	13.58.13.6	036.34.35.7	0
E11	Tigray	Tembein	Abergelle	Titay-Hagum	1478	13.25.49.3	038.59.46.6	61
E12	Amhara	Semenwolo	Qobo	Gobiye	1607	11.59.29.2	039.38.20	115.75
E13	Tigray	West Tigray	Tahtay-Adiabo	Qushet	1041	14.34.34.4	037.44.25.7	0

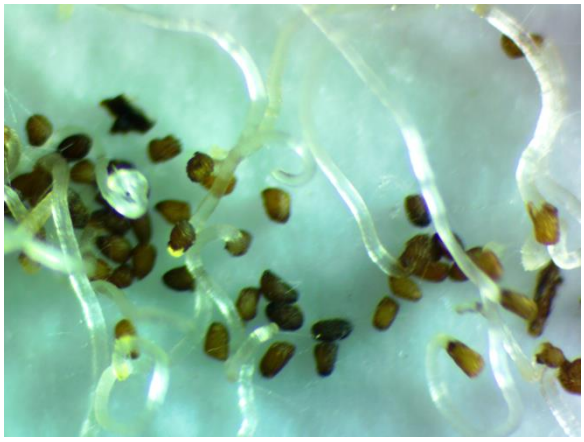
E14	Tigray	Rayaazebo	Alamata	Alamata	1468	12.29.49.7	039.37.30.3	124.25
E16	Amhara	Dese	Wuchale	Chisabalima	1617	11.29.02.4	039.07.14.6	0
E17	Amhara	Oromiya	Jile-Timuga	Merewa	1476	10.19.33.3	033.53.06.3	11.25
E18	Tigray	West Tigray	Haftay-Humera	Maykedira	625	14.10.32.7	036.39.22.5	30
E19	Amhara	North Shoa	Kewet	Abayatir	1373	09.51.11.0	040.01.42.3	2
E20	Tigray	Tembein	Abergelle	Yechila	1455	13.30.09.8	039.02.25.8	0
E21	Tigray	Raya-Azebo	Alamata	Timuga(waja)	1501	12.20.18.4	039.35.17.5	0
E22	Tigray	West Tigray	Tahtay-Adiabo	Gemhalo	1040	14.32.12.4	037.46.36.0	78
E23	Amhara	North Wolo	Qobo		1503	12.06.09.3	039.36.56.6	27.5
E24	Amhara	North Shoa	Kewet	Mariye	1263	09.59.21.4	033.55.26.1	67.5
E25	Tigray	West Tigray	Tahtay-Adiabo	Simret	1079	14.24.22.8	037.52.11.6	46.75
E26	Tigray	West Tigray	Haftay-Humera		619	14.14.46.7	036.39.46.2	53.25
E27	Tigray	Raya-Azebo	Alamata	Alamata	1513	12.21.53.3	039.34.36.2	10.5
E28	Tigray	West Tigray	Tahtay-Adiabo	Qushet	1040	14.39.40.1	037.45.37.8	0
E29	Tigray	West Tigray	Haftay-Humera	Maykedira	635	14.10.27.4	036.36.11.3	2.5
E30	Amhara	Oromiya	Artuma-Fursi	Hulatukuye	1460	10.33.46.8	039.55.50.5	0
E31	Tigray	West Tigray	Haftay-Humera		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	North Wolo	Raya-Azebo	Kebele 08	1441	12.06.00.3	039.38.52.7	0
E34	Tigray	West Tigray	Haftay-Humera	Maykedira	621	14.0457.6	036.34.23.2	0
E35	Tigray	West Tigray	Haftay-Humera	Maykedira	642	14.02.11.9	036.34.2.21.7	56
E36	Tigray	Raya-Azebo	Alamata	Alamata	1494	12.25.46.4	039.35.05.7	28

E37	Amhara	North Shoa	Bekewot	Debrinajegol	1281	09.56.29.4	033.57.52.4	21.75
E38	Amhara	Oromiya	Jile-Timuga	Merewa	1421	10.17.38.8	033.58.03.3	17.5
E39	Tigray	West Tigray	Haftay-Humera		628	14.14.14.6	036.40.52.5	64.25
E40	Amhara	Oromiya	Artuma-Fursi	Jarakichini	1457	10.30.54.2	039.58.47.7	4.75
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
E45	Tigray	West Tigray	Haftay-Humera	Maykedira	704	13.55.22.6	036.33.08.9	0
E46	Amhara	North Wollo	Raya-Azebo	Kebele 07	1425	12.05.26.2	039.39.26.8	2.25
E47	Amhara	Oromiya	Jile-Timuga	Merewa	1407	10.17.57.3	033.53.29.3	0

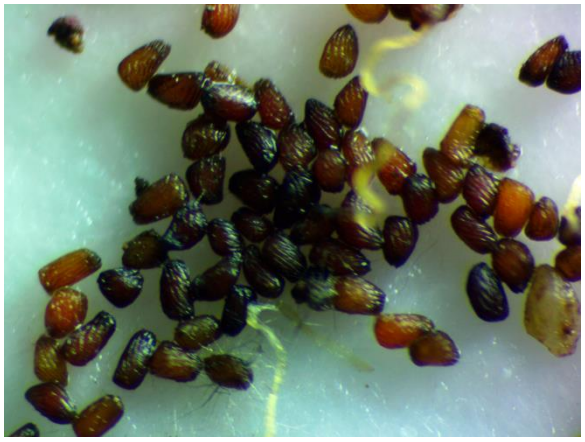
Appendix 2. Physico-chemical properties of soils from which the target bacterial isolates were obtained

Soil code	AV P (PPM)	% TN	CEC meq/ 100gm soil	pH	OC (%)	% clay	% silt	% sand	K meq/ 100g soil	Ca meq/ 100g soil	Mg meq/ 100g soil	Na meq/ 100g soil	Cu in ppm	Fe in ppm	Mn in ppm	Zn in ppm	acidity
E14	11.2	0.1	27.6	7.8	0.9	11.3	32.5	56.3	1.0	42.3	3.8	0.1	0.7	20.8	2.0	0.7	slightly alkaline
E25	4.4	0.1	33.8	8.4	2.0	56.3	27.5	16.3	1.1	40.4	12.7	0.2	1.9	38.7	3.0	0.1	strongly alkaline
E29	141.7	0.1	62.5	6.9	4.3	36.5	37.0	26.5	1.0	82.0	27.0	0.9	1.2	109.9	5.4	5.8	neutral
E30	261.8	0.1	69.3	6.9	4.1	32.5	40.0	27.5	0.9	93.1	24.1	1.1	1.0	112.5	6.8	7.8	neutral
E34	5.1	0.0	49.4	7.9	2.0	58.8	15.0	26.3	1.1	66.5	20.5	0.1	0.8	20.1	6.2	0.0	slightly alkaline
E39	4.0	0.1	59.5	8.5	2.3	61.3	15.0	23.8	0.9	91.5	24.5	0.5	0.7	15.3	2.8	0.0	strongly alkaline

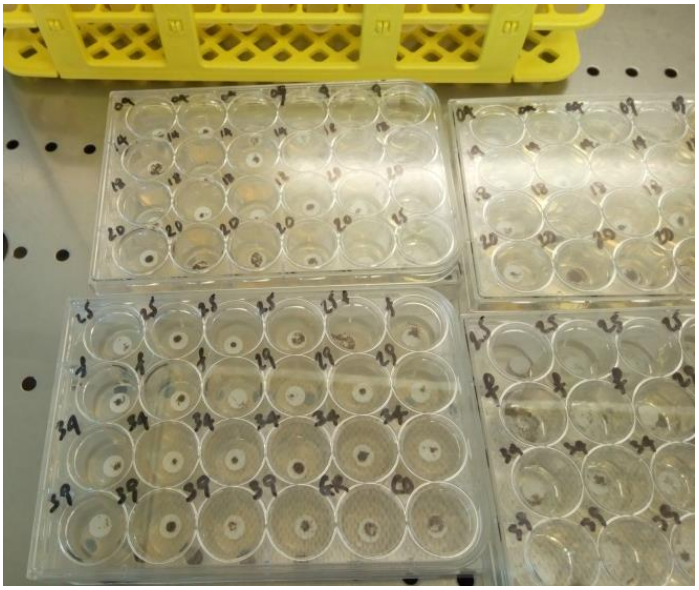
Appendix3. Germinated and decayed *Striga* seeds.



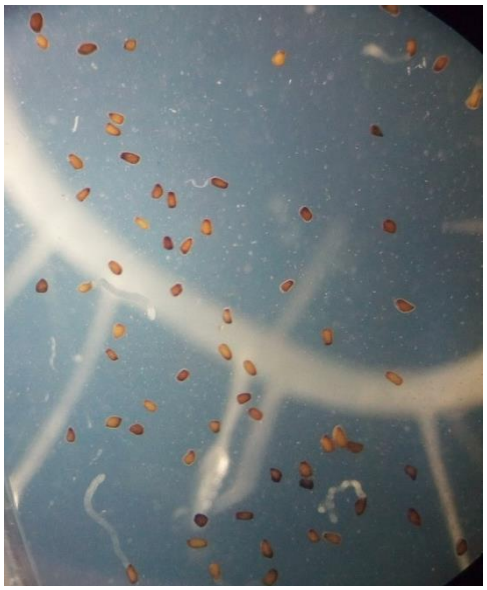
A



B



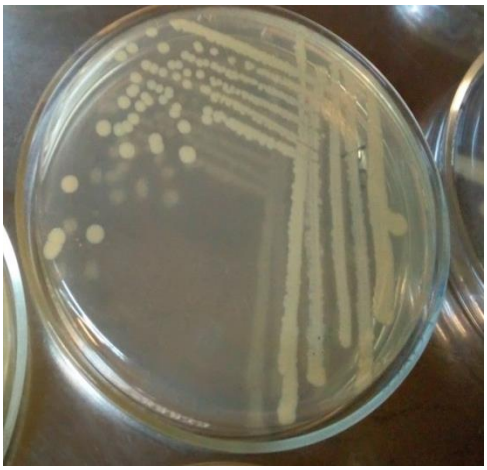
C



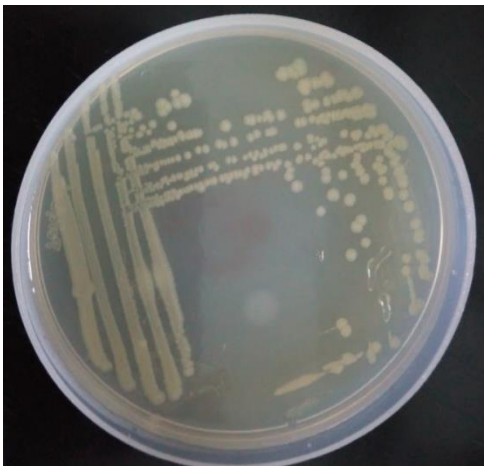
D

A; germinated *Striga* seeds, B; non-viable *Striga* seeds, C; tissue culture plate test and D; agar plate test

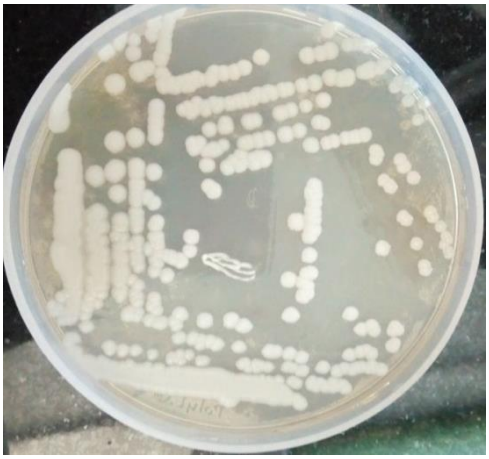
Appendix 4. Colonies sample bacterial isolates



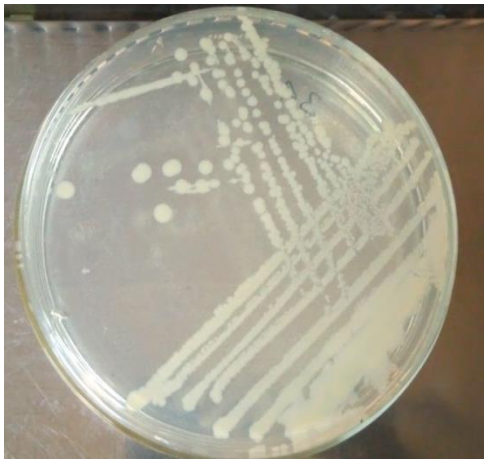
A



B

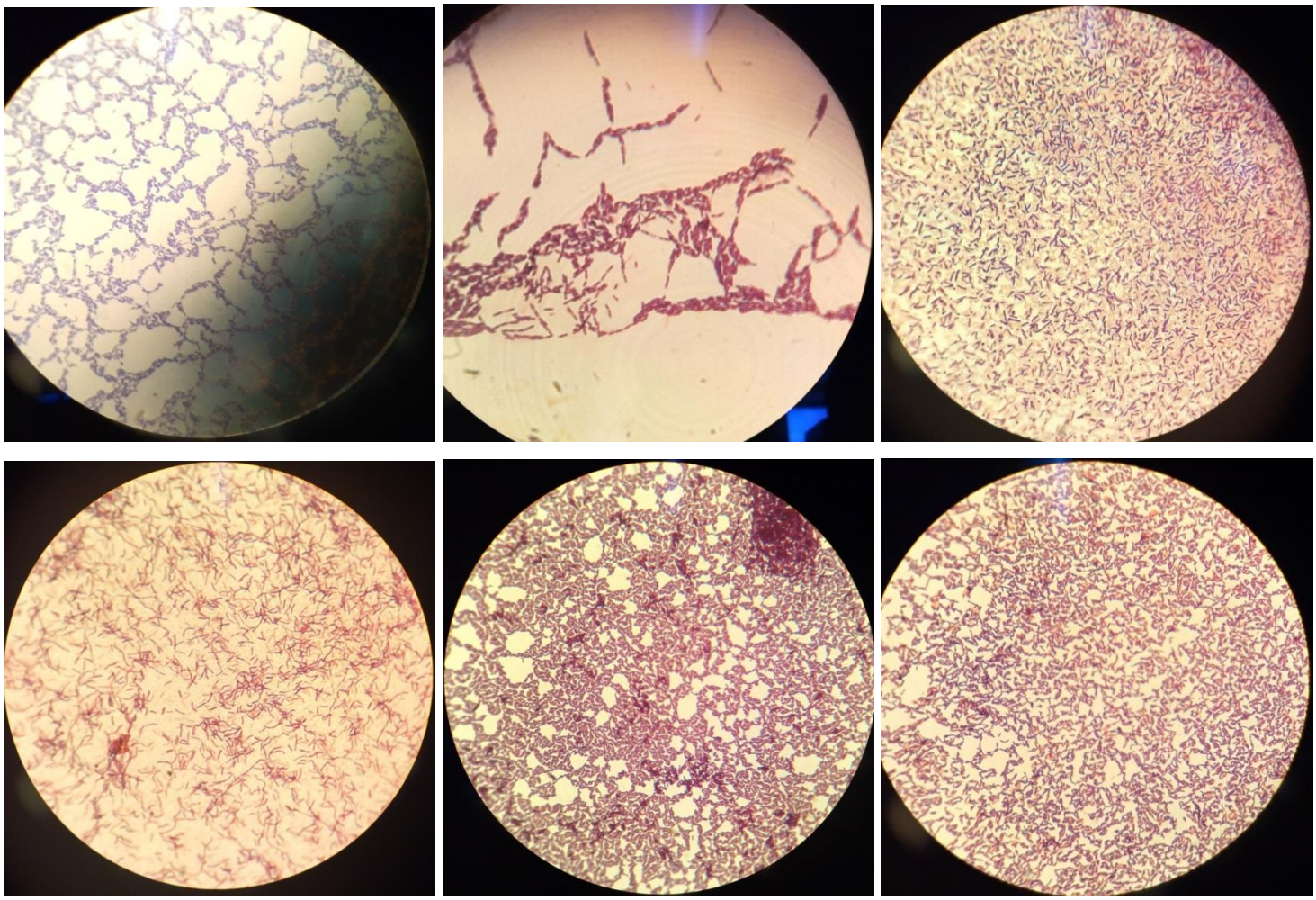


C



D

Appendix 5. Gram stain result of some isolates



Appendix 6. Pictures of biochemical tests.

A

B

C

* A; Catalase test result, B; citrate test result and C; motility test results.