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Screening and Identification of Potential *Striga* [*Striga hermonthica (Del.)*] Suppressing Rhizobacteria Associated with Sorghum [*Sorghum bicolor* (L.) Moench] in Northern Ethiopia

Urgesa Tsega Tulu[1*], Teklehaimanot Haileselassie[2], Sewunet Abera[1] and Taye Tessema[3]

[1] *Ethiopian Institute of Agricultural Research, Agricultural Biotechnology Research Center, Holeta, Ethiopia*
[2] *Addis Ababa University, Institute of Biotechnology, Addis Ababa, Ethiopia*
[3] *Ethiopian Institute of Agricultural Research, Addis Ababa Ethiopia, Ethiopia*
* Corresponding author, E-mail: urgesatsega@gmail.com

Abstract

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the globally important cereal crops well adapted to Sub-Saharan Africa (SSA) agro-ecology. However, the productivity of sorghum is hindered by both abiotic and biotic factors including drought, *Striga*, insect pests, poor soil fertility, and diseases. Among the constraints, *Striga* (genus), also called witch weed, is the most important production problem in the area. Although there were various control methods practiced for years, none of these became practically effective to eradicate *Striga*, neither they are easily accessible for small holder farmers, while still some are not environmentally friendly. Therefore, this study was designed with the objective of identifying potential Striga suppressing rhizobacteria associated with sorghum. Treatment of *S. hermonthica* seeds with isolates E19G12, E29G2b and E19G10 resulted in the lowest *S. hermonthica* seed germination of 0%, 1%, and 2.7% respectively, which were significantly lower than any of the treatment. Mean germination percentage ranged from 9 to 59.7 and 0 to 27 in the absence and presence of host plant, respectively. The results showed a statistically significant germination inhibition ($P < 0.001$). Finally, shortlisted most effective isolates E19G6a, E19G9, E19G6b, E19G10, E19B, E19G12, E29G2a and E29G7 were morphologically and biochemically identified to belong to the genera of *Pseudomonas*, *Klebssiella*, *Bacillus* and *Entrobacter*. The result of the study demonstrated the existence of promising soil-borne bacteria that could be exploited as a bioherbicides to control *Striga* infestation on sorghum provided that broader samples from various parts of the country are explored.

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Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) belongs to the *Poaceae* family and it is one of the globally important cereal crops. It is widely grown in the semi-arid tropics, where frequent drought is experienced. It is the fifth in line of production after maize, rice, wheat, and barley and feeds over 500 millions of people in the developing world[1–3]. Ethiopia is the fourth top sorghum producing country in the world following the United States of America, Nigeria, and Mexico[4]. Out of the total cereal production of the country, sorghum accounts for 18.5% with a productivity of about 2.8 tons per hectare[5].

However, due to abiotic and biotic constraints, sorghum potential productivity is being compromised. Among the abiotic factors are low soil fertility, drought, and salinity. Agriculturally important biotic constraints include the hemi-parasitic weed *Striga*, panicle diseases, stem borers, and insects[6]. In Ethiopia, sorghum production challenges associated with both biotic and abiotic constraints vary from region to region. However, drought and *Striga* (*Striga hermonthica*) are the most important problems across the country[7].

Several of the *Striga* control approaches widely investigated and developed include cultural, chemical, biological, genetic or breeding for resistance and a combination of more than one of these[8–10]. Many of these methods are either not practically successful or not economically feasible for low-income farmers in SSA[11].

Small-scale farmers particularly in Northern region of Ethiopia need easy, accessible, and effective *S. hermonthica* management strategies that are compatible with their production practices. Soil borne bacteria have potential to perturb the early stages of *Striga* and *Orobanche* growth by reducing their incidence by 90 to 100 percent[12]. It has been also showed that soil- borne fluorescent *Pseudomonad* strains suppressed the germination of *S. hermonthica* and *Orobanche* seeds[13]. Moreover, a few pathogenic bacteria were found to be effective to control *S. hermonthica* and replace commercial chemical herbicides[14]. Furthermore,[15] showed the significant suppression of the key stages of *Striga* development by *Bacillus* strains. The study by[16] identified microbes that were potent against *S. hermonthica* and proposed their use in the reduction of *S. hermonthica* seed bank in infested soils. The communication between microbes and *S. hermonthica* depends on signal transduction, the expression of pathogenicity, and virulence factors of the microbe[16]. Thus, inoculation of microbes such as rhizobacteria could minimize the competition of cereal crops with weeds and may reduce the use of chemical herbicides and could benefit agriculture contributing to increased crop yields.

However, isolation, characterization, and utilization of specific microbial agents capable of causing *S. hermonthica* seed decay has not been much exploited in Ethiopia. Therefore, the aim of this study was to identify specific rhizobacteria that have the potential of suppressing *Striga* infestation on sorghum.

Materials and Methods

Description of study area

The experiment was carried out in Microbial Biotechnology and *Striga* Bioassay Laboratories at National Agricultural Biotechnology Research Center (NABRC), Holeta. NABRC is located at 9°3'N latitude and 38°30'E longitude, 34 km away from Addis Ababa, in the central part of Ethiopia, West Shoa Zone of Oromia Regional State.

Source of experimental materials

Soil sample

Soil samples were collected from three sorghum growing fields (sites) in northern region of Ethiopia during the 2018 main crop season. These were Artuma Fursi district in Oromia zone of Amhara region (site 1), Kewet district in Semien Shoa Zone of Amhara region (site 2), and Qaftay Humera in the district West Tigray zone of Tigray region (site 3) with medium, low and high *Striga* infestation, respectively (Fig. 1). In each site, the soil sample was collected from four random spots in four quadrants after locating the fields using Global Positioning System (GPS) coordinates and recording the altitude for each site (Supplementary Table S1). Soil samples were collected using a sterile shovel at a depth of 20–30 cm and put into a labeled clean plastic bag and transported to the greenhouse facility at National Agricultural Biotechnology Research Center, Holeta.

Sorghum seeds

Seeds of different sorghum germplasm that are known to be *Striga* susceptible, *Striga* resistant, drought tolerant, widely used, released, and local land races were used in this study (Table 1). The seeds were stored at the National Agricultural Biotechnology Research Center cold room by the national Integrated *Striga* Control (ISC) project under Ethiopian Institute of Agricultural Research.

Striga seeds

S. hermonthica seeds used for this experiment were collected from *S. hermonthica*-infested sorghum in farmers' fields in Ethiopia, Tigray Region, Central Zone Abergele District Titay Hagum Kebele during 2018 main cropping season [altitude (m): 1466; latitude (East): 13.25'51.8"; longitude (North): 038.59'50.3"].

Planting sorghum on the soil in the greenhouse

Seeds of sorghum that were well matured and with good morphological characteristics were selected and surface sterilized in 1.5% bleach for 30 minutes. The seeds were then allowed to germinate for about 30 hours in an incubator set to 30 °C. Seedling was transferred to a pot and grown on the soils collected from various sorghum growing sites in greenhouses. The management including watering and weeding was made accordingly until it was set three leaves ready for harvesting rhizosphere soil samples.

Rhizosphere soil collection

Rhizosphere soil sample collection was made following the method described in[17]. After setting the third leaf, the sorghum was uprooted and vigorously shaken by hand for five min until non-adhering soil was completely removed. Rhizo-

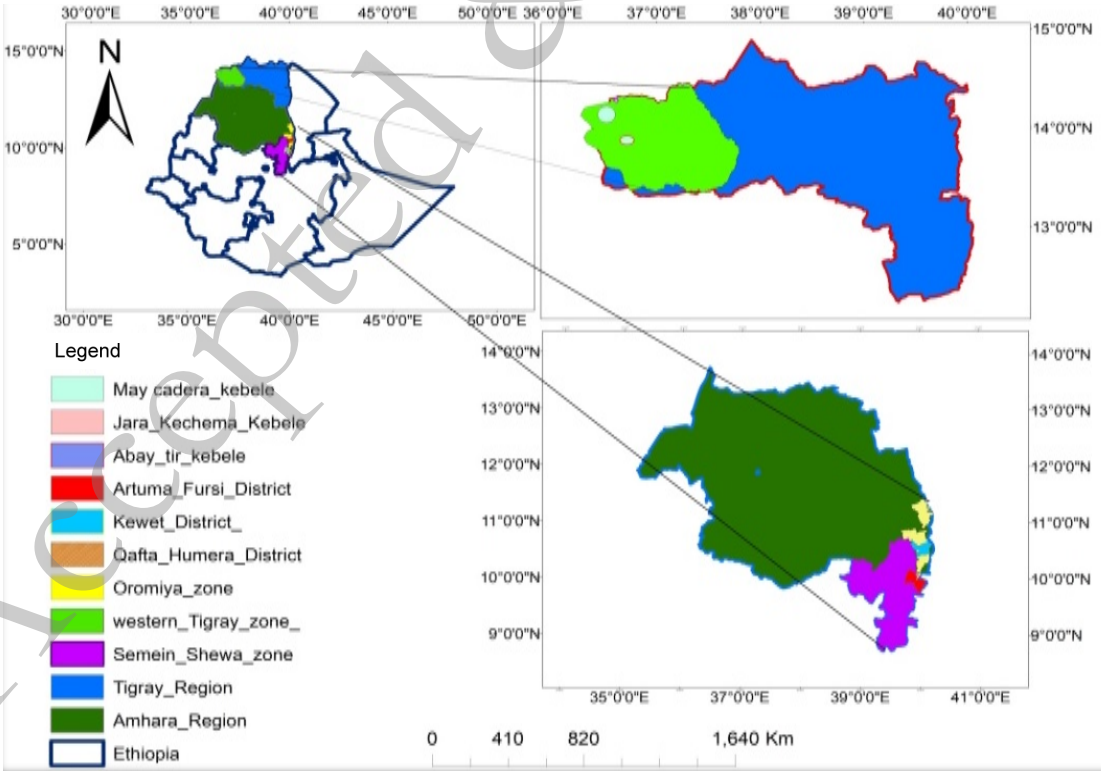

Fig. 1 Map showing soil sample collection areas.

Table 1. Sorghum genotype selection for greenhouse planting and isolation of bacteria

Code	Name	Source	Character	Selection criteria
G1	ETSL101847	Tigray	Local land race	Land race and widely used
G2	ETWS 90754	Amhara	Wild type	Wild type
G3	ETWS 91242	Beneshangul	Wild type	Wild type
G4	Framida	Purdue University	*Striga* resistance	*Striga* resistant and widely used
G5	ETSL100046	Land race	LGS	Land race and LGS
G6	ETSL101853	Land race	HGS	Land race, widely used and HGS
G7	Misikir	MI_Drought_Score	Drought tolerant	Drought tolerant
G8	S35	ICRISAT	Stay green	Stay green or Drought tolerant
G9	Shanqui red	China	*Striga* susceptible variety	HGS and *Striga* susceptible variety
G10	SR5-Ribka	IBC	*Striga* resistant and fusarium compatibility	*Striga* resistant and fusarium compatibility
G11	SRN39	Purdue University	Striga resistance	*Striga* resistant and widely used
G12	Teshale	ICRISAT	Best released susceptible varieties	Widely used

Note: LGS = low germination stimulant; HGS = high germination stimulant, G = genotype.

sphere soil was collected by removing the sorghum's soil parts with a sterile blade and shaking the roots for 10 min in 50 ml falcon tubes containing 35 ml sterile distilled water to remove the adhering soil. The soil suspensions were then incubated to homogenize the soil content on a shaker (300 rpm, 90 min, and 25 °C) before being centrifuged at room temperature for 10 min to concentrate soil particles in the pellet.

Rhizosphere bacteria isolation

Rhizosphere bacteria were isolated by serial dilution technique. One gram of each soil pellet was suspended, each in 90 ml sterile distilled water in a 50 ml falcon tubes and mixed thoroughly overnight using a mechanical shaker at 110 rpm, until completely dispersed. Then a 100 µl aliquot was transferred with sterile pipettes to 9 ml sterile 0.85% saline solution in a test tube. A serial dilution (up to 10^{-8}) was prepared. From each $10^{-4,}$ $10^{-5,}$ and 10^{-6} serial dilutions, 0.1 ml of an aliquot was spread on nutrient agar on Petri-dishes (90 mm), for each dilution in triplicate. Plates were incubated at 28 °C for 24 hours. Representative types of bacterial colonies were further purified by sub-culturing on fresh medium and used for downstream work or stored in 35% glycerol at −80 °C[17–19].

In vitro screening of rhizoabcterial isolates for their biocontrol traits

Rhizobacteria isolates were first screened for production of HCN followed by screening HCN positive isolates for IAA production. Common producers of HCN and IAA isolates were selected for further evaluation of their effects on *S. hermonthica* germination inhibition *in vitro*.

Hydrogen Cyanide (HCN) Production

Hydrogen cyanide production by the bacterial isolates was tested qualitatively using the methods followed by[20] with slight modification in incubation period. The bacterial isolate cultures were streaked on Trypto Soya Agar amended with 4.4 g/l glycine. Whatman filter papers were sterilized and soaked in 2% sodium carbonate in 0.5% picric acid solution was placed in the top of each plate. Plates were sealed with parafilm and incubated for 4 days at 28 °C. The change in the color of Whatman filter paper from yellow to light brown, brown or reddish brown was observed as an indication of weak, moderate or strongly hydrogen cyanide producers, respectively.

Indole Acetic Acid (IAA) Production

The ability to produce IAA of the isolate was detected from the culture of the bacterial isolates following the procedure described by[21]. Briefly, pure colonies from a 24-hr. culture were inoculated into nutrient broth supplemented with 2 % tryptophan and in the absence of tryptophan (control), and incubated at 28 °C for 48 hrs. Five milliliter culture was removed from each tube and centrifuged at 12,000 x g for 15 min. Two milliliter aliquot of the supernatant was transferred to a fresh tube. This was then treated with 2 ml salkowsky reagent (1 ml 0.5 M FeCl in 50 ml $HClO_4$) and incubated at room temperature for 25 min. Development of pink color indicates positive result for IAA production.

In vitro evaluation of the effects of selected isolates on Striga germination

Each common HCN and IAA producing isolates was evaluated for their *Striga* germination and seed decay activity in *Striga* bioassay laboratory using Agar Gel Assay (AGA) and Whatman filter paper. To do this, *S. hermonthica* seed germination test was conducted to determine its viability prior to use in in *vitro* evaluation of the effects of rhizobacterial isolates on the seed in the absence and presence of susceptible hosts. However, the seed had to be exposed to the right environmental conditions for the optimum period of time to break dormancy and ready for germination. Hence, the *Striga* seed was conditioned by incubating at 29 °C for 10 - 14 days. In each case, *S. hermonthica* seed was treated with each isolate and germination percentage computed to see the germination inhibitory activity of the isolates[22,23].

Determination of germination percentage of S. hermonthica

S. hermonthica seed surface sterilization and preconditioning was made according to the protocol used by[23]. First, seeds were surface sterilized in 75% ethanol under a hood in a 50 ml flask for 2 minutes and rinsed three times with sterile double distilled water. This was followed by washing the seed with activated metricide (fungicide) for 2 minutes and rinsed three times with sterile double distilled water. Finally, 14.5 ml ddH_2O and 1.5 ml of Benomyl solution (conditioning solution) was added to the flask. The flask was wrapped with aluminum foil and incubated at 30 °C for 10 days for preconditioning.

After 10 days of preconditioning, about hundred sterilized *S. hermonthica* seeds were transferred into a sterile glass fiber disc on a Petri plate lined with moist Whatman filter paper. Three glass fiber discs on each plate containing preconditioned *S. hermonthica* seeds were germinated by adding 20 µl of 0.1 ppm GR24 and incubated for 2 days at 28 °C[24]. Negative

controls containing preconditioned *S. hermonthica* seeds were added to sterile distilled water. The numbers of germinated and non-germinated *S. hermonthica* seeds were counted using a binocular microscope fitted with a digital camera (Power Shot A640, Canon Inc., China). Germination percentage of *Striga* was determined by counting the total number of seeds on each disc and germinated *Striga*[23,16].

Evaluation of the effects of the Isolates on S.hermonthica seed germination in the absence of host Plant

The isolates were evaluated for their ability to reduce *Striga* germination by using GR-24, a synthetic germination stimulant. *Striga* seed surface sterilization and preconditioning was done as described in a previous section. About 100 preconditioned *Striga* seeds were added to glass fiber disc placed in Petri plate lined with double sterile filter papers and moistened with 3 ml of sterile ddH_2O. The experiment was replicated 3 times each (three glass fiber discs per Petri plate). The seeds on the disc were treated with 100 µl of three days old bacterial suspensions in broth. In the control treatment, blank broth was added to discs containing preconditioned *S. hermonthica* seeds. The Petri plates were sealed with parafilm and incubated at 30 °C in the dark for 48 h.

After 48 h, 20 µl of 0.1 ppm GR24 was added to keep the germination uniform except for the effect of isolates and further incubated overnight at 29 °C. The number of total *S. hermonthica* seeds and the number of germinated/ inhibited per replicate was recorded under a stereomicroscope fitted with a camera[16]. Germination percentage for each replicate was calculated using the formula described in[23].

$$\text{Germination percentage (GP)} = \frac{\text{Number of germinated Striga seeds}}{\text{Total number of Striga seeds}} \times 100$$

Evaluation of the effects of the selected isolates on S. hermonthica seed in the presence of susceptible host

Striga conditioning was made as explained in the above section, but in this case, it was embedded in agar (bacto agar) solution after 5 days. By using a glass Pasteur pipette, a drop of preconditioned *Striga* seeds were added to the center bottom of a sterile plate in the conditioning flask. The seeds were treated with 0.5 ml of three days old of bacterial suspension in broth and kept for 30 min. In the control treatment, seeds were treated with blank nutrient broth media. Each treatment was replicated three times and arranged in RCD in an incubator at 30 °C. 0.7% (g/l) agar solution was prepared and autoclaved for 15 min and then allowed to cool in containment room water bath to 50 °C. The liquid agar was directly poured over the *striga* under hood until the agar reaches the sides of the plate and the *striga* seeds were distributed evenly across the plate. Plates were allowed to cool for 10 minutes before covering and placed in a dark at 30 °C in incubator for 10 days from the conditioning start date of the *Striga* seed.

Simultaneously with *Striga* conditioning, surface sterilization was made on susceptible sorghum seeds called Teshale using 1.5% bleach (containing a drop of Tween-20) and agitated three times for 30 minutes. The bleach solution was then poured off and rinsed 2 times with sterile ddH_2O. The seeds were then soaked overnight to imbibe in 5 ml of a 5% (w/v) Captain solution. Next day, the Captain slurry was poured off under a laminar flow hood and rinsed with 5 ml sddH_2O. Then, the seeds and water were poured into labeled sterile Petri dishes, each containing two Whatman filter paper (90 mm) circles and incubated until radicles emerged[23].

Next day, the germinated sorghum was gently picked up with sterile forceps and planted 1 cm from the edge of the plate pointing to the center of the plate in agar in which the *Striga* seeds were already embedded. The plates were incubated at 30°C in an incubator where they remained for 3 days.

After 3 days, a 2 × 2.5 cm area measured was made along the main sorghum root 2 cm from the kernel at the back side of the agar plate using a thick water-resistant marker pen. This area is with high probability of *Striga* seeds coming into contact with sorghum root exudates. Total and germinated *Striga* seeds in each area were counted under a stereomicroscope and germination percentage computed using the methods described in the previous section[25,15].

Morphological and biochemical characterization and identification of the most effective rhizobacteria isolates

The most efficient bacterial isolates with production of HCN and IAA and corresponding inhibitor of *S. hermonthica* indicated by low mean germination percentage were selected. These isolates were morphologically and biochemically characterized using the method followed by[26] and[27] as described below.

Morphological characterization

The efficient bacterial isolates were characterized by growing on nutrient plate for 24 hr at 28 °C. Best candidate of bacterial isolates were observed under stereomicroscope for colony size, shape, color, arrangement and gram reaction. For Gram staining, slide was cleaned with detergent and marked by codes of isolates. With the help of sterile wire loop, single colony of bacterial culture was made on clean glass slide and air dried and heat fixed. Then smear was covered with crystal violet for 1min and slide was washed with drop of distilled water. Smear was covered with 2 drops of iodine solution for 30 seconds and slide was washed with alcohol and then distilled water. The smear was covered with 1 drop of safranin for 1min and then washed by distilled water, air dried and observed under microscope.

Biochemical characterization

Each efficient bacterial isolate was tested for sugar utilization, production of methyl red, indole and catalase. This would help to identify the isolates at genus level.

Sugar utilization test

The ability of the isolates to utilize carbohydrate sugars as a sole carbon source was determined in broth media containing specific sugar (glucose, fructose and sucrose) and Bromocrsol purple (0.4 g/l). A 96 deep well ELISA plate filled with 1ml broth was inoculated by 0.1 ml of fresh culture in triplicate including control. The culture was incubated at 28 °C for 24 hrs and observed for the formation of yellow color as positive results.

Methyl red test

Broth containing (5 g of each Peptone, Glucose, Potassium phosphate and 1000 ml distilled water; pH = 7) was prepared and steam sterilized using autoclave. In test tubes, 1.5 ml of the broth was poured and each was inoculated with test organism, and then incubated at 28 °C for 48 hrs. Four drops of methyl red indicator was added to each tube and gently shaken for 30 seconds. The tubes were kept for 15 minutes and observed for

color change (where, positive test = bright red and negative test = yellow to orange)

Catalase test

The nutrient agar slants were inoculated with test isolates. An inoculated nutrient agar slant was kept as control. The cultures were incubated at 28 °C for 24 hrs. A loop full of bacterial culture was kept on a clean slide with the isolate label. A drop of 3% hydrogen peroxide was added on a slide. The culture was then observed for the gas bubble formation.

Statistical analysis

All the experimental units were arranged in CRD. Data on effects of selected isolates in *S. hermonthica* seeds germination was recorded. R software version 3.5.3 was used to perform analysis of variance (ANOVA) for all measured data. Tukey's test was used to compare and separate the means for significance level at 5%.

Results

Bacteria isolation and screening for *Striga* suppressive traits (HCN and IAA)

A total of 117 bacteria were isolated from rhizosphere of 12 sorghum varieties grown on soil collected from three different sorghum growing regions in Ethiopia (Supplementary Table S2). The isolates were first tested for their qualitative hydrogen cyanide production on a nutrient agar plate. From these, only 47 (40.2%) of the isolates were found to produce HCN with different levels (low producers, medium producers and strong producers).

Forty seven isolates capable of producing HCN were again tested for IAA production, another weed suppressive trait of rhizobacteria. Accordingly, 6 (12.8%) isolates were strong producers, 9(19%) were moderate producers and 7(14.9%) were low or weak producers and 25(53.2%) were not producers of IAA at all.

From both test, 21 isolates were common producers of HCN and IAA. These include E19G1, E19G3, E19G6a, E19G9, E19G11a, E19G6b, E19G10, E19B, E19G7, E19G11b, E19G12, E29G2a, E29G11, E29G2b, E29G9, E29G7, E40G1a, E40G5, E40G1b, E40G10 and E40G12. These isolates were selected for further evaluation of their effects on *S. hermonthica* germination inhibition *in vitro*. Majority of the selected isolates did vary in their HCN and IAA production abilities. Some strong producers of HCN were comparably moderate and weak producers of IAA and vice versa. A few isolates, however, showed similarity in their HCN and IAA production. Two isolates, namely E19G12 and E29G7 were strong producers of both HCN and IAA in common.

Determination of germination percentage of S. hermonthica

In this study, the germination test for *S. hermonthica* resulted in 63% germination upon conditioning the seeds for 10 days and treating with GR-24, a synthetic germination stimulant.

In vitro evaluation of the effects of selected isolates on GR-24 induced germination of S. hermonthica

In this study, bacterial isolates were evaluated for their effects on *Striga* germination/inhibition *in vitro*. The results of the assay showed that significant differences ($p < 0.001$) were observed between some isolates on the effects of rhizobacteria isolates on GR-24 induced *S. hermonthica* germination in the filter paper (Figures 2 and Figure 3). Regardless of considerable variation in their inhibition effects, all isolates showed a significant reduction in germination percentage compared to the control (broth treatment). But, the extent of germination inhibition varies from 9 to 59.7 mean germination percentage.

Evaluation of the effects of selected isolates on S. hermonthica seed germination in the presence of susceptible host

This was an activity done as an alternative to the greenhouse evaluation to see whether there are similar or different trends compared to the evaluation using GR-24 as a stimulant. The study indicated that the germination of *Striga* in the presence of host plant was lower than that of GR- 24 induced germination in all treatments. There was significant difference

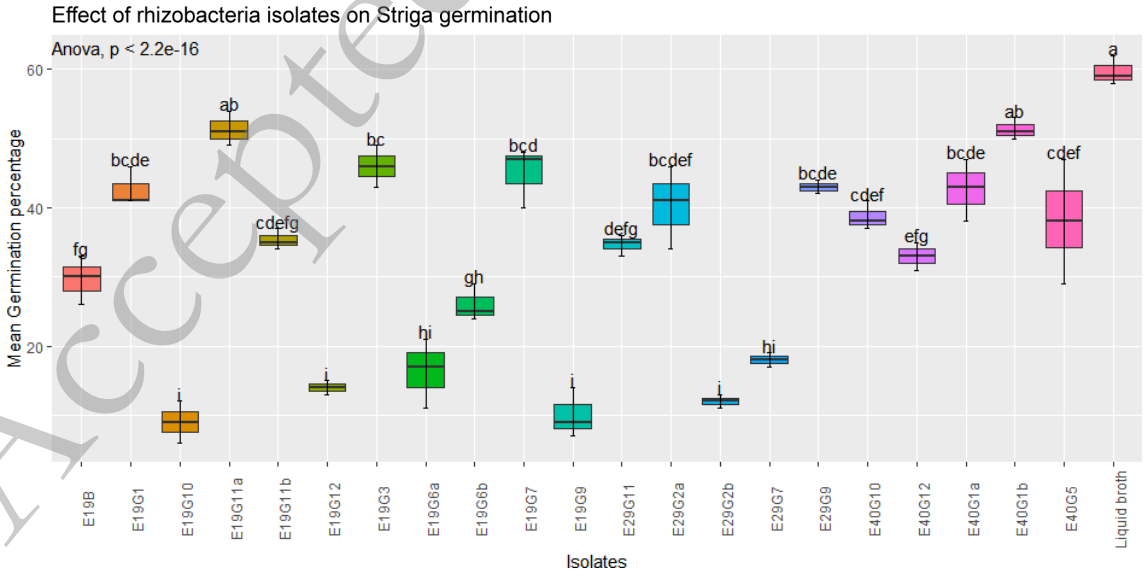

Fig. 2 Effect of rhizobacteria isolates on GR-24 induced S. hermonthica germination in filter paper assay. Values are means of combined data of three replicates each. Means followed by the same letter are not statistically different at $p \leq 0.05$ according to the Tukey-test.

Fig. 3 Glass Fiber Filter Paper based *S. hermonthica* germination assay

($p < 0.001$) in mean germination percentage among isolates (Figures 4 and Figure 5).

A few isolates showed increased germination of *S. hermonthica* seeds, while many of them showed a significant suppression of *S. hermonthica* seed germination ($p < 0.001$) compared to control treatment (broth). The highest germination percentage (27%) were recorded in the control (blank broth treated seeds), followed by isolates E19G9 (24%) and E40G5 (20%). Treatment of *S. hermonthica* seeds with isolates E19G12, E29G2b and E19G10 resulted in the lowest *S. hermonthica* seed germination of 0% 1%, and 2.7% respectively, which were significantly lower than any of the treatment (Figure 4). Germination inhibition followed an almost similar pattern to the treatments without the presence of host plant except that treatment with the synthetic stimulant GR-24 caused an elevated germination percentage compared to treatment in the presence of host plant sorghum.

Regarding mean germination percentage in the absence of host plant, eight isolates, namely, E19G6a, E19G9, E19G6b, E19G10, E19B, E19G12, E29G2b and E29G7 showed significant inhibition of *S. hermonthica* germination as indicated by low mean germination percentage, 16, 10, 26, 9, 29.7, 14, 12 and 18, respectively. Similarly, some isolates that have indicated high *Striga* germination inhibition in the absence of host plants also showed reduced germination in the presence of host plants although not correspondently or in the same pattern. There was also no consistent pattern in all isolates and parameters evaluated in association with various sorghum genotypes from where they have been isolated and the three soil types. But, majority of the bacterial isolated from the soil of low *Striga* infested site (E29) potentially inhibited *Striga* germination in the absence of host plant.

Morphological and biochemical characterization and identification of the most effective rhizobacteria isolates

Finally, upon *in vitro* evaluation, eight efficient rhizosphere bacteria isolates with different *Striga* suppressive effects were further morphologically and biochemically characterized for gram reaction, colony color, size, shape, margin, elevation, sugar utilization ability, catalase and methyl red test. Accordingly, 6 (75 %) of the rhizosphere bacteria inhibiting *Striga* germination were found to be gram negative, 2 (25%) gram positive, 4 (50%) glucose positive, 7 (87.5%) fructose positive, 2 (25%) sucrose positive, 6 (75%) methyl red positive, 6 (75%) catalase positive (Table 2 and Table 3).

Based on the comparative analysis of various morphological and biochemical characteristics, the bacterial isolates are identified to fall under four genera: *Bacillus*, *Pseudomonas, Enterobacter* and *Klebsiella*. Among the bacterial genera, four were *Pseudomonas,* two *Bacillus*, one *Enterobacter* and one *Klebsiella* (Table 3).

Discussion

The potential of growth suppressive effects of rhizobacteria and their possible use as a biological control options in the management of *S. hermonthica* have been investigated to be agriculturally important to boost crop productivity[28,16]. A group of microorganisms with potential as biological control agents of weeds are the deleterious rhizosphere inhabiting bacteria (DRB) characterized as nonparasitic rhizobacteria colonizing plant root surfaces and being able to suppress plant

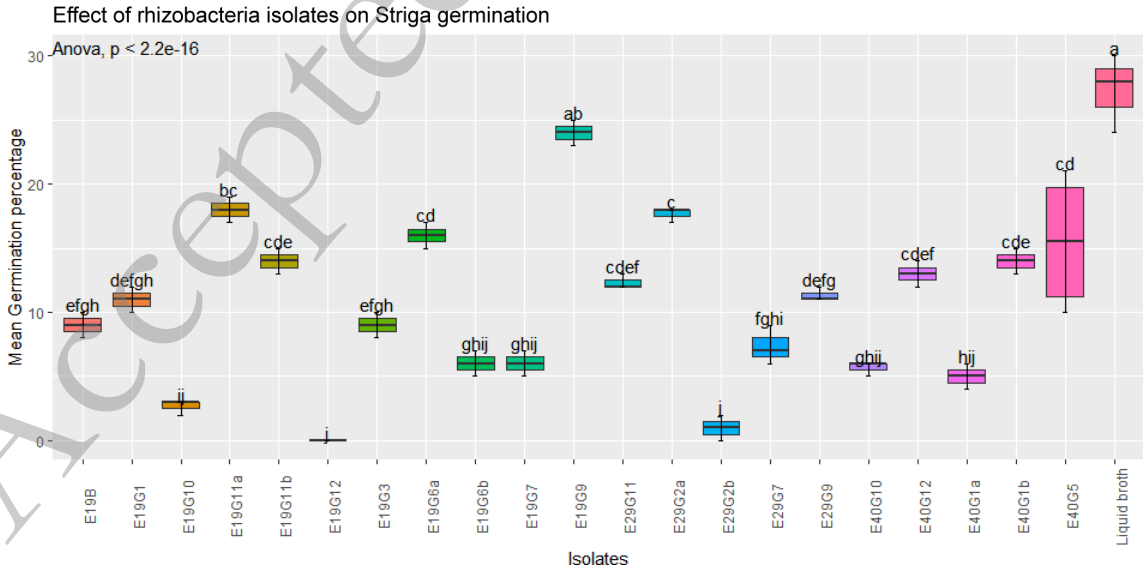

Fig. 4 Effects of rhizobacterial isolates on *S. hermonthica* seed germination in the presence of susceptible host plant.Values are means of combined data of three replicates each. Means followed by the same letter are not statistically different at p≤0.05 according to the Tukey-test.

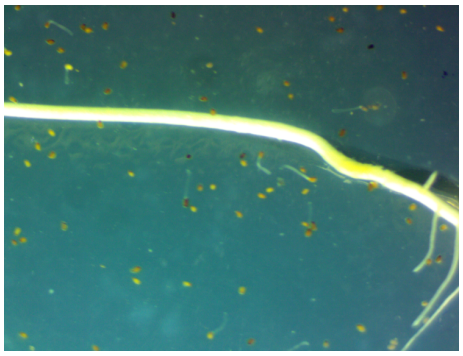

Fig. 5 *Striga* seed germination using agar gel assay.

growth[29,30,31]. Rhizosphere is the zone at the interface of soil-plant roots that harbors the most complex microbial communities[30,32]. The deleterious activity toward weed seed viability and seedling growth by most microorganisms under study for biological control is due to the production of phytotoxins. The common metabolites produced in the rhizosphere of plants that can be phytotoxic at higher than physiologic concentrations include the auxins and hydrogen cyanide[33]

HCN producing rhizobacteria have been known to act as biocontrol agents against weeds[34] In this study, 117 rhizobacteria isolates were tested for HCN production and 47(40.2%) were capable of producing HCN with different levels. Out of which, 11(23.4%) isolates were strong producers, 15(31.9%) were moderate producers and 21(47) % were low or weak producers of hydrogen cyanide.[35] conducted a similar study on weed germination inhibition potential of rhizosphere *Pseudomonas* and obtained 37 percent capability of HCN production of isolates and this capacity was different among the strains. According to the study by[36], rhizobacteria such as *Pseudomonads* are known for their ability to produce HCN, but the quantity produced varies widely among species and strains of the bacterium.

It has been reported by[37] and[34] that glycine is a direct precursor of HCN found in root exudates though several factors significantly influence its production across bacteria. For example, the level of HCN produced in root-free soil by *P. putida* and *A. delafieldii* generally increased with higher amounts of supplemental glycine, with *P. putida* typically generating more HCN at a given glycine level[38,39]. Studies have shown that HCN is a potential inhibitor of enzymes involved in metabolic processes like respiration, CO_2 and nitrate assimilation, and carbohydrate metabolism. Hence, this gas is known to negatively affect root metabolism and root growth[34,40]. Furthermore, cyanide interacts with the protein plastocyanin, which inhibits the photosynthetic electron transport[36].

Many authors reported about the potential of cyanogenic rhizobacteria for weed suppression by producing HCN and the role they play in biological control of weed[41]. Cyanide producing rhizobacteria are specific in their actions and they do not generally negatively affect the host plants. A major group of rhizobacteria producing secondary metabolite hydrogen cyanide and with potential for biological control is the *Pseudomonas*[42,43]. Rhizosphere bacteria particularly, *Pseudomonas spp* have the ability to reduce weed growth and they were proved to produce HCN. *Pseudomonads* isolated from rhizosphere of velvet leaf were able to reduce velvetleaf viability and emergence significantly[44,45].

The production of the IAA phytohormone is another common trait of rhizobacteria[46,47]. Indole-3-acetic acid (IAA) is the major naturally occurring auxins which influences the root and shoot growth of the plant, stimulating ethylene production, cell division and differentiation. The rate of production of

Table 2. Morphological characterization and identification of the most effective rhizobacteria isolates

Isolates	Morphological characterization					
	Pigment	Shape	Size	Elevation	Margin	Gram staining
E19G6a	white	Circular	Medium	Raised	Entire	–
E19G9	white	Circular	Medium	Raised	Entire	–
E19G6b	brown	Circular	Medium	Raised	Entire	+
E19G10	white	circular	Medium	Raised	Entire	–
E19B	brown	Irregular	large	raised	flat	–
E19G12	brown	Circular	Medium	Raised	Entire	+
E29G2a	white	Irregular	Large	raised	Mucoid	–
E29G7	white	Circular	Medium	Raised	Entire	–

+= Positive for a given test, - = Negative for a given test under consideration

Table 3. Biochemical characterization of the most effective rhizobacteria isolates

Isolates	Biochemical tests					
	Glucose	Fructose	Sucrose	Catalase	Methyl red	Tentative identification
E19G6a	+	+	–	+	+	*Pseudomonas sp.*
E19G9	–	–	–	+	+	*Pseudomonas sp.*
E19G6b	–	+	–	+	+	*Bacillus sp.*
E19G10	+	+	+	–	–	*Klebsiella sp.*
E19B	+	+	–	+	+	*Pseudomonas sp.*
E19G12	–	+	–	+	+	*Bacillus sp.*
E29G2a	–	+	+	–	–	*Entrobacter sp.*
E29G7	+	+	–	+	+	*Pseudomonas sp.*

– = no sugar utilization, catalase and methyl red negative, += sugar utilization, catalase and methyl red positive

ethylene is directly proportional to the concentration of IAA[48,49]. It has been noted that 80% of rhizospheric bacteria produce IAA by metabolizing L - tryptophan[50]. Rhizosphere-inhabiting soil microbes synthesize and release auxins as secondary metabolites because of rich supplies of substrates exuded from plant roots. Some microbes produce auxins in the presence of enough precursor molecules such as tryptophan[46,51].

The current study has shown that 46.8% of the tested isolates were capable of producing IAA. Similar study has been conducted by[52] on growth promotion of rhizobacterial isolates from the rhizosphere of sorghum and grasses in Ethiopia and South Africa and found 73% production of IAA in tested isolates in the presence of tryptophan. The authors further noted the tendency of decreasing IAA concentration in the absence of tryptophan. The lower number of IAA producers in this study than the previous report could be due the difference in sorghum varieties from where the rhizobacteria isolated and other factors in the soil.

Similarly, the report on the study of the effects of rhizobacteria on plants indicated their use as bioherbicides to control weeds[53]. Rhizosphere microorganisms mediated suppression of plant growth during interaction is linked to the secretion of secondary metabolites from microorganisms[54]. Detection of *Enterobacter sp*. found significant amount of IAA secretion due to the presence of an increased activity of tryptophan deaminase, an enzyme which produces IAA from its precursor molecule tryptophan[55]. The negative effect of IAA is associated with the elevated levels of IAA production[56] For example,[57] demonstrated the role of accumulated production of IAA by *P. putida* and its effects in inhibition on plant growth. The increased IAA production stimulates biosynthesis of ethylene by the enzyme aminocyclopropane-1-carboxylate (ACC)[58]. IAA producing *Enterobacter sp also* showed to inhibit lettuce plant growth and enhanced ethylene synthesis[59].

Prior to conducting any germination assay, seed viability test and knowing its percentage of germination are fundamentally important to use it in the subsequent experimentation. In the current study, *S. hermonthica* seed viability test resulted in 63% germination percentage on conditioning the seed in benomyl solution for 10 days and treating the seed with a synthetic germination stimulant GR-24. This agrees with the report by[23] which suggested that the germination percentage of *Striga* seed has to be at least 30% for downstream application of the seed and[18] indicated 55-57% germination induction by GR-24 in seed condition in water in testing Striga seed germination.

Isolation of weeds inhibiting rhizobacteria was made from sorghum rhizosphere and the mechanism involved in weed inhibition of *Striga* seed germination inhibition was undertaken to identify potential *Striga* suppressive rhizobacteria associated with the host plant sorghum. *In vitro* evaluation of the effects of inoculation of bacterial isolates on the inhibition of *S. hermonthica* was studied under laboratory bioassay. This technique was developed for the selection of bacteria inhibitory to the germination of *S. hermonthica* seeds in such a way that adequate contact between the bacteria and *S. hermonthica* seeds was ensured without the bacterial culture medium itself inhibiting *S. hermonthica* seed germination**.** The study focused on germination inhibition at the early stage of *S. hermonthica* and generated information to develop reliable and accessible Striga control strategies for small holder farmers.

There was considerable variation in the inhibition of *Striga* germination by bacterial isolates obtained from sorghum rhizosphere grown on soil collected from different sites. The lowest (9%) and highest (59.7%) germination percentage was observed in E19G10 and broth (control treatment), respectively. A similar study on rhizobacterial strains for suppression of germination of *S. hermonthica* by[13] found a wide range of results (13–50% germination of *S. hermonthica* seeds). Our study indicated the potential of rhizosphere bacteria in inhibiting the early stages of *S. hermonthica* development. This helps to reduce many of the damage caused by *Strigas* before emerging above the ground.

The study showed variations in the inhibition of *Striga* germination by isolates obtained from sorghum rhizosphere grown on soil collected from different sites. For example, many of the bacteria isolated from soil E19 (Amhara Region, Oromo Special Zone) significantly reduced the germination percentage of *S. hermonthica*. Soil E19 was obtained from site 1, where there was low *Striga* infestation. The low infestation of *Striga* in the field from where soil E19 was obtained may be associated with *Striga* germination inhibition by the bacterial population and other factors in the vicinity. Some soils are known to be suppressive to *Striga*, and their suppression was linked to the microbial populations[60].

On the other hand, isolating E19G11a from the same soil E19 but isolated from the rhizosphere of *Striga* resistant SRN-39 did not significantly reduce *Striga* germination. The enhanced germination of *Striga* by this isolate may be again explained by the nature of the microbiome of the soil. This is consistent with the finding of[13] in which few isolates increased germination of *S. hermonthi*ca seeds, others had no effect on seed germination, while some showed a significant suppression of *S. hermonthica* seed germination compared with the check (no bacterium). Furthermore,[61] suggested that both inhibition and promotion of *Strig*a germination can be attributed to microbial action and this can be achieved by manipulation of ethylene biosynthesis, ethylene action, or by promotion of ethylene metabolism or that of its immediate precursor ACC (1-aminocyclopropane-1-carboxylic acid). Generally, there was no consistent pattern in *Striga* germination inhibition of the soils collected from the three sites. However, many of the isolates collected from site 1(E19) resulted in low mean germination percentage regardless of the sorghum variety from where the bacteria were isolated.

The use of weed management strategies involving chemical herbicides generally alters soil structure going alongside with changes in the microbial community[41]. Using soil microorganisms to control weeds is an alternative method to herbicides that may reduce dependence on chemical herbicides and increase the use of environmentally sound practices that are easily available to small holder farmers. The soil microbiome plays an important role in the establishment of weeds and invasive plants with which they are associated and build up close relationships. For example, sorghum seedlings [*Sorghum bicolor* (L) Moench] of different genotypes differ in association with soil microorganisms[62].

Evaluation of the effects of isolates from various sorghum varieties grown on soil collected from different sorghum growing regions in Ethiopia was also conducted in the presence of a host plants called Teshale variety (sorghum) using Agar Gel Assay (AGA). This method helps to overcome the limitations

experienced during field evaluation in establishing a uniform environment to study host-parasite interaction, as it happens in a controlled environment laboratory. The method also allows observation of host-parasite interaction at various stages of *Striga* life cycle[23].

In this study, the lowest (0%) and highest (27%) mean germination of *S. hermonthica* seeds was obtained in the presence of the host plant. This is much higher than the finding of[13] who obtained the lowest (13%) reduction in germination percentage of *S. hermonthica* inoculated with bacterial isolates in the presence of host plant sorghum. Similarly,[18] studied the use of rhizobacteria to control *S. hermonthica* and observed the inhibition of *Striga* germination by bacterial isolates. The germination inhibition of bacterial isolates could be associated with a direct effect of the isolates on the seed or indirectly via the production of chemicals that are toxic to seeds, inhibitors/promoters of ethylene biosynthesis or its action[63,64,12].

Furthermore, the study demonstrated that the control (broth) treatment in the presence of host plant resulted in the highest mean germination percentage, but it was still lower than GR- 24 induced germination percentage of seeds in the absence of host. This finding agreed with the study by[17] on the effectiveness of GR-24 in *S. hermonthica* seed germination stimulatory activity.

Morphological and biochemical characterization of the most effective shortlisted isolates *in vitro* evaluation finally resulted in four different genera of bacteria: *Pseudomonas, Bacillus, Klebssiella* and *Eentrobacter* (Table 3). A variety of rhizobacteria, including *Bacillus*[65], *Pseudomonas*[66], *Azospirillum*[67] species are commonly found in the rhizosphere of crops. The study indicated that majority of the isolates were strong producer of HCN and IAA and deduced to belong to the *Pseudomonas* genera. This agrees with the report by[43] that HCN production is found to be a common trait of *Pseudomonas* (88.89%) and *Bacillus* (50%) in the rhizospheric soil and plant root nodules.

Pseudomonas is among the common plant root inhabiting soil bacteria[68]. Rhizobacetria genera including *Pseudomonas sp., Klebsiella oxytoca* and *Enterobacter sakazakii* were also shown to inhibit *S. hermonthica* seed germination[69].

Conclusion

The results of this study have revealed that there are novel rhizobacteria with a great potential of inhibiting *Striga* seed germination resulting in reduction of parasitic infestation on sorghum. This potential can be exploited by isolating and characterizing rhizospheric bacteria associated with sorghum and evaluating their *Striga* suppressive effects. The suppression effects of rhizobacteria on *Striga* seed could be associated with microbial production of phytotoxic secondary metabolites and inhibitory chemicals such as HCN and IAA that could induce a biocontrol effect. Many of the isolates with most effective *Striga* suppression were obtained from low *Striga* infested field (E19) indicating that rhizospheric bacteria could contribute to the reduction in parasitic infestation. It has been also shown that, regardless of the level of inhibition, all rhizobacterial isolates suppressed *Striga* germination up on *in vitro* evaluation in the presence and absence of host plant sorghum. The most effective *Striga* suppressive isolates were identified to be fallen under four bacterial genera and majority of them were belong to the *Pseudomonas* genus. The isolates are good candidates for addressing *Striga* associated constraints in sorghum production where there is a low input for small holder farmers in our country.

Authors' Contributions

Urgesa Tsega Tulu, Sewunet A., and Teklehaimanot H. designed this study. Urgesa Tsega Tulu performed the experiment and did the statistical analysis. Urgesa Tsega Tulu, Teklehaimanot H. and Taye T. were involved in the writing of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request

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Conflict of interest

The authors declare that they have no conflict of interest.

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References

1. Shapiro, C. A. and Charles S. Wortmann, C. S. 2006. Corn response to nitrogen rate, row spacing, and plant density in Eastern Nebraska. *Agronomy Journal* 98:529–534
2. FAO. 2012. Database of agricultural production. FAO Statistical Databases (FAOSTAT). https://www.fao.org/3/i3137e/i3137e.pdf
3. Spallek, T., Mutuku, M. and Shirasu, K. 2013. The genus *Striga*: a witch profile. *Molecular Plant Pathology* 14:861–869
4. FAOSTAT. 2017. Food and Agriculture Organization of the United Nations, statistic division. fao. org, http://faostat.fao.org/ (Accessed: 15May 2018)
5. CSA (Central Statistics Agency). Report on area and production of crops of central statistics agency. 2018. Central Statistics Agency, Addis Ababa, Ethiopia. https://www.scirp.org/reference/referencespapers?referenceid=2952450
6. Wortmann, C. S. , Mamo, M. , Abebe, G. , Mburu, C. , Kayuki, K. C. , Letayo, E. , and Xerinda, S. 2006. The Atlas of Sorghum Production in Five Countries of Eastern Africa. University of Nebraska, Lincoln, USA
7. Rich, P. J., Grenier, C. and Gebissa Ejeta. 2004. *Striga* resistance in the wild relatives of sorghum. *Crop Science* 44:2221–2229
8. Ejeta, G. 2007. Breeding for *Striga* resistance in sorghum: exploitation of intricate host parasite biology. *Crop Science* 47:216–227

9. Ayongwa, G. C., Stomph, T. J., Belder, P., Leffelaar, P. A., and Kuyper, T. W. 2011. Organic matter and seed survival of *Striga hermonthica* - Mechanisms for seed depletion in the soil. *Crop Protection* 30:1594–1600
10. 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. *Frontiers in Plant Science* 7:1–8
11. Bozkurt, M., Muth, P., Parzies, H., and Haussmann, B. 2014. Genetic diversity of East and West African *Striga hermonthica* populations and virulence effects on a contrasting set of sorghum cultivars. *International Journal of Weed Biology, Ecology and Vegetation Management* 55:71–81
12. Hassan, M. M., Abdelgain, M. E. and Babiker, A. G. T. 2009. Effects of Bacterial Strains and Isolates on in Situ Germination, Subsequent Developmental *Stage* of *Striga hermonthica* onto Sorghum Roots. *Advances in Environmental Biology* 5:3263–3269
13. Ahonsi, M. O., Berner, D. K., Emechebe, A. M. and Lagoke, S. T. 2002. Selection of rhizobacterial strains for suppression of germination of *Striga hermonthica* (Del.) Benth. seeds. *Biological Control* 24:143–152
14. Pilgeram, A. L. and Sands, D. C. , 2010. Bioherbicides. *Industrial Applications*. pp. 395-405
15. Mounde, L. G. 2015. Understanding the role of plant growth promoting bacteria on sorghum growth and biotic suppression of Striga infestation, Thesis, University of Hohenheim, Stuttgart.
16. Neondo, J. O. 2017. Exploring Biological Control and Transgenic Weed Management Approaches against Infestation by Striga hermonthica in Maize, Thesis, JKUAT, Kenya.
17. Barillot, C. D., Sarde, C. O., Bert, V., Tarnaud, E. and Cochet, N. 2013. A standardized method for the sampling of rhizosphere and rhizoplan soil bacteria associated to a herbaceous root system. *Annuals of Microbiology* 63:471–476
18. Babalola, O. O., Berner, D. K. and Amusa, N. A. 2007. Evaluation of some bacterial isolates as germination stimulants of *Striga hermonthica*. *African Journal of Agricultural Research* 2:27–30
19. Hassan, M. M. and 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* 11:263–270
20. Bakker, A. W. , Schippers, B. 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and Pseudomonas spp. mediated plant growth stimulation. *Soil Biology and Biochemistry* 19:451–457
21. Thakuria, D., Talukdar, N. C., Goswami, C., Hazarika, S., Boro, R. C. and Khan, M. R. 2004. Characterization and screening of bacteria from rhizosphere of rice grown in acidic soils of Assam. *Current Science* 86:978–985
22. Berner, D. K., Ikie, F. O. and Green, J. M. 1997. ALS-inhibiting herbicide seed treatments control Striga hermonthica in ALS-modified corn (*Zea mays*). *Weed Technology* 11:704–707
23. Rich, P. J. and Gobena, D. 2016. Laboratory Manual for Screening *Striga* Resistance in Sorghum, Purdue University, USA.
24. Gafar, N. Y., Hassan, M. M., Ahmed, M. M., Osman, A. G., Abdelgani, M. E. and Babiker, A. E. 2015. In vitro study of endophytic bacteria, carbohydrates and their combination on early developmental stages of Striga hermonthica (Del.) Benth. *Advances in Environmental Biology* 10:66–74
25. Mohamed, A. H., Housley, T. L. and Ejeta, G. 2010. An *in vitro* technique for studying specific *Striga* resistance mechanisms in sorghum. *African Journal of Agricultural Research* 5:1868–1875
26. Hemraj, V., Diksha, S. and Avneet, G. 2013. A review on commonly used biochemical test for bacteria. *Innovare Journal of Life Science,* 1:1–7
27. Prescott, L. M. and Harley, J. P. 2002. Harley Prescott: Laboratory Exercises in Microbiology, Fifth edition. The McGraw–Hill Companies, New York. pp. 466-502.
28. Atera, E. A. , Itoh, K. , Azuma, T. and Ishii, T. 2012. Response of NERICA rice to *Striga hermonthica* infections in western Kenya. *International Journal of Agriculture and Biology* 14:271–275
29. Kremer, R. J. and Kennedy, A. C. 1996. Rhizobacteria as biocontrol agents of weeds. *Weed Technology* 10:601–609
30. Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C. and Moënne-Loccoz, Y. 2009. The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil* 321:341–361
31. Mendes, R., Garbeva, P. and Raaijmakers, J. M. 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiology Reviews* 37:634–663
32. Schlaeppi, K. and Bulgarelli, D. 2015. The plant microbiome at work. *Molecular Plant-Microbe Interactions* 28:212–217
33. Kremer, R. , 2007. Deleterious rhizobacteria. *Plant-associated Bacteria*, pp. 335-357
34. Schippers, B., Bakker, A. W., Bakker, P. A. H. M. and Van Peer, R. 1990. Beneficial and deleterious effects of HCN-producing *Pseudomonads* on rhizosphere interactions. *Plant and Soil* 129:75–83
35. Heydari, S. , Rezvani-Moghadam, P. and Arab, M. 2015. Hydrogen cyanide production ability by *Pseudomonas fluorescence* bacteria and their inhibition potential on weed germination. *Competition for Resources in a changing world: New drive for rural development, Tropentag, Hohenheim*
36. Kremer, R. J. and Souissi, T. 2001. Cyanide production by rhizobacteria and potential for suppression of weed seedling growth. *Current Microbiology* 43:182–186
37. Knowles, C. J. 1976. Microorganisms and cyanide. *Bacteriological Reviews* 40:652–680
38. Castric, P. A. 1977. Glycine metabolism by Pseudomonas aeruginosa: hydrogen cyanide biosynthesis. *Journal of Bacteriology* 130:826–831
39. Owen, A. and Zdor, R. 2001. Effect of cyanogenic rhizobacteria on the growth of velvetleaf (Abutilon theophrasti) and corn (Zea mays) in autoclaved soil and the influence of supplemental glycine. *Soil Biology and Biochemistry* 33:801–809
40. Grossmann, K. 2010. Auxin herbicides: current status of mechanism and mode of action. *Pest Management Science:formerly Pesticide Science* 66:113–120
41. Trognitz, F. , Hackl, E. , Widhalm, S. and Sessitsch, A. 2016. The role of plant–microbiome interactions in weed establishment and control. *FEMS Mmicrobiology Ecol* 92:1–15
42. Kremer, R. J. and Kennedy, A. C. 1996. Rhizobacteria as biocontrol agents of weeds. *Weed Technol* 10:601–609
43. Kamei, A. , Dolai, A. K. and Kamei, A. 2014. Role of hydrogen cyanide secondary metabolite of plant growth promoting rhizobacteria as biopesticides of weeds. *Global Journal of Science Front Research* 14:109–12
44. Lakshmi, V., Kumari, S., Singh, A. and Prabha, C. 2015. Isolation and characterization of deleterious *Pseudomonas aeruginosa* KC1 from rhizospheric soils and its interaction with weed seedlings. *Journal of King Saud University Science* 27:113–119
45. Begonia, M. F. T. and Kremer, R. J. 1994. Chemotaxis of deleterious rhizobacteria to velvetleaf (Abutilon theophrasti Medik.) seeds and seedlings. *FEMS Microbial Ecology* 15:227–235
46. Arshad, M. and Frankenberger, W. T. 1991. *Microbial production of plant hormones. In: The Rhizosphere and Plant Growth*, eds. Keister, D. L. and Cregan, P. B. , Kluwer Academic Publishers, University of California, Riverside. pp. 327-334
47. Damam, M. , Moinuddin, M. K. and Kausar, R. 2016. Isolation and screening of plant growth promoting actinomycetes from rhizosphere of some forest medicinal plants. *International Journal of Chemtech Research* 9:522–528
48. Shaik, I., Janakiram, P., Sujatha, L. and Chandra, S. 2016. Isolation and identification of IAA producing endosymbiotic bacteria from *Gracilaria corticata* (J. Agardh). *International Journal of Bioassays*

5:5179–5184

49. Vargas, L. K. , Volpiano, C. G. , Lisboa, B. B. , Giongo, A. , Beneduzi, A. and Passaglia, L. M. P. 2017. *Potential of rhizobia as plant growth-promoting rhizobacteria. In: Microbes for Legume Improvement*, eds. Zaidi, A. , Khan, M. S. , Musarra, J. , Springer, Cham, pp. 153-174
50. Patten, C. L. and Glick, B. R. 1996. Bacterial biosynthesis of indole-3-acetic acid. *Canadian Journal of Microbiology* 42:207–220
51. Kremer, R. J. 2006. *The role of allelopathic bacteria in weed management. In: Allelochemicals: Biological Control of Plant Pathogens and Diseases*, eds. Inderjit and Mukerji, K. G. , Dordrecht, Springer, pp. 143-155
52. Idris, A. , Labuschagne, N. and Korsten, L. 2009. Efficacy of rhizobacteria for growth promotion in sorghum under greenhouse conditions and selected modes of action studies. *The Journal of Agricultural Science*, *147*(1), pp. 17-30
53. Boyette, C. D. and Hoagland, R. E. 2015. Bioherbicidal potential of *Xanthomonas campestris* for controlling *Conyza canadensis*. *Biocontrol Science and Technology* 25:229–237
54. Barazani, O. Z. and Friedman, J. 1999. Allelopathic bacteria and their impact on higher plants. *Critical Reviews in Plant Science* 18:741–755
55. Bandurski, R. S. , Cohen, J. D. , Slovin, J. P. and Reinecke, D. M. 1995. *Auxin biosynthesis and metabolism. In: Plant Hormones*, eds. Davies, P. J. , Dordrecht, Kluwer Academic Publishers, pp. 39-65
56. Sarwar, M. and Frankenberger, W. T. 1994. Influence of L-tryptophan and auxins applied to the rhizosphere on the vegetative growth of *Zea mays* L. *Plant and Soil* 160:97–104
57. Patten, C. L. and Glick, B. R. 2002. Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. *Applied Environmental Microbiology* 68:3795–3801
58. Kende, H. 1993. Ethylene biosynthesis. *Annual Review of Plant Biol* 44:283–307
59. Park, J. M. , Radhakrishnan, R. , Kang, S. M. and Lee, I. J. 2015. IAA producing *Enterobacter sp.* I-3 as a potent bio-herbicide candidate for weed control: a special reference with lettuce growth inhibition. *Indian Journal of Microbiology* 55:207–212
60. Ciotola, M., Watson, A. K. and Hallett, S. G. 1995. Discovery of an isolate of Fusarium oxysporum with potential to control *Striga hermonthica* in Africa. *Weed Research* 35:303–309
61. Berner, D., Carsky, R., Dashiell, K., Kling, J. and Manyong, V. 1996. A land management-based approach to integrated Striga hermonthica control in sub-Saharan Africa. *Outlook on Agriculture* 25:157–164
62. Funnell-Harris, D. L., Pedersen, J. F. and Marx, D. B. 2008. Effects of sorghum seedlings and previous crop on soil fluorescent Pseudomonas spps. *Plant Soil* 311:173
63. Babiker, A. G. T. , Gebissa Ejeta, Butler, L. G. and Woodson, W. R. 1993b. Ethylene biosynthesis and strigol-induced germination of *Striga asiatica*. *Physiologia Plantarum* 88:359–365
64. Babiker, A. G. T. 2007. *Striga*: The spreading scourge in Africa. *Regulation of Plant Growth and Development* 42:74–87
65. Idris, H. A., Labuschagne, N. and Korsten, L. 2007. Screening rhizobacteria for biological control of *Fusarium* root and crown rot of sorghum in Ethiopia. *Biological Control* 40:97–106
66. Qessaoui, R. , Bouharroud, R. , Furze, J. N. , El Aalaoui, M. , Akroud, H. , Amarraque, A. , Van Vaerenbergh, J. , Tahzima, R. , Mayad, E. H. and Chebli, B. 2019. Applications of new rhizobacteria *Pseudomonas* isolates in agroecology via fundamental processes complementing plant growth. *Science Reports* 9:1–10
67. Roesch, L. F. W., Camargo, F. A., Bento, F. M. and Triplett, E. W. 2008. Biodiversity of diazotrophic bacteria within the soil, root and stem of field-grown maize. *Plant and Soil* 302:91–104
68. Babalola, O. O. , Osir, E. O. and Sanni, A. I. 2002. Characterization of potential ethylene-producing rhizosphere bacteria of *Striga*-infested maize and sorghum. *African Journal of Biotechnology* 1:67–69
69. Babalola, O. O. and Odhiambo, G. D. 2008. Effect of inoculation with *Klebsiella oxytoca* '10 mkr 7'on *Striga* suicidal germination in *Zea mays*. *World Applied Science Journal* 3:57–62