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Research Article

Isolation and Characterization of *Striga hermonthica* Seed-Bank Depleting Bacteria from *Striga* Infested Sorghum Growing Areas of Ethiopia

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Abstract

Striga hermonthica is a very serios parasitic weed of (*Sorghum bicolor* L. Moench). It attack host plants by living as hemi-parasite and attaching small sucker root system to host plant. A study was undertaken to evaluate the effect of bacterial isolate 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* morphologically.

Keywords

Striga Hermonthica, Germination, Stimulant, Seed-Decay, Viability, Parasitic-Weed

1. Introduction

Sorghum bicolor (L.) Moench is an important crop for 500 millions of people in Sub-Saharan Africa which is mainly cultivated in drier areas, especially on shallow and heavy clay soils [28]. 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, re-

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spectively [12]. It is the third largest cereal crop in Ethiopia and is produced in most parts of the country, especially in drought prone areas [11, 10]. 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 [4]. 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 [21]. Hence, *Striga* infestation is one of the most yield limiting constraints of sorghum causes up to 100% yield loss [18, 23].

So far, cultural and chemical methods as well as breeding for host resistance have been practiced to control *Striga* [5]. 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 [6]. *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 [1, 3, 9]. 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 [3, 7].

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 [22]. 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 [25].

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. It was 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 [17].

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 [5]. Therefore, the current study was initiated in order to determine the suitability of soil bacteria to control *Striga* infestation.

2. Materials and Methods

2.1. 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, Wollo, Abergelle and Humera in Amhara, Shiraro, area in Tigray and western Harary in Oromia regions.

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. 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.

2.2. Assessment of *Striga* Seed Bank Depleting Bacterial Communities

2.2.1. *Striga* Seed Conditioning

Striga seeds were surface sterilized by using the procedure stated in [2]. 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 to control fugal growth during conditioning. Incubated at 30°C for 14 days. Every 2- 3 days, benomyl was removed and changed by fresh benomyl solution [20]. 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.

2.2.2. *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°C under dark condition.

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°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°C for 8 days to observe whether the seed is viable or not [19]. 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.

2.3. Isolation of *Striga* Seed Bank Depleting Bacteria

35 *Striga* seeds extracted from selected soils at week 4 were dispersed over on nutrient agar medium in aseptic condition. After 24 hours incubation at 30°C each colony from the plates were transferred to nutrient broth in sterile Eppendorf tube. 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.

2.4. 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 [8]. The suspension was added to 100 ml nutrient broth medium and incubated overnight on the incubator shaker.

2.5. In-Vitro Evaluation of Bacterial Isolates for *Striga* Seed Germination Stimulation

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 having 24 wells 15.22 mm diameter and a volume of 3ml. 6mm diameter glass fiber discs 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. Microbial isolates from nutrient broth were added to each *Striga* seed containing 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°C. After 4 days of incubation the data were recorded by observing *Striga* seeds under stereo microscope with a magnification of germinated *Striga* seeds were recorded [19].

2.6. *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.

2.7. 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°C. 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.

2.8. 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°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.

Finally, all targeted isolates were classified in to genera level by using Bergey's manual of bacteriology [24].

2.9. 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.

3. Results and Discussion

3.1. 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, [3] 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.

Table 1. *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

3.2. 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, [27] 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, [22] 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 [25]. 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 [16].

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

3.3. 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, [15] also isolated 211 bacterial isolates from 80 soil samples that are capable of inhibiting GR-24 stimulated *Striga* seed germination. On the other hand, [3] 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

3.4. 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).

It was 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% [13, 29]. On the other hand [3] 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

Isolate	Germination	Germination by GR	Isolate	Germination	Germination by GR	Total no. of seeds
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.

3.5. 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 [3]. [26] also reported that GR-24 was the best parasitic plant germination stimulant chemical and used for soil seedbank depleting.

3.6. 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. [22] reported that antibiosis and enzymatic properties of some potential bio-control isolates of some *Bacillus* spp. recorded high antibiosis, enzymatic and seed decay values. [25] 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.

3.7. 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 [14].

Similarly, [1] 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

Isolate	Mean *Striga* seed germinated*	Isolate	Mean *Striga* seed germinated
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.

3.8. 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 [24].

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 [22]. 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 [1]. 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 [3].

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% [15].

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*

Isolate	Colony shape, margin and Surface characteristics respectively	Cell shape	Gram stain	Motility	Catalase	Citrate	Urease	Genera
GS45	Irregular, smooth, smooth	Rod	-	-	+	+	-	*Klebsiella*

4. Conclusion and Recommendation

4.1. 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.

4.2. 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.

Abbreviations

CFU	Colony Forming Units
GR 24	Growth Regulator (Synthetic)
H_2O_2	Hydrogen Per Oxide
OD	Optical Density
ppm	Parts Pre Mzillion

Author Contributions

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Mamo Bekele: Writing – original draft

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Taye Tessema: Funding acquisition, Project administration, Supervision

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] 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.

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

[3] 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.

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

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

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

[7] 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.

[8] 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.

[9] 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.

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

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

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

[13] Gafar NY, Hassan MM, Rugheim AME, Osman AG, Mohamed IS, Abdelgani ME and 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.

[14] 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.

[15] 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.

[16] 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.

[17] 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.

[18] 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.

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

[20] 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.

[21] 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.

[22] 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.

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

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

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

[26] 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.

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

[28] 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.

[29] 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.