

Screening for potential *Striga hermonthica* fungal and bacterial biocontrol agents from suppressive soils in Western Kenya

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Abstract *Striga hermonthica* is a hemiparasitic weed that causes huge grain yield losses to small-scale farmers in Africa. Effective biocontrol agents against *S. hermonthica* can sustainably mitigate these losses. This study characterized the biocontrol potential of culturable fungal and bacterial isolates from *S. hermonthica* suppressive soils of western Kenya. These isolates were screened for their ability to produce antibiotic compounds and extra cellular enzymes and also their ability to cause *S. hermonthica* seed decay. Genomic DNA of the selected bacterial and fungal isolates was extracted and partial characterization of 16S rRNA and 18S rRNA genes performed respectively. Analysis show that antibiosis and enzymatic

properties of potential biocontrol isolates correlated positively. Isolate KY041696 recorded high antibiosis, enzymatic and seed decay values. This study also revealed that bioactive bacterial isolates belonged to *Bacillus*, *Streptomyces* and *Rhizobium* genera. In this study, no fungal isolate caused *S. hermonthica* seed decay. This study therefore provides baseline information on the potential biocontrol microbes against *S. hermonthica* in Western Kenya that could be exploited further in the management of the weed.

Keywords Exudate · Biocontrol agent · Antibiosis · Extra-cellular enzymes

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Introduction

Striga hermonthica infestation in cereal fields results in about 30–90% yield loss hence emerging as a major constraint to cereal production in Sub-Saharan Africa (Musyoki et al. 2015). This great loss is due to production of numerous viable *S. hermonthica* seeds (Mbuvi et al. 2017). Lifecycle of this weed is synchronized with that of its host such that the parasite seeds only germinate in the presence of a host resulting in significant crop damage and more parasite seed accumulation (Ahonsi et al. 2002; Mbuvi et al. 2017).

The prevailing weed management strategy against *S. hermonthica* relies on cultural, chemical and manual control techniques (Nzioki et al. 2016). These options are rarely adopted by farmers because they are

expensive, take long to have an impact and their application does not mitigate subterranean host damage (Nzioki et al. 2016). While biological weed control can be effective, it is sometimes uneconomical and produces inconsistent results at different location due to lack of biocontrol agent adaptability (Teka 2014; Pereg and Mcmillan 2015). To date, there is no known effective and sustainable *S. hermonthica* management option that is widely adopted by small-scale farmers in Western Kenya (Avedi et al. 2014). For *S. hermonthica*, it is important to consider biocontrol agents that are effective and well adapted to the region of application (Atera et al. 2012; Musyoki et al. 2015).

The mechanism for biocontrol action is believed to be through direct antibiosis, competitive exclusion, interference with pathogen signaling and/or induction of plant resistance mechanisms (Compant et al. 2012). Most biocontrol agents are specific in action, do not contaminate the environment through residues and are more acceptable and affordable than fertilizers or genetically engineered crops (Whipps 2001; Heydari and Pessaraki 2010). For example, *Pseudomonas fluorescens* and *Pseudomonas putida* have been reported to significantly inhibit *S. hermonthica* seed germination under screen-house experiments (Babalola et al. 2007). In the case of fungal biocontrol option, *Fusarium oxysporium* f. sp. *Strigae* (Foxy2) has been shown to reduce emergence of *S. hermonthica* and *Striga asiatica* plants through destruction of appressorium, hyaline tissue, xylem vessels or cortical parenchyma (Avedi et al. 2014). However, there is no known report of a biocontrol agent with potential to cause *S. hermonthica* seed decay. The use of a combination of compatible biocontrol agents with divergent modes of action is likely to yield better results than the use of any single biocontrol agent (Midthassel et al. 2016).

This study analyzed the bioactivity of culturable bacterial and fungal isolates from *S. hermonthica* suppressive soils of Western Kenya with the intention of designing maize probiotics capable of suppressing *S. hermonthica* or inducing *S. hermonthica* seed decay. The findings of this study form the basis of robust bioprospection efforts to identify microbial isolates that could be exploited as *S. hermonthica* biocontrol agents in the region.

Materials and methods

Description of study sites, sample collection criteria and processing

Samples were collected from 16 sites in six counties prone to *S. hermonthica* infestation in Western Kenya (supplementary material, Table S1). A purposive sampling technique was used to collect diseased *S. hermonthica* plant samples together with their respective rhizosphere soil samples. Diseased *S. hermonthica* plants that appeared to be wilting, drying or had soft rot, complete blight of the stem and/or black floral parts and growing in close proximity with healthy host plants were collected. About 500 g of respective rhizosphere soils were also taken from depth of up to 20 cm. Samples were transported in cool boxes to the molecular biology laboratory at Institute for Biotechnology Research (IBR), Kenya. Plant samples were surface sterilized according to Kwaśna and Bateman (2007) protocol. Ten grams of each soil sample was used to prepare serial dilutions (up to 10^{-5}) as described by Babalola et al. (2007).

Isolation and purification of bacterial and fungal isolates

Pieces of pre-sterilized explants (macerated into 2 mm-length size) and aliquots of 0.1 ml of each soil dilutions were inoculated on five different media. Peptone pentachloronitrobenzene agar (PPA), Potato dextrose agar (PDA) Spezieller Nährstoffarmer Agar (SNA), nutrient agar (NA) and Babalola et al. (2007) media were used to isolate bacteria and fungi from the samples following Babalola et al. (2007) and Kwaśna and Bateman (2007) protocols. For each media type, five media treatments (replicated six times) based on pH values (pH 3, 6, 7, 8 and 11) were prepared. Emerging bacterial colonies were streaked on fresh nutrient agar media while emerging fungal colonies were cultured on PDA, SNA and Carnation Leaf Agar (CLA) to obtain pure colony. The emerging bacterial and fungal isolates were assigned coded names (supplementary material, Table S2). Gram staining and spore/mycelia staining techniques were used to morphologically characterize bacterial and fungal isolates, respectively.

Fungal spore production

Fungal isolates were further grown on PDA, SNA and CLA for 14 days to allow for fungal sporulation. Fifteen milliliters of sterile distilled water was added to the collected spores/mycelia and the mixture thoroughly ground. The mixture was filtered using an 80 µm filter sieve and the spore concentration standardized to 10^6 spore ml^{-1} using haemocytometer.

In the pre-selection assays, isolates that grew on media with different pH levels and also recorded high antibiosis and enzymatic values were used in the *S. hermonthica* biocontrol screening assay. Bacterial isolates BUT101, BUT501, BUS202, BUS203 and SIA102 grew very fast (based on periodical Optical Density measurement) and were highly susceptible to inhibition by ampicillin, tetracycline, centramaxazole, streptomycin, kanamycin, gentamicin, and sulphamohoxazole and chloramphenicol antibiotics. These characteristics were used to select them as test microorganism in the antibiosis experiment.

Antibiosis test

Interaction of bacterial isolates (four points of inoculation per Petri plate) and test microorganisms (BUT101, BUT501, BUS202, BUS203 and SIA102) formed bacterial antibiosis treatments replicated three times. Standardized bacterial suspensions were made based on 0.5 McFarland Turbidity Standard (Nuneza et al. 2015). Aliquots of 100 µl of test microorganism were spread-plate onto Mueller–Hinton Agar (MHA). Sterile Whatman No.1 filter paper discs (1 cm diameter) were saturated with isolates as disc inoculants (four discs per plate replicated six times). The plates were sealed with parafilm and incubated at 37 °C for 24 h and the emerging zones of inhibition measured. Similarly, a fungal spore paste, prepared by mixing 10^6 spores per ml suspension with one drop of triton X-100 was used to saturate fungal inoculant discs. Three replicates, each consisting of four discs per isolate, were inoculated onto Muller Hinton agar media previously spread with test microbe (BUT101, BUT501, BUS202, BUS203 and SIA102), incubated at 30 °C and monitored routinely after every 24 h. The emerging zones of inhibition were measured after seven days.

Bacterial and fungal extracellular enzymatic assays

Minimum salt media (MSM) according to Khalil's (2011) protocol amended with a special carbon source (based on target enzyme) were prepared. All tests (four points of inoculation per Petri plate replicated three times for each isolate) were carried out at incubation conditions of 30 °C (fungus) and 37 °C (bacteria) for 48 h.

For cellulase and xylanase enzyme tests, isolates were inoculated on solidified 1% (v/v) MSM fortified with 1% (w/v) carboxymethyl cellulose and 1% (w/v) Xylan, respectively (Teather and Wood 1982; Gesse and Gashe 1997). Isolates producing cellulase and xylanase enzymes after 48 h were identified by flooding the Petri plates with 0.1% aqueous Congo red for 15 min followed by repeated washing with 1 M NaCl. Visible zones of clearance were measured and used to compare extra cellular enzyme activity profile. For amylase activity, isolates were cultured on solidified medium consisting of 1% (w/v) soluble starch and 1% (v/v) MSM salts. After 48 h, Petri plates were flooded with 1% Lugol's iodine to detect the presence of clear halos around amylase-producing isolates. Protease activity was assessed by inoculating isolates on Zilda et al.'s (2012) solid media. The occurrence of clear zone after 48 h indicated production of extracellular protease enzyme. Pectinase activity of isolates was evaluated using Huang et al.'s (2012) protocol. After 48 h, plates were flooded with 1% Lugol's iodine to detect the presence of clear halos as indicator for production of extra cellular pectinase enzyme.

Conditioning of *S. hermonthica* seeds and determination of their germination viability

Bunches of 10 mg *S. hermonthica* seeds (approximately 1500 seeds) were surface-sterilized and pre-conditioned according to Lenzemo et al.'s (2009) protocol. Two hundred sterilized *S. hermonthica* seeds from each bunch were aseptically transferred into a Petri plate lined with moist Whatman GFA filter paper, wrapped with aluminum foil and incubated at 28 °C for 11 days for pre-conditioning. Three Petri plates of preconditioned *S. hermonthica* seeds of each bunch were germinated by adding 3 ml of 0.1 ppm GR24 and incubated overnight at 28 °C. The number of germinating *S. hermonthica* seeds was determined

using a Leica MZ7F stereomicroscope fitted with a DFC320FX camera. The remaining *S. hermonthica* seeds from bunches that had >69% average germination were used in the *S. hermonthica* seed decay experiments.

Evaluation of the bioherbicidal activity of selected isolates

A total of sixteen pre-selected isolates were screened for their ability to induce *S. hermonthica* seed decay that consequently resulted in the reduction of the number of germinating *S. hermonthica* seeds after exposure to GR24. Two controls comprising of pre-conditioned *S. hermonthica* seeds to which 3 ml of sterile distilled water was added (negative control) and *S. hermonthica* seeds treated with 3 ml of 0.1 ppm GR24 (positive control) were included in the experiment. Petri plates used in this assay were lined with two sterile Whatman GFA filter papers. In the bacterial assay, six replicates each consisting of 100 pre-conditioned *S. hermonthica* seeds per Petri plate were carefully spread on the bottom filter paper and covered with the second filter paper. Ten ml of 10^{-1} serial dilution of standardized bacterial suspension was used to wet the top filter paper. The Petri plates were aseptically sealed with parafilm inside a laminar flow hood and incubated at 28 °C in the dark for 48 h. After 48 h, the top filter paper was carefully removed, 3 ml of 0.1 ppm GR24 aseptically added and further incubated overnight at 28 °C.

In a separate experiment, standardized fungal spore concentrations (10^6 spores ml^{-1}) were evaluated for their ability to cause *S. hermonthica* seed decay (Kroschel et al. 1996). Bunches of 10 mg *S. hermonthica* seeds that had been pre-conditioned for four days were carefully and aseptically spread on Whatman GFA filter paper and covered with another filter paper in Petri plates. Ten ml of standardized fungal spore suspensions were used to wet the top filter papers that had been sprinkled with 0.05 g of PDA. The Petri plates were sealed with parafilm and incubated in the dark at 28 °C for ten days. After incubation, the top filter paper was carefully and aseptically removed and seeds were washed with sterile distilled water through two sterile filter sieves with mesh size of 315 and 80 μm . The first sieve facilitated the separation of *S. hermonthica* seeds and fungal spores from the mycelium while the second

sieve enabled the separation of *S. hermonthica* seeds from fungal spores. One hundred *S. hermonthica* seeds of each treatment were, under sterile condition, transferred onto Whatman GFA filter paper moistened with 3 ml of 0.1 ppm GR24 in a Petri plate (replicated six times) and incubated overnight in the dark at 28 °C.

In both bacterial and fungal screening assays, the Petri plates were analyzed under a Leica MZ7F stereomicroscope fitted with a DFC320FX camera, for induction of *S. hermonthica* seed decay. The number of decaying *S. hermonthica* seed per replicate was recorded.

Partial 16S rRNA characterization

Pre-selected bacterial isolates were grown in Luria broth at 37 °C for 48 h. The cultures were then centrifuged at $13,000 \times g$ for 1 min and the supernatant discarded. DNA extraction from the pelleted bacterial cells was performed according to Sambrook and Russell (2001) protocol and stored at 4 °C. The 16S rRNA gene sequence was PCR-amplified using bacterial primer pair 8F (5'-AGRCTTTGATCCTGGCTCAG-3') and 1492R (5'-CGGCTACCTTGTTACGACTT-3') for all selected bacterial isolates. Amplification was carried out in a 30 μl mixture containing 3 μl of 10X PCR buffer, 4 μl of 2.5 mM dNTPs, 2.5 μl of 8F forward primer (5 pmol), 2.5 μl of 1492R reverse primer (5 pmol), 0.4 μl of 5 U μl^{-1} Taq polymerase, 1.5 μl of DNA template and 16.1 μl PCR grade water. The amplification was performed as follows: initial denaturation at 94 °C for 5 min, 30 cycles each of denaturation at 94 °C for 30 s, primer annealing at 55 °C for 30 s, chain extension at 72 °C for 1.5 min, and a final extension at 72 °C for 8 min.

The PCR amplicons (≈ 1.5 kb) were checked by gel electrophoresis, labeled and shipped to Macrogen, South Korea for sequencing. Retrieved sequences were submitted to National Center for Biotechnology Information (NCBI) GenBank database and assigned accession numbers ranging from KY038852 to KY038856 and KY041695 to KY0041697. The sequences were aligned using BioEdit sequence alignment editor software and their closely related database sequences retrieved using Basic Local Alignment Search Tool (BLAST) algorithm search program of NCBI (<https://www.ncbi.nlm.nih.gov/blast/>). Sequence alignments and construction of

phylogenetic tree was done using MEGA 7 (Kumar et al. 2016).

Partial 18S rRNA characterization

Pre-selected fungal isolates were revived on PDA medium and DNA extraction done using Brandfass and Karlovsky (2008) protocol. The 18S rRNA gene sequence was amplified using primer pair, 566F, 5'-CAG CAG CCG CGG TAA TTC C-3' and 1200R, 5'-CCC GTG TTG AGT CAA ATT AAG C-3' that amplify approximately 650 bp DNA fragment from the V4 and V5 regions. PCR was performed using BIOLINE 2X ReadyMix Taq polymerase kit (CAT. No. BIO-2541) at the following conditions: initial denaturation at 95 °C for 15 min, 35 cycles each of denaturation at 95 °C for 45 s, primer annealing at 60 °C for 45 s, chain extension at 72 °C for 1 min, and a final extension step at 72 °C for 10 min.

The PCR amplicons were checked by gel electrophoresis, labeled and shipped to Macrogen in South Korea for sequencing. The obtained partial 18S rRNA gene sequences were deposited in the NCBI GenBank database with accession numbers ranging from KY04168 to KY04176. These sequences were used to search and retrieve closely related sequences from the largest database tailored for fungal ITS sequences UNITE (<https://unite.ut.ee/>). The sequences were aligned using BioEdit sequence alignment editor software and their closely related database sequences retrieved using BLAST algorithm search program of NCBI. Sequence alignments and construction of phylogenetic tree was done using MEGA 7 (Kumar et al. 2016).

Statistical analysis

Diameters of emerging zones of inhibition and clearance (considered as indicators of efficiency) in the antibiosis and extra cellular enzymatic experiments were measured. The percentage of decaying *S. hermonthica* seeds in all the treatments were subjected to arcsine square root transformation function prior to statistical analysis. General linear model (PROC GLM) procedure of SAS software version 9.1 was used to perform analysis of variance (one-way ANOVA) for all the measured data with bacterial/fungal isolate as the independent variable. Tukey's honest

significant difference (HSD) test was used to compare and separate the means of diameter of zones of inhibition and clearance as well as the mean number of *S. hermonthica* seed decay. The outputs of data analyses were presented as mean \pm SE in table and graph forms. Correlation profiles of zones of inhibition, zones of clearance and number of *S. hermonthica* seed decay with respect to selected isolates were visualized as hierarchical clustering heatmaps generated by BiodiversityR script (R programming language and the vegan package) based on Manhattan metric using R version 3.3.1 software (R Development Core Team 2012; Oksanen et al. 2016).

Results

Sixteen pre-selected isolates that showed significant antibiosis and enzymatic activity were evaluated in this study. Comparative assessment of bacterial antibiosis property showed that isolates SM5ISS (KY041696) exhibited significantly high antibiosis properties against BUT101, BUT501, BUS201 and SIA102 while isolate SM12ISP (KY038856) posted remarkably high antibiosis effect against BUS202 and BUS203 (Table 1). Analysis of bacterial extra cellular enzyme activity revealed that most isolates expressed significant biodegradative activity (Table 1). For instance, isolate SM63C (KY038855) recorded remarkably high extra cellular activity for xylanase, cellulase, protease and amylase enzymes. Pure culture of isolates SM5ISS (KY041696), SM5ISP (KY041697), SM12ISP (KY038856) and SMS15 (KY038852) expressed the same pectinase activity while isolates SM103ISS (KY038854), SM63C (KY038855) and SM8F2 (KY041695) did not secrete this enzyme (Table 1).

Analysis of antibiosis properties of fungal isolates showed that isolate SH12ISS (KY048169) expressed significantly high antibiotic effect against all test organisms used (Table 1). Evaluation of fungal extra cellular lytic enzyme activity showed that isolate SH_S13C (KY048174) recorded significantly high extra cellular activity for amylase, pectinase and xylanase tests (Table 1). Cellulase assay results showed that isolate SH12ISS (KY048169) recorded significantly high cellulase activity (Table 1). Analysis of proteolytic activity revealed that all isolates recorded significantly equal expression profile for protease enzyme activity as revealed in Table 1.

Table 1 The mean values of diameter (cm) of zones of inhibition (antibiosis) and zones of clearance (extra-cellular enzyme activity) of pure culture of selected isolates

Isolate	Antibiosis test										Extra cellular enzyme tests					
	BUT101	BUT501	BUS201	SIA102	BUS202	BUS203	Pectinase	Xylanase	Cellulase	Protease	Amylase					
Bacterial isolates	SM5ISS	6.4 ± 0.3 ^a	11.0 ± 0.5 ^a	6.0 ± 0.0 ^a	8.4 ± 0.3 ^a	0.0 ± 0.0 ^b	0.0 ± 0.0 ^b	1.4 ± 0.0 ^a	2.4 ± 0.1 ^a	0.7 ± 0.0 ^b	0.0 ± 0.0 ^d	2.0 ± 0.1 ^a				
	SM5ISP	5.0 ± 0.3 ^b	8.4 ± 0.2 ^{ab}	0.0 ± 0.0 ^c	7.3 ± 0.3 ^{bc}	0.0 ± 0.0 ^b	0.0 ± 0.0 ^b	1.5 ± 0.0 ^a	1.7 ± 0.1 ^b	0.0 ± 0.0 ^c	0.6 ± 0.1 ^c	1.8 ± 0.0 ^a				
	SM63C	4.0 ± 0.2 ^{bc}	8.0 ± 0.6 ^{ab}	0.0 ± 0.0 ^c	7.0 ± 0.5 ^{cd}	0.0 ± 0.0 ^b	0.0 ± 0.0 ^b	0.0 ± 0.0 ^b	2.6 ± 0.1 ^a	1.4 ± 0.1 ^a	2.9 ± 0.1 ^a	1.9 ± 0.1 ^a				
	SM8F2	0.0 ± 0.0 ^d	9.4 ± 0.1 ^{ab}	6.3 ± 0.2 ^a	7.7 ± 0.3 ^b	0.0 ± 0.0 ^b	0.0 ± 0.0 ^b	0.0 ± 0.0 ^b	1.1 ± 0.1 ^c	1.3 ± 0.1 ^a	1.8 ± 0.1 ^b	0.0 ± 0.0 ^c				
	SM103ISS	3.0 ± 0.1 ^c	7.0 ± 0.6 ^{bc}	0.0 ± 0.0 ^c	6.3 ± 0.3 ^{ef}	0.0 ± 0.0 ^b	0.0 ± 0.0 ^b	0.0 ± 0.0 ^b	1.6 ± 0.1 ^b	0.8 ± 0.1 ^b	2.7 ± 0.1 ^a	0.0 ± 0.0 ^c				
	SM12ISP	0.0 ± 0.0 ^d	5.9 ± 0.2 ^{bc}	0.0 ± 0.0 ^c	6.0 ± 0.0 ^f	6.3 ± 0.3 ^a	7.3 ± 0.3 ^a	1.3 ± 0.1 ^a	0.7 ± 0.0 ^d	0.6 ± 0.0 ^b	0.0 ± 0.0 ^d	0.9 ± 0.0 ^b				
	SM133C	0.0 ± 0.0 ^d	7.0 ± 0.5 ^{bc}	0.0 ± 0.0 ^c	6.3 ± 0.3 ^{ef}	0.0 ± 0.0 ^b	0.0 ± 0.0 ^b	1.3 ± 0.1 ^a	0.6 ± 0.0 ^d	0.6 ± 0.0 ^b	0.7 ± 0.0 ^c	0.8 ± 0.1 ^b				
	SMS15	0.0 ± 0.0 ^d	4.2 ± 0.2 ^c	4.1 ± 0.1 ^b	6.7 ± 0.6 ^{de}	0.0 ± 0.0 ^b	0.0 ± 0.0 ^b	1.3 ± 0.1 ^a	0.7 ± 0.0 ^d	0.7 ± 0.0 ^b	0.0 ± 0.0 ^d	0.9 ± 0.0 ^b				
	F _{7,16}	153.00	8.40	901.83	86.07	11907.00	15987.00	240.00	133.25	33.97	221.33	257.07				
	P	0.0001	0.0002	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001				
	Fungal isolates	SH12ISS	7.6 ± 0.2 ^a	12.0 ± 0.1 ^a	9.2 ± 0.1 ^a	10.0 ± 0.2 ^a	7.0 ± 0.1 ^a	8.3 ± 0.1 ^a	2.3 ± 0.1 ^{bc}	2.5 ± 0.1 ^{ab}	11.3 ± 0.2 ^a	2.7 ± 0.1 ^a	3.6 ± 0.2 ^b			
		SH9ISP	6.1 ± 0.2 ^b	6.4 ± 0.1 ^d	7.0 ± 0.2 ^c	5.1 ± 0.2 ^c	4.0 ± 0.1 ^c	5.5 ± 0.1 ^c	2.3 ± 0.0 ^{bc}	2.6 ± 0.1 ^{ab}	3.6 ± 0.1 ^b	2.7 ± 0.0 ^a	3.4 ± 0.1 ^{bc}			
		SH_JSS01F	5.9 ± 0.1 ^b	9.4 ± 0.1 ^b	8.1 ± 0.2 ^b	9.1 ± 0.2 ^b	6.0 ± 0.1 ^b	6.5 ± 0.1 ^b	1.9 ± 0.1 ^{de}	2.4 ± 0.1 ^b	3.3 ± 0.2 ^{bc}	2.6 ± 0.1 ^a	4.3 ± 0.2 ^a			
		SH8HSP	5.4 ± 0.1 ^c	8.7 ± 0.1 ^c	6.1 ± 0.1 ^d	8.5 ± 0.0 ^b	5.6 ± 0.0 ^b	6.4 ± 0.1 ^b	2.5 ± 0.1 ^{ab}	2.6 ± 0.1 ^{ab}	3.3 ± 0.1 ^{bc}	2.5 ± 0.1 ^a	2.6 ± 0.1 ^c			
		SH_S17b	4.5 ± 0.1 ^d	6.2 ± 0.1 ^{de}	4.6 ± 0.1 ^e	3.0 ± 0.1 ^d	5.6 ± 0.1 ^b	6.4 ± 0.1 ^b	1.8 ± 0.1 ^e	1.8 ± 0.0 ^e	2.5 ± 0.1 ^d	2.6 ± 0.1 ^a	2.8 ± 0.1 ^{de}			
SH_S13C		3.2 ± 0.1 ^e	6.0 ± 0.1 ^{de}	3.9 ± 0.1 ^f	2.2 ± 0.0 ^e	4.4 ± 0.1 ^c	5.9 ± 0.1 ^{bc}	2.8 ± 0.1 ^a	2.7 ± 0.1 ^a	3.3 ± 0.1 ^{bc}	2.6 ± 0.1 ^a	4.3 ± 0.1 ^a				
SH2ISS		2.9 ± 0.2 ^e	3.4 ± 0.1 ^f	4.1 ± 0.2 ^f	5.1 ± 0.2 ^c	4.0 ± 0.1 ^c	6.5 ± 0.1 ^b	2.1 ± 0.2 ^{cde}	2.7 ± 0.1 ^a	3.1 ± 0.1 ^c	2.5 ± 0.2 ^a	3.7 ± 0.1 ^b				
SH152		2.9 ± 0.1 ^e	5.7 ± 0.1 ^e	4.1 ± 0.1 ^f	2.6 ± 0.1 ^{de}	4.1 ± 0.1 ^c	5.5 ± 0.1 ^c	2.2 ± 0.1 ^{bcd}	2.4 ± 0.1 ^b	3.2 ± 0.1 ^{bc}	2.8 ± 0.1 ^a	3.1 ± 0.1 ^{cd}				
F _{7,16}		513.52	588.86	587.87	540.28	36.04	41.63	22.40	25.66	976.07	2.34	68.45				
P		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0756	0.0001				

Means with SE for each antibiosis/extra-cellular enzyme activity followed by the same letter in the same column are not statistically different as indicated by Tukey's HSD test ($p \leq 0.05$)

Empirical estimates of the ability of bacterial isolates to cause pre-germination *S. hermonthica* seed decay are presented in Fig. 1. Given that the average viability score of the *S. hermonthica* seeds used in this study was 69%, bacterial isolate SM5ISS (KY041696) recorded significantly high (45%) *S. hermonthica* seed decay (Fig. 1). However, no fungal isolate induced any remarkable change on the physiological appearance of the preconditioned *S. hermonthica* seeds (seed decay).

Analysis of the relationship between antibiosis properties, extra cellular enzyme activity and *S. hermonthica* seed decay estimates of bacterial isolates showed that these isolates clustered into four morphometric clades. Isolates SM5ISS (KY041696) and SM12ISP (KY038856) formed solitary clades while each of the remaining two clades comprised of three bacterial isolates. Isolates SM8F2 (KY041695), SM133C (KY038853) and SMS15 (KY038852) formed a clade while isolates SM5ISP (KY041697), SM103ISS (KY038854) and SM63C (KY038855) formed the last clade (Fig. 2). Isolate SM5ISS (KY041696) exhibited a remarkable strong positive relationship profile between antibiosis, extra cellular enzymatic activity and *S. hermonthica* seed decay (Fig. 2). In contrast, isolate SM133C (KY038853) recorded a relatively weak relationship profile between antibiosis, extra cellular enzymatic activity and *S. hermonthica* seed decay (Fig. 2). Analysis of the relationships among the fungal isolates with respect to antibiosis and extracellular estimates

showed that, isolate SH12ISS (KY048169) formed a unique solitary clade while the rest clustered together (Fig. 3).

Phylogenetic analysis of bacterial isolates revealed that the isolates clustered into three main genus clades namely *Bacillus*, *Streptomyces* and *Rhizobium* (Fig. 4). The *Bacillus* clade had the highest number of screened isolates distributed in four sub clades (Fig. 4). The deduced fungal phylogenetic tree profile revealed that all the screened isolates were closely affiliated to members of the genera *Aspergillus* and *Trichoderma* (Fig. 5).

Discussion

Most microbes used in biological control of crop pests, weeds and diseases secrete a myriad of metabolites that act on the pathogen by either depriving the pathogens of nutrients and space, lysing cell and/or blocking specific functions related to pathogen growth or inducing host plant resistance (Compant et al. 2012; Zhao et al. 2013). In this study, bacterial isolate KY041696 and fungal isolate KY048169 exhibited significantly high antibiosis and extra cellular enzymatic properties, which probably confers an exclusive competitive survival advantage to them over non antibiotic-producing microbes in the same environment (e.g. Kämpfer 2006). The extra cellular enzymes produced by isolates are presumably responsible for the lysis of cell wall components of the phytopathogen

Fig. 1 Proportion (%) of decaying pre-conditioned *S. hermonthica* seeds after exposure to microbial isolates. Error bars corresponds to SE, while bar with the same letters indicate means that are not significantly different as per Tukey's HSD test ($p \leq 0.05$)

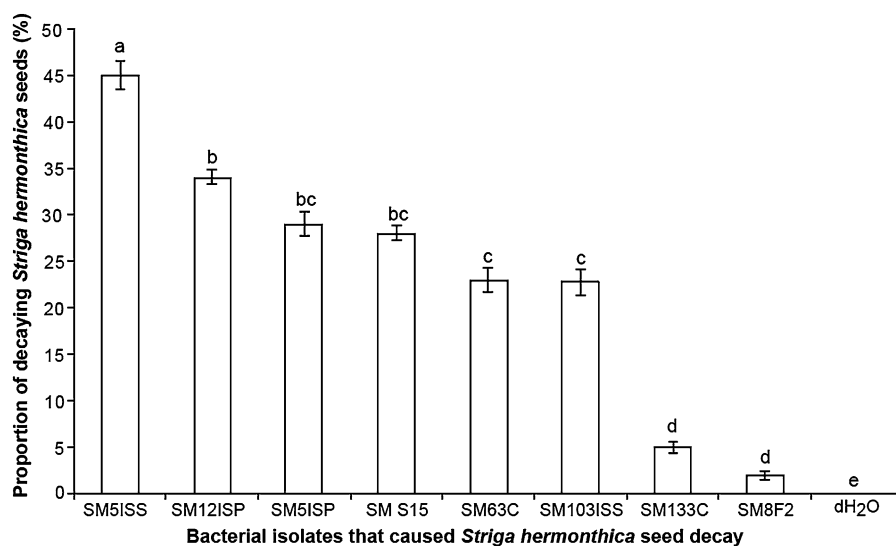
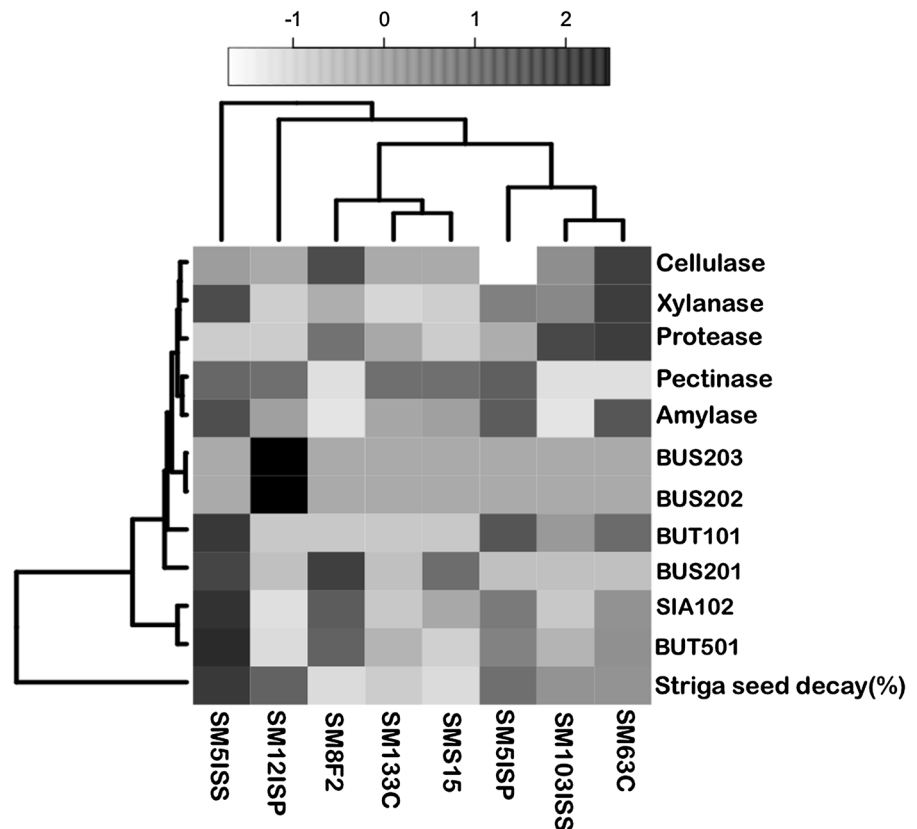


Fig. 2 Hierarchical clustergram (Manhattan metric) of bacterial isolates generated using mean values of antibiosis, enzymatic activity and proportion of decaying *S. hermonthica* seeds. The *scale bar* indicates the quantified significant strength of the assayed morphometric descriptor. *Black* and *white* colours indicate the highest and the least recorded significant mean values, respectively at $p \leq 0.05$



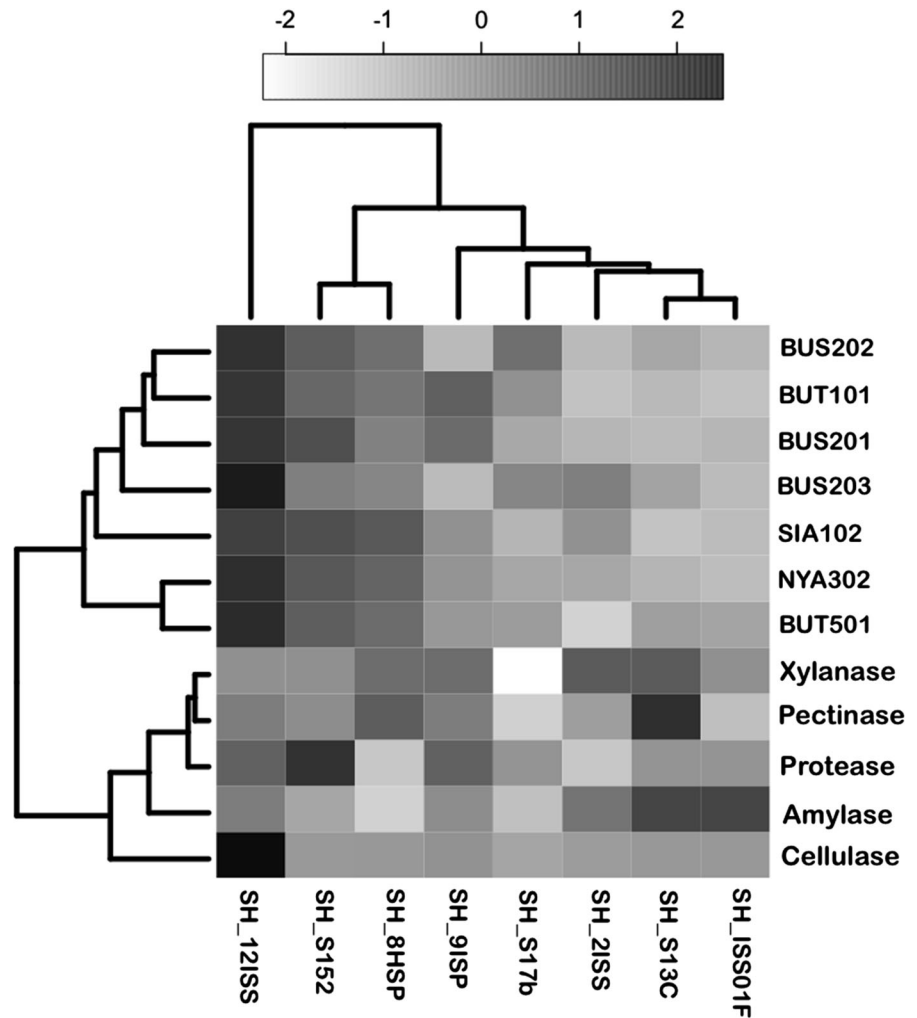
or hyperparasitism expressed against *S. hermonthica* in the form of seed decay (Sicua et al. 2015).

This study revealed that most of the bacterial isolates that caused *S. hermonthica* seed decay had close genetic relationship with members of the genus *Bacillus* with only a single bacterial isolate having close genetic affiliation to *Streptomyces* and *Rhizobium*. The huge diversity characterizing the *Bacillus* species at the taxonomic level is also noticeable for their metabolic features and this probably explains their inherent ability to decay *S. hermonthica* seed. For instance, some *Bacillus subtilis* and *Bacillus amyloliquefaciens* strains have been investigated and found to be environmentally safe biological control agents with excellent colonization capacity and remarkable versatility in protecting plants from phytopathogenic fungi (Zhao et al. 2014; Torres et al. 2015). Earlier studies have also shown that *Bacillus* spp. are spore-forming bacteria and hence can easily be stored and transported as stable biological control agent products (Choudhary and Johri 2009; Hobley et al. 2013). This study established that isolates

KY038852 and KY038854 are genetically related to members of *B. subtilis*. Previous studies reported that some *B. subtilis* strains, used in environmental bioremediation and industrial application, secreted different hydrolases which enabled them to use external cellulosic and hemicellulosic substrates present in plant cell walls (Borriss et al. 2011). Findings by Borriss et al. (2011) possibly accounts for the reason why isolates KY038852 and KY038854 have ability to cause *S. hermonthica* seed decay.

Borriss et al. (2011) demonstrated that *B. amyloliquefaciens* subsp. *plantarum* is able to colonize plant roots and produce plant growth hormone known as indole-3-acetic acid. Other studies have also established that *B. amyloliquefaciens* have the ability to produce numerous antimicrobial and bioactive metabolites such as surfactin, iturin and fengycin which have well-established in vitro activity (Liu et al. 2014). The production of these compounds highlights *B. amyloliquefaciens* and its close relative in the study (KY041696) as good candidates for the development of biocontrol agents (Liu et al. 2014). *Bacillus*

Fig. 3 Hierarchical clustergram (Manhattan metric) of fungal isolates generated using mean values of antibiosis and enzymatic activity. The *scale bar* indicates the quantified strength of the assayed morphometric descriptor. *Black* and *white* colours in the heatmap indicate the highest and the least recorded significant mean values respectively at $p \leq 0.05$



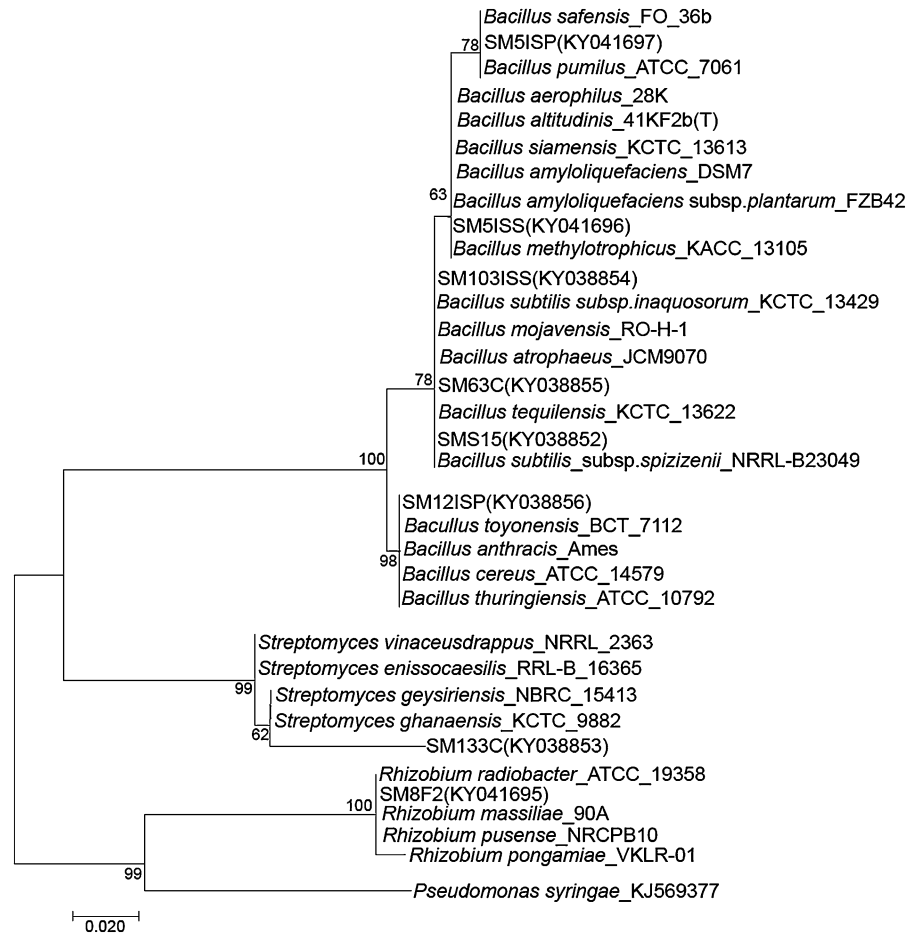
atrophaeus strains on the other hand have capacity to adapt and use several external nutrients as energy sources (Sella et al. 2008). This probably explains why isolate KY038855 which is closely related to *B. atrophaeus* caused *S. hermonthica* seed decay.

Soil microorganisms have been reported to alter soil pH and also modify the equilibrium of many chemical and biochemical reactions in the rhizosphere (Li et al. 2015). The biological activities of these microorganisms in the soil largely mediates solubilization of both macro and micronutrients (biofertilizers) thereby making them available for plant uptake at the root surface (Li et al. 2015). One such important agricultural microorganism is *B. methylotrophicus*. This strain is believed to have ability to solubilize insoluble phosphorus and inhibit mycelial growth of

phytopathogenic fungi (Mehta et al. 2013). In this study, isolate KY041696 clustered together with *B. methylotrophicus* in the same phylogenetic clade. This study also established that isolate KY041695 is genetically affiliated to members of the *Rhizobium* genus. Members of the genus *Rhizobium* have been reported to be nitrogen-fixing rhizobacteria with some members having capacity to solubilize insoluble phosphorus (Ahemad and Kibret 2014; Pereg and Mcmillan 2015). This therefore implies that isolates KY041696 and KY041695 possibly have the capacity to solubilize insoluble phosphorus and make it available to plants.

Other studies have shown that low rhizosphere phosphorus content not only affects soil productivity/fertility but also impairs proper plant growth and

Fig. 4 Phylogenetic tree profile of the selected bacterial isolates together with their closest genetically related species based on partial 16S rRNA sequence characterization. The scale bar refers to 0.02 substitutions per nucleotide position. Bootstrap values obtained with 1000 resampling are referred to as percentages at all branches



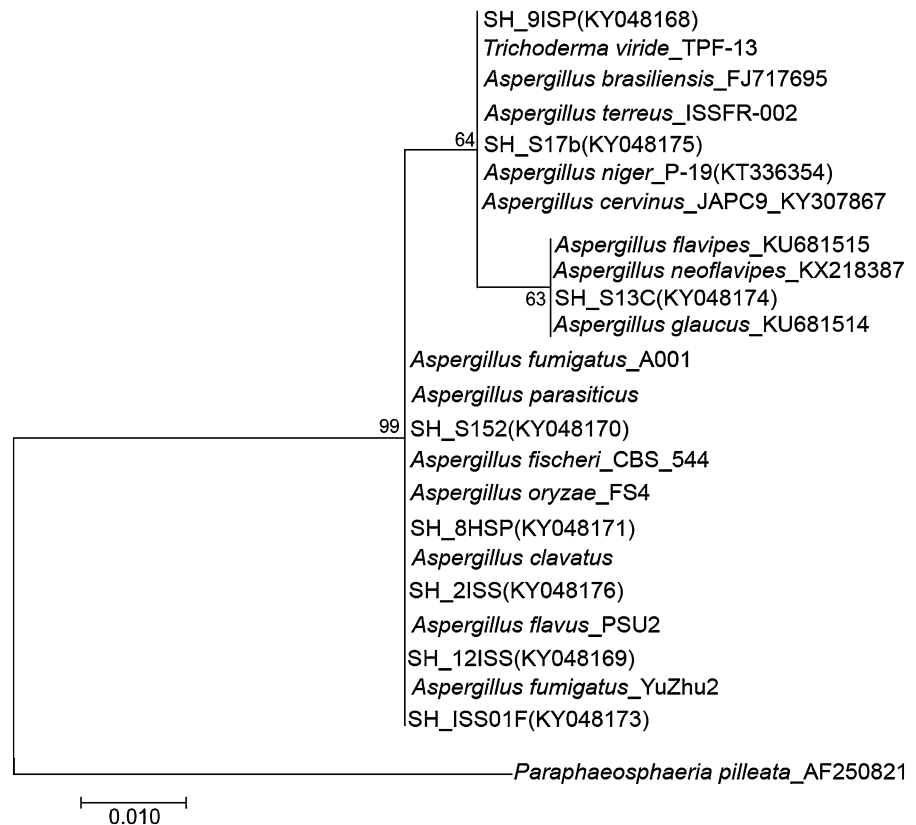
exacerbates *S. hermonthica* infection (Jamil et al. 2012). This tripartite relationship is strong in farms under cereal production (Ransom 2000). Most cereals growing in phosphorus and nitrogen deficiency soils produce natural compounds referred to as strigolactones (Yoneyama et al. 2013). These compounds are essential recognition signals that aid germination of root parasitic weeds such as *S. hermonthica* (Yoneyama et al. 2013). This therefore implies that any biological strategy aimed at increasing rhizosphere phosphorus content ultimately ameliorates *S. hermonthica* suppression rates in infested farms. This probably rationalizes the classification of soils with isolates KY041696 and KY041695 as *S. hermonthica* suppressive soil.

The presence of *Streptomyces* strains among the screened isolates is consistent with other research findings which indicate that *Streptomyces* do exist in soils of arable land (Kämpfer 2006; Tarkka et al.

2008). The success of these filamentous bacteria in terrestrial environments is attributed to their ability to produce array of catabolic enzymes that degrade biopolymers (Tarkka et al. 2008). The potential of this isolate to control *S. hermonthica* cannot be underestimated because members of *Streptomyces* have been shown to have versatile biodegradative activity (Sousa et al. 2008). Ample evidence indicates that *Streptomyces* are quantitatively and qualitatively important in the plant rhizosphere where they influence plant growth and protect plant roots against invasion by root pathogens (Tarkka et al. 2008).

Although no single fungal isolate caused *S. hermonthica* seed decay, their role as biofertilizer in rhizosphere underscores their importance in *S. hermonthica* suppression (Singh et al. 2011). In this study, isolate KY048168 is genetically related to *Trichoderma viride*. *Trichoderma* is widely used as biocontrol agent against phytopathogenic fungi, and as a

Fig. 5 Phylogenetic tree profile of the selected fungal isolates together with their closest genetically related species based on partial 18S rRNA sequence characterization. The scale bar refers to 0.01 substitutions per nucleotide position. Bootstrap values obtained with 1000 resampling are referred to as percentages at all branches



biofertilizer because of its ability to establish mycorrhiza-like association with plants (Saba et al. 2012; Pereg and McMillan 2015). The key factor to the ecological success of this genus is the combination of very active mycoparasitic mechanisms plus effective defense strategies induced in plants (Saba et al. 2012). Major mechanisms involved in the biocontrol activity of *Trichoderma* spp. are competition for space and nutrients, production of diffusible and/or volatile antibiotics and hydrolytic enzymes like chitinase and β -1,3-glucanase (Saba et al. 2012; Lakshmanan and Sadasivan 2016). These hydrolytic enzymes partially degrade the pathogen cell wall and leads to its parasitization (Saba et al. 2012). Several mechanisms by which *Trichoderma* influences plant development have been proposed and these include: production of phytohormones, the solubilization of sparingly soluble minerals, induction of systemic resistance in the host plant, reduction in pollutant toxicity (organic or heavy metal), and the regulation of rhizospheric microflora (Li et al. 2015).

All the screened fungal isolates in this study have genetic affiliation to *Aspergillus*. *Aspergillus* species are a fascinating group of fungi exhibiting immense ecological and metabolic diversity (Rank et al. 2010). Several members of *Aspergillus* have been found to be involved in phosphorus solubilization and bioremediation processes in the rhizosphere (Singh et al. 2011). However given the association with aflatoxin, which is a cancer causing chemical, utilization of *Aspergillus* species as a biological control agent especially in maize fields needs to be carefully scrutinized to avoid grain contamination. The close genetic affiliation to genus *Aspergillus* by most of the screened fungal isolates cultured from *S. hermonthica* suppressive soils underlines their role in *S. hermonthica* suppression. It is interesting that in the sampled region, *F. oxysporium* f. sp. *strigae* which has been widely associated with *S. hermonthica* suppression was not isolated (Nzioki et al. 2016; Avedi et al. 2014).

This study has demonstrated that soil microbes with potential of being used as biocontrol agents produce

several extra-cellular lytic enzymes and antibiotic compounds. However additional research is needed to determine the functions and mode of action of the virulent enzymes of these microbes needed for development of novel biological control remedies of *S. hermonthica*.

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Compliance of ethical standards

Conflict of interest The authors have no conflict of interest to declare.

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Johnstone Omukhulu Neondo has completed his PhD research study titled 'Exploring biocontrol and transgenic weed management approaches against infestation by *Striga hermonthica* in maize' at Institute for Biotechnology research of Jomo Kenyatta University of Agriculture and Technology, Nairobi Kenya. He was tasked with responsibility of carrying out laboratory experiments of both bioprospection for striga biocontrol agents as well as genetic transformation of selected local maize varieties with PAP gene.

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