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In vitro study of endophytic bacteria, carbohydrates and their combination on early developmental stages of *Striga hermonthica* (Del.) Benth.

Nadia Yasseen Gafar, Mohammed Mahgoub Hassan, Magdoline Mostafa Ahmed, Awad Galal Osman, Migdam Elsheikh Abdelgani and Abdelgabar Eltayeb Babiker

[1]*Environment and Natural Resources and Desertification Research Institute, National Centre for Research, Khartoum, Sudan*

Address For Correspondence:
Mohammed Mahgoub Hassan, Environment and Natural Resources and Desertification Research Institute, National Centre for Research, Khartoum, Sudan
E-mail: mohkadis@yahoo. Com

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ABSTRACT

*Striga hermonthica*is a serious parasitic weed on cereal crops which causes severe damage to its host. Three endophytic bacterial isolates (isolated from sugarcane crop) and two carbohydrates, glucose and sucrose, were evaluated in a laboratory, for effects on the parasite germination and haustorium initiation. The results indicated that bacterial isolates ISO20, ISO29 and ISO30,significantly inhibited germination as compared to the controls, irrespective of conditioning status of *Striga* seed. The two carbohydrates, alone or in combination with bacterial isolates, were evaluated on *Striga* germination. Results revealed that each of the two carbohydrates, irrespective of the concentration, reduced germination significantly by 29 - 100 %,as compared to the control. The depressive effects of the carbohydrates on *Striga* germination increased with increasing concentrations, in response to GR24. Furthermore, glucose was more suppressive to *Striga* germination than sucrose. The combination of glucose and sucrose sustained the highest inhibitory effects (63 - 67 %) as compared to each carbohydrate alone and the controls. However, the combination between bacterial isolates plus glucose or sucrose was the most inhibitory than each bacteria or carbohydrate alone. They reduced germination by 79 and 100 % in response to GR24 as compared to conditioning media. Moreover, both carbohydrates reduced haustorium initiation, significantly in response to DMBQ. Furthermore, seeds conditioned in sucrose or glucose at the highest concentration (10 g/100 ml) and similarly treated with DMBQ did not form haustoria. Bacterial isolates were identified using morphological and biochemical tests. The establishment of the inhibitory effect of the most promising bacterial isolateISO20(*Gluconacetoacter* spp.)on early developmental stages of *Striga* is a step towards utilizing such bacteria as biocontrol agents against *S. hermonthica.* In conclusion: carbohydrates enhanced the efficacy of the bacteria as a suppressor of the parasite and reducer of the parasite debilitating effects.

KEYWORDS: *bacterial isolates, carbohydrates, Striga, germination, haustorium*

INTRODUCTION

Sorghum is the second most important cereal crop after maize in Sub-Saharan Africa. It is the main staple food for about 300 million people who live in the semi-arid tropics [1]. In Sudan, sorghum is the most important cereal crop in terms of production and consumption. Although the crop's importance and the long experience in its cultivation, sorghum yield is very low (0.4 t/ha,) [2] compared to its potential. The low productivity can be attributed mainly to the use of traditional low-yielding varieties, limited or no use of fertilizers, and poor management practices. Parasitic plant species, *Striga*, are obligate root parasites which depend on sorghum for the supply of carbon, nutrients and water. Problems with parasitic weeds are compounded by many factors. For

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example, many regions in Africa and South Asia where these parasitic weeds are endemic are inhabited by small landholder farmers who are unable to adopt expensive chemical control or to use modern agricultural practices. The complex bio-ecology of *Striga* and its close physiological interaction with its host plants are the main problems that limit the development of positive control measures that can be accepted and used by subsistence farmers. Control measures involve rotation with trap crops, intercropping with legumes, use of *Striga*-tolerant varieties, early planting, fertilizers, herbicides, resistant varieties, solarization, and even hand weeding [3]. In many infested areas, integrated control is practiced and has been effective [4].Moreover, the most effective strategy is to reduce the soil seed bank and/or inhibit the parasite at an early growth stage (e.g. germination and radicle elongation). In that sense, 'suicidal germination' is one of the most attractive strategies to reduce the soil seed bank [5].Biological control is considered as a potential cost-effective and environmentally safe means for reducing weed populations in crops, forests, or rangelands where low profit margins prevent large herbicide expenditure [6].Plant growth promoting rhizosphere are known to increase root system uptake properties of rhizobacteria colonized crops by facilitating ion nitrate adsorption, Phosphate solubilization, and iron chelation [7]. The present investigations were undertaken to evaluate the effects of selected *bacterial isolates* and carbohydrates, on early developmental stages of the parasite.

MATERIALS AND METHODS

A series of laboratory experiments were undertaken to examine the efficacy of bacterial isolates and carbohydrates on early developmental stages of *Striga hermonthica*. In all experiments, treatments were arranged in a factorial design with randomized complete design (RCD) with four replicates. All laboratory experiments were conducted at the Department of Alternatives to Pesticides and Biological Control, Environment and Natural Resources Research Institute (ENRRI), National Center for Research (NCR), Khartoum, Sudan.

Plant materials:
Striga hermonthica seeds:

Striga hermonthica seeds were collected from parasitic plants growing under sorghum in 2008 at Abu Nama Research Station Farm, Sinnar, Sudan.

Test solutions:
GR24 stock solution:

The strigolactone analogue GR24 was provided by professor Zwanenberg, B. the University of Nimijhen, the Netherlands. A stock solution (10 ppm) of GR24 was prepared by dissolving 1 mg in 1 ml of acetone and completing to volume (100 ml) with sterile distilled water. The solution was kept refrigerated at 4ºC for further use.

DMBQ stock solution:

2, 6- dimethoxybenzoquinone (DMBQ) was provided by Prof. Sugimoto, Y. from Kobe University, Japan. A stock solution (100 µM) was prepared by dissolving 1.68 mg in 1 ml acetone and completing to volume (100 ml) with sterile distilled water.

Carbohydrates:

Glucose and sucrose were purchased from the local market. A stock solution of each carbohydrate was prepared by dissolving 50 g in 50 ml distilled water (1g/ml).

Laboratory experiments:
Isolation of endophytic bacteria:

Root and stem samples were collected from a sugarcane fields and immediately brought to the laboratory for isolation. The method employed by Islam et al [7] and Rashedul et al., (8) were used for the isolation of endophytic bacteria as shown stepwise below:

Preparation of growth medium:

Sold and semi-solid LGI-P media were used for the growth of endophytic bacteria.

Inoculations in the semi-solid medium:

For isolation of endophytic bacteria, the two portions of sugarcane (root and stem) were used. Under aseptic condition, one gram of sugarcane roots surface sterilized (as described above) was transferred to a sterile mortar with 10 ml sterile distilled water and 10 mg of silicon carbide (600 mesh), then gently crushed by a sterile pestle.

Furthermore, stem juice samples were also taken. Aliquot, (0.25 ml) from each sample root and stem juices were inoculated separately into tubes containing 1 ml of semisolid LG1-P medium. The tubes were incubated at room temperature for about 3 - 4 days for the formation of subsurface pellicles. All cultures were streaked on LG1-P (plates) for purification.

A total of 14 endophytic bacteria isolates were screened in the laboratory for their ability to inhibit GR24 - induced germination of *S. hermonthica* seeds.

Striga hermonthica seeds surface disinfection, conditioning and germination:

Striga seeds were collected and clean by placement in a measuring cylinder (1000 ml) containing tap water. Floating materials containing debris and immature light seeds were discarded. The seeds were washed several times with tap water to free them from sand. Then seeds were surface disinfected by socking in 70 % ethanol for 2 min. with continuous agitation and rinsed three times with sterile distilled water. Subsequently, the seeds were immersed in 1 % sodium hypochlorite (NaO_2Cl) for 2 min. and rinsed three times with sterile distilled water. The NaO_2Cl solution was obtained by dilution of commercial (5 %) sodium hypochlorite (Bleach) solution. Subsequently, the seeds were thoroughly washed with sterilized distilled water. The seeds were plotted dry on Whatman filter paper (No.1) under a laminar flow hood, then kept in sterilized vial for further studies.

Striga seeds were conditioned as described by Babiker *et al.*[9]. Glass fiber filter papers (GF/C) discs (8 mm diameter) were cut, wetted thoroughly with water and placed in an oven at 100 ºC for 1 h to be sterilized. The discs, placed in 9 cm Petri dishes lined with glass fiber filter papers (GF/C), were moistened with 5 ml distilled water, or diluted media inoculated or not inoculated with the respective bacterium isolates or strains. About 25 - 50 surface disinfected *S. hermonthica* seeds were sprinkled on each of the glass fiber discs in each Petri dish. The dishes, sealed with parafilm were placed in black polythene bags and incubated at 30 ºC in the dark for 10 days. The disc containing *Striga* seeds were dapped on normal filter paper (No. 1) to remove excess water and then transferred to sterile Petri dishes. Each disc was treated with 20 µl of GR24 at 0.01 and/or 0.1 ppm. Then the seeds were re-incubated and examined for germination 24 h later using a stereomicroscope.

Effects of two carbohydrates on S. hermonthica seeds germination:

The aim of this experiment was to study the effects of two carbohydrates (glucose and sucrose) on *Striga* seed germination in response to GR24 during and after conditioning. Glucose and sucrose with different concentrations (5, 10, 15, 20, 25 and 30 g/100 ml) were used in this experiment. *Striga* seeds were conditioned in distilled water or carbohydrates with their different concentrations as previously described, then discs containing *Striga* seeds were treated with 20 µl of GR24 (0.01and 0.1 ppm) or distilled water as previously described. The seeds were re-incubated and examined for germination 24 h. later using a stereomicroscope.

Effects of selected bacterial isolates and carbohydrates on S. hermonthica germination:

This experiment was conducted to study the effects of the three endophytic bacterial isolates (ISO20, ISO29 and ISO30), selected on basis of the results of the preliminary screening, and two carbohydrates (glucose and/or sucrose), were evaluated using the previously described procedure for ability to suppress *Striga* seeds germination. *Striga* seeds were conditioned as previously described. The discs containing *Striga* seeds were treated with 20 µl of GR24 at 0.01 and 0.1 ppm or distilled water. The seeds were re-incubated and examined for germination as described above.

Effects of carbohydrates and GR 24 mixture on germination of Striga seeds pre-conditioned in bacterial isolates:

S. hermonthica seeds conditioned in water for 10 days as previously described were treated with three endophytic bacterial isolates (ISO20, ISO29 and ISO30), separately for 5 days. The discs containing *Striga* seeds in bacterial isolates were treated with aliquots 20µl of the combination of each carbohydrate (15g/100 ml) and GR24 (0.1ppm) in ratio (1:1v/v). The seeds were re-incubated and examined for germination as was described above.

Effects of carbohydrates on Striga haustorial initiation:

Two carbohydrates (glucose and sucrose) with different concentrations (0.31, 0.63, 1.25, 2.5, 5 and 10 g/100 ml) were evaluated for their effects on *Striga* haustorial initiation as previously described. *Striga* seed discs were conditioned in presence or absence of the two carbohydrates with different concentrations. After 10 days, discs were treated with 20 µl of GR24 at 0.1 ppm to induce germination. Then dishes containing the discs were re-incubated for 48 h. at 30 ºC. As previously described, the pairs of discs were treated with 40 µl of DMBQ at 10 and 20 µM. The Petri dishes were re-incubated in the dark at 30 ºC for 24 h. and examined for haustorial initiation.

Statistical data analysis:

In all laboratory experiments, treatments were arranged in a randomized complete design with 4 replicates. Data on percentage germination and haustorial initiation were calculated for each disc, transformed to arcsine and subjected to analysis of variance (ANOVA). Means were tested for significance by the Duncan Multiple Range Test at $P \leq 0.05$ [10].

Identification of selected isolates:

Cultural, morphological and biochemical tests were made. Identification of endophytic bacterial isolates was done using API20 NE system (bioMe´rieux/France). This experiment was conducted at the Department of Microbiology, Veterinary Research laboratory, Soba, Sudan.

Result:

Effects of carbohydrates on Strigaseeds germination in response to GR24 (during and after conditioning):

Striga seeds conditioned in distilled water for 10 days and then treated with GR24 at 0.01 or 0.1 ppm displayed the highest germination (49- 48 %) (Table 1). Results displayed that the two carbohydrates (glucose and sucrose), irrespective of their concentrations reduced germination significantly as compared to the control. Germination of seeds imbibed in the highest concentration (30 g/100 ml) of each glucose or sucrose and treated with GR24 at 0.01 ppm was completely suppressed (100 %). While at the lowest concentration (5 g/100 ml) of glucose and sucrose, germination of *Striga* was reduced significantly by 49 and 37 %, respectively as compared to the control, irrespective of GR24 concentration. GR24 applied to seeds conditioned in water induced the highest germination (61 and 55 %) (Table 2). *Striga* seeds conditioned in water for 10 days and then treated with carbohydrates at different concentrations for 3 days, displayed reduced significantly germination in response to GR24 as compared to the control. The highest concentration (30 g/100ml) of glucose applied to conditioned seeds reduced germination significantly in response to GR24 at both concentrations as compared to the control. It reduced germination by 52 and 45 %, respectively. Germination of seeds conditioned in sucrose at 30 g/100 ml exhibit decreased germination, irrespective of GR24 concentrations. It reduced germination by 44 %. Moreover, sucrose at the lowest concentration (5 g/100ml) had no significant effects on *Striga* seed germination, irrespective of GR24 concentrations. Both carbohydrates reduced radical length at 20 g/100 ml. Generally, the depressive effects of the two carbohydrates decreased with increasing GR24 concentrations. Furthermore, glucose was more suppressive on *Striga* seed germination than sucrose.

Effects of selective bacterial isolates and carbohydrates, applied during conditioning on S. hermonthica seeds germination in response to GR24:

GR24 at 0.01 and 0.1 ppm. applied to seeds conditioned in water induced 80 and 83 % germination, respectively (Table 3). However, LGI-P broth medium reduced seeds germination significantly, irrespective of GR24 concentration as compared to the water control. Among bacterial isolates, isolate ISO20, was the most inhibitory. It reduced germination significantly by 10 and 18 % in response to GR24 as compared to LGI-P medium and water, respectively. *Striga* seeds conditioned in glucose or sucrose alone or in combinations displayed 37, 43 and 23 % germination, respectively in response to GR24. The combination of glucose and sucrose sustained the highest inhibitory effect (63 - 67 %) as compared to each carbohydrate alone and the controls. The highest inhibitory effect was achieved by the combinations between each bacterial isolate with either glucose or sucrose. In addition, the combination between each bacterial isolate with glucose and sucrose reduced germination by 79 and 100 % in response to GR24 as compared to conditioning media.

Effects of selective bacterial isolates and carbohydrates, applied at termination on S. hermonthica seeds germination treated with carbohydrates and GR24:

Striga seeds conditioned in water and treated with GR24 (0.01 ppm) alone or in combination with either glucose or sucrose (15g/100 ml) displayed 51, 43 and 44% germination, respectively (Table 4). However, LGI-P broth medium reduced germination in response to GR24 alone or in combination with each carbohydrate, albeit not significantly as compared to the water control. *Striga* seeds conditioned in presence of glucose or sucrose showed reduced germination significantly in response to GR24 alone or in combination with each carbohydrate as compared to water control. Generally, glucose was more inhibitory than sucrose, in response to GR24 alone or in combinations with carbohydrates. Moreover, in among all bacterial isolates, *Striga* seeds conditioned in bacterial isolate ISO20 and treated with GR24 alone or in combinations with either glucose or sucrose displayed 50, 18 and 18 % germination, respectively. They reduced germination by 1, 58 and 59 %, respectively as compared to the water control. However, *Striga* seeds inoculated with bacterial isolate ISO30 and treated with GR24 alone or in combinations with either carbohydrate displayed induced germination, albeit not significantly as compared to LGI-P broth medium.

Effects of carbohydrates on haustorial initiation in response to DMBQ:

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DMBQ (10 and 20 µM), applied to *Striga* germilings resulting from seeds conditioned in water displayed 64 and 71 % haustorium initiation, respectively (Table 5). DMBQ applied to *Striga* germilings resulting from seeds conditioned in glucose at 0.31, 0.36 and 1.25 g/100 ml reduced haustorium initiation significantly to 18, 37 and 39 %, respectively as compared to the control. However, germilings from seeds conditioned in glucose at the highest concentrations (2.5 to 10 g/100 ml) displayed no haustorium initiation in response to DMBQ as compared to the control. Conditioning in sucrose at 0.31 to 5 g/100 ml reduced hautorium significantly by 9 to 85 % in response to DMBQ as compared to control. However, at the highest concentration of sucrose (10 g/100 ml) haustorium initiation completely inhibited in response to DMBQ as compared to the water control. In the present study, the bacterial isolates *Bacillus* spp. and acetic acid bacteria that showed reduction in *Striga*haustorium initiation may be due to production of catalase enzyme.

Discussion:

About 35% of the carbon for *Striga* plant growth comes from the host photosynthate. *Striga* is reported to perturb the hormonal balance of its host. Sharp reductions in Indol Carbohydrates constitute 5 to 25% of the organic matter in most soils [11].

Carbohydrates are degraded by microorganisms to metabolizable substances, which are the most readily available food for soil organisms [12].The present study revealed thatglucose at highest concentration was the most inhibitory. Similar results were reported by Tomilov *et al*., [13]. Results displayed that the depressive effect of glucose and sucrose on *Striga* germination increased with increasing carbohydrates concentrations. Similar findings were reported by Hassan *et al.*, [14]. Furthermore, Chae*et al*. [15] reported that other carbohydrates such as fructose, D-fructose 1:6-diphosphate and L-sorbose, promoted slight germination of *Striga* seeds at lower concentrations. The combination between isolates and carbohydrates increased the inhibitory effects on *Striga* seeds germination, may be through production of chemical (acetic acid) or perturbation of ethylene biosynthesis. The results are consistent with a model in which both germination and subsequent morphogenesis in *Striga* are associated with exogenous and endogenous phytohormones [9]. In the present study, sucrose, irrespective to the concentrations, was drastically decreased *Striga* germination. It is known that sugars act as important signaling molecules like phytohormones throughout all stages of plant development [16]. For example, as a sugar-inducible carbohydrate-related metabolism, a supply of sucrose, glucose, or fructose to *Arabidopsis* induces expression of a gene for β-amylase [17]. In fact, sugar metabolism in germinating seeds of *S. hermonthica*may slightly differs from that in germinating seeds of broomrapes because there was less planteose in *S. hermonthica* seeds than in broomrape seeds18]. The differences in sugar use during germination of root parasitic weeds in Orobanchaceae may be attributed to their ability to photosynthesize. Hemi-parasites are capable of fixing carbon themselves, whereas holo-parasites obtain all of their reduced carbon from their hosts [19]. It may be possible that the presence of foreign substances inside the host vessels activates the release of the carbohydrate into the infected vessels, an additional defense mechanism, similar to that known to be associated with wilt diseases [20].

Plants generally store starch or lipids in seeds as a source of energy for germination. However, seeds of *S. hermonthica* lack detectable amount of starch [18] suggesting that the dry seeds of root parasitic weeds may contain little or no starch. Sucrose can act as a signal and regulates many genes involved in growth and development [21, 22].Hassan *et al* [14] reported that *Striga* seeds germination was affected by sucrose and glucose. They reduced germination by 20 and 60%, respectively as compared to the control. The depressive effects on *Striga* germination increased with increasing sugar concentrations. The sucrose level in *S. hermonthica* seeds drastically decreased during germination, and there was a significant accumulation of monosaccharides at the later stage of germination [18].

Similarly Dor *et al*. (2007) reported reduced germination and loss of viability in seeds of several *Orobanche* species due to toxins produced by *Fusarium oxysporuf.sp*. *orthocrars*. Previous reports indicated that germination of *S. hermonthica* and *S. asiatica* needs both ethylene biosynthesis and action [9] and that GR24 reduces abscisic acid (ABA) and increased Gibberellins (GAs) and cytokinins (CKs) levels in germinating *S. hermonthica* seeds [23]. Soil borne pathogens have a number of practical advantages in the control of root parasite such as *Orobanche*. Bacteria involved in plant-microbe interactions have been reported to synthesize hormones such as ABA, GA and IAA [24]. The functional role of hormone synthesis by microorganisms is not fully understood. Bacterial isolates and strains were found to produce phytotoxins [20].

These substances proved to be very phytotoxic against *Striga* seeds even at lower concentrations. González *et al.*,[26] reported that acetic acid bacteria Acetobacter progression in grapes produces high level of acetic acid. Plants generally store starch or lipids in seeds as a source of energy for germination. In the present study result displayed that, the combination of each bacterial isolate and glucose and sucrose reduced germination by 79 and 100 % in response to GR24 as compared to conditioning media.

Carbohydrates supply carbon sources for microbial activities that contribute to mineral nutrient production in soil. Sugars act as a source of energy for microbes, supporting nutrient mineralization in C limiting tropical soils. Solomon *et al*.,[27] reported that the clay fraction is enriched with microbial derived carbohydrates

whereas coarse and fine sand fractions of soil are enriched with plant derived carbohydrates. Some studies have indicated that when arbuscular mycorrhizal (AM) fungal spores absorb glucose, the supply of this carbon source facilitates the biosynthesis of amino acids, including arginine as the main amino acid for new germ tube and hypha growth. This is in turn improves N absorption[28].A supply of exogenous glucose to the AM fungal spores generated a significant enhancement in the uptake of exogenous N sources, with more than 3 times more free amino acids being produced than those supplied with only exogenous CO_2. Meanwhile, arginine was the most abundant free amino acid produced and it was incorporated into the proteins of AM fungal spores to serve as an N storage compound [29].Therefore, soil carbohydrates and their relationships with soil nutrients in different land use practices may provide vital information regarding the availability of limiting nutrients in natural and managed ecosystems in the tropics [30]. The present study revealed that, the highest concentration of sucrose at 10 g/100 ml was completely inhibited haustorium initiation in response to DMBQ as compared to the water control. The bacterial isolates *Bacillus* spp. and acetic acid bacteria that showed reduction in *Striga* haustoria may be due to production of catalase enzyme. Differential production of the enzyme catalase, which disproportionate H_2O_2 to H_2O and molecular oxygen, by bacterial isolates would lead to differential production of DMBQ and hence differential reduction in haustorium initiation [31; 32]. The degraded products from host cell walls, as carbohydrates, can act as the endogenous elicitors of defense responses [33].Carbohydrates, sucrose or glucose, may be also involved in the osmoregulation of *Striga* germination. In conclusion: carbohydrates enhanced the efficacy of the bacteria as a suppressor of the parasite and reducer of the parasite debilitating effects.

Table 1: Effects of carbohydrates, applied during conditioning on *S. hermonthica* seeds germination in response to GR24

	Germination (%)													
		Carbohydrates concentrations (g/100 ml)												
GR24 (ppm)	Water	Glucose						Sucrose						mean
		5	10	15	20	25	30	5	10	15	20	25	30	
0.01	(49)	(24)	(17)	(14)	(10)	(0)	(0)	(28)	(24)	(24)	(23)	(10)	(0)	(17)
	57	18	9	6	4	0	0	23	17	16	16	4	0	13
0.1	(48)	(25)	(18)	(17)	(10)	(0)	(0)	(33)	(30)	(28)	(23)	(11)	(0)	(19)
	55	19	10	9	4	0	0	30	25	23	16	5	0	15
Mean	$(48)^{f}$	$(25)^{de}$	$(18)^{c}$	$(16)^{bc}$	$(10)^{b}$	$(0)^{a}$	$(0)^{a}$	$(30)^{e}$	$(27)^{de}$	$(26)^{de}$	$(23)^{d}$	$(10)^{b}$	$(0)^{a}$	
	56	18	10	7	4	0	0	26	21	20	16	4	0	

Values between parenthesis ( ) indicate arcsine transformed data.
Means within a row followed by the same letter (s) are not significantly different according to Duncan Multiple Range Test at $P \leq 0.05$.
S.E. for carbohydrates (± 2.8) S.E. for concentration (± 1.1) S.E. for interaction (± 4)

Table 2: Effects of carbohydrates, applied at termination on *Striga* seeds germinationin response to GR24

	Germination (%)													
		Carbohydrates concentrations (g/100 ml)												
GR24 (ppm.)	Water	Glucose						Sucrose						mea n
		5	10	15	20	25	30	5	10	15	20	25	30	
0.01	(51)	(44)	(41)	(34)	(33)	(27)	(25)	(55)	(47)	(42)	(26)	(25)	(24)	(36)
	61	49	43	31	30	22	18	67	54	45	20	18	18	36
0.1	(48)	(47)	(45)	(43)	(42)	(36)	(26)	(45)	(45)	(44)	(44)	(32)	(31)	(41)
	55	53	50	47	46	38	20	50	50	48	48	29	26	43
Mean	$(49)^{f}$	$(46)^{ef}$	$(43)^{def}$	$(38)^{cde}$	$(38)^{cd}$	$(32)^{abc}$	$(25)^{a}$	$(50)^{f}$	$(46)^{ef}$	$(43)^{def}$	$(35)^{bc}$	$(28)^{ab}$	$(28)^{ab}$	
	58	51	47	39	38	30	19	58	52	47	34	24	22	

Values between parenthesis ( ) indicate arcsine transformed data.
Means within a row followed by the same letter (s) are not significantly different according to Duncan Multiple Range Test at $P \leq 0.05$.
S.E. for carbohydrates (±3.5) S.E. for concentration (±1.3) S.E. for interaction (±4.9)

Table 3: Effects of selected bacterial isolates and carbohydrates, applied during conditioning on *S. hermonthica* seeds germination in response to GR24

	Germination (%)																	
	Conditioning Media					Combination of Isolates and Carbohydrates (15g/100 ml)												
						Isolates + Glucose			Isolates + Sucrose			Isolates + Glucose and Sucrose						
GR24 (ppm.)	W[1]	B[2]	ISO 20	ISO 29	ISO 30				ISO 20	ISO 29	ISO 30	ISO 20	ISO 29	ISO 30	ISO 20	ISO 29	ISO 30	
						Glucose	Sucrose	Glucose +Sucrose										mean
0.01	(63)	(57)	(50)	(53)	(61)	(35)	(40)	(21)	(0)	(0)	(0)	(0)	(4)	(5)	(0)	(0)	(9)	(23)
	80	70	59	63	77	34	41	13	0	0	0	0	2	2	0	0	3	26
0.1	(66)	(60)	(55)	(57)	(65)	(39)	(46)	(23)	(0)	(12)	(3)	(12)	(11)	(9)	(0)	(0)	(15)	(28)
	83	75	68	70	80	39	52	15	0	6	1	6	5	0	0	0	7	30
Mean	$(65)^{k}$	$(59)^{ij}$	$(53)^{h}$	$(55)^{hi}$	$(63)^{jk}$	$(37)^{f}$	$(43)^{g}$	$(23)^{e}$	$(0)^{a}$	$(6)^{bc}$	$(1)^{ab}$	$(6)^{bc}$	$(7)^{cd}$	$(7)^{cd}$	$(0)^{a}$	$(0)^{a}$	$(12)^{d}$	
	81	72	64	66	78	36	46	14	0	3	1	3	3	3	0	0	0	

W[1]: Water,B[2]: LGI-P broth medium. Values between parenthesis ( ) indicate arcsine transformed data. Means within a row followed by the same letter (s) are not significantly different according to Duncan Multiple Range Test at $P \leq 0.05$. S.E. for carbohydrates and isolates (±2.5) S.E. for concentration (±0.9) S.E. for interaction (±3.5)

Table 4: Effects of selected bacterial isolates on *Strigahermonthica* seeds germination treated with carbohydrates and GR24 (after conditioning):

GR24 (ppm.)	Germination (%)							
	Conditioning media		Carbohydrates (15g/100 ml) and bacterial isolates					
	W[1]	B[2]	Glucose	Sucrose	ISO20	ISO29	ISO30	Mean
0.01	(51)	(49)	(23)	(25)	(50)	(53)	(54)	(44)
	60	57	15	18	59	64	66	48
0.01+G15	(43)	(31)	(23)	(25)	(18)	(38)	(40)	(31)
	47	27	15	18	14	38	42	29
0.01+S15	(44)	(35)	(23)	(25)	(18)	(23)	(44)	(30)
	48	34	15	17	12	16	49	27
Mean	$(46)^{d}$	$(39)^{c}$	$(23)^{a}$	$(25)^{ab}$	$(29)^{b}$	$(38)^{c}$	$(46)^{d}$	
	52	40	15	18	28	39	52	

W[1]: Water, B[2]: LGI -P broth medium. Values between parenthesis ( ) indicate arcsine transformed data.
Means within a row followed by the same letter (s) are not significantly different according to Duncan Multiple Range Test at P $\leq$ 0.05.
S.E. for bacteria (±3.3) S.E. for concentration (±1.8) S.E. for interaction (±4.7)

Table 5: Effects of carbohydrates on *Striga*haustorium initiation in response to DMBQ:

DMBQ µM	Haustorium (%)													
		Carbohydrates concentrations g/100ml												
		Glucose						Sucrose						
	Water	0.31	0.63	1.25	2.5	5	10	0.31	0.63	1.25	2.5	5	10	mean
10	(53)	(39)	(29)	(25)	(0)	(0)	(0)	(46)	(31)	(25)	(18)	(6)	(0)	(21)
	64	40	23	18	0	0	0	52	27	18	12	4	0	20
20	(57)	(51)	(41)	(33)	(0)	(0)	(0)	(54)	(35)	(31)	(26)	(11)	(0)	(26)
	71	60	44	29	0	0	0	65	34	27	19	7	0	27
Mean	$(55)^{i}$	$(45)^{g}$	$(35)^{f}$	$(29)^{de}$	$(0)^{a}$	$(0)^{a}$	$(0)^{a}$	$(50)^{h}$	$(33)^{f}$	$(28)^{d}$	$(22)^{c}$	$(8)^{b}$	$(0)^{a}$	
	67	50	33	24	0	0	0	59	30	22	16	5	0	

Values between parenthesis ( ) indicate arcsine transformed data.
Means within a row followed by the same letter (s) are not significantly different according to Duncan Multiple Range Test at P $\leq$ 0.05.
S.E. for carbohydrates (±1.7) S.E. for concentration (±0.7) S.E. for interaction (±2.4).

Conclusions:

1. Bacterial isolates perturb early growth stages of *S. hermonthica*. Circumstantial evidence suggests that the bacterial action on *Striga* may be mediated through phytohormones.
2. Carbohydrates especially at the higher doses decreased *Striga* germination and haustoria.
3. Bacterial isolates in combination with carboydrates (used as substrate) increased the efficacy of microorganisms in reducing germination and haustoria of *Striga*.
4. Future research should focuses on : i) screen various inexpensive compounds and local materials as physiological precursors for the respective phytohormones ii) establish the identity of the phytohormones produced by individual bacterium and bacterial isolate.

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