Annals of
Plant Sciences
Volume 9, Issue 10 (2020) pp. 4049 - 4059
ISSN: 2287-688X
Research Article

Impact of *Trichoderma harzianum* and bacterial strains against *Striga hermonthica* in Sorghum

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Abstract: A series of laboratory and green house experiments were conducted to investigate the efficacy of *Trichoderma harzianum* fungi, bacteria (*Flavobacterium, Bacillus megatherium var. phosphaticum* (BMP) and *Azomonas*) on *Striga hermonthica* early developmental stages, incidence and sorghum growth under laboratory and greenhouse conditions. The first laboratory experiment results showed that application of BMP + *Flavobacterium* significantly inhibited *S. hermonthica* seeds germination during and after conditioning in response to GR24 concentrations as compared to medium control. In the second laboratory experiment, application of the filtrate of *T. harzianum* alone or in combinations with bacteria significantly inhibited *S. hermonthica* germination and haustorium initiation as compared to the corresponding control. From greenhouse experiment results, *S. hermonthica* emergence significantly reduced by *T. harzianum* and insignificantly by the combination of *Flavobacterium* + BMP + *T. harzianum*. *T. harzianum* followed by the combination of *Flavobacterium* + BMP gave the highest increment in plant height. *T. harzianum* significantly increased sorghum number of leaves as compared to the infested control. The combination of *Flavobacterium* + BMP + *T. harzianum* gave the highest number of leaves. The combination *Flavobacterium* + BMP gave the highest sorghum shoot dry weight, followed by *T. harzianum*. While the highest sorghum root dry weight was obtained from the combination of *Flavobacterium* + BMP + *T. harzianum*. Generally, the combination of *T. harzianum* + *Flavobacterium* + BMP reduced *S. hermonthica* infestation and enhanced sorghum growth in comparison to the infested control.

Keywords: *Bacillus megatherium*; Flavobacterium; *Striga hermonthica*; *Trichoderma harzianum*.

Introduction

Striga spp. are obligate root parasitic weeds that severely reduce sorghum production in Africa (Babiker *et al*., 2007). Although several *S. hermonthica* management strategies have been used, but a very little success has been achieved (Othira *et al*., 2012). This weed is more damageing and debilitating under drought and low soil fertility conditions (Oswald, 2005). *Striga* produces large amounts of seeds that remain viable for more than 20 years in the soil and only germinate in the existence of a suitable host (Mbuvi *et al*., 2017). The continued lack of fertilizer application and the heavy infestation of sorghum and maize fields with *S. hermonthica* have elicited fruitless weed control efforts in the past (Omondi *et al*., 2014).

Most microbes used in biological control of crop pests, weeds and diseases secrete numerous metabolites that act on the pathogen by

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either depriving the pathogens of nutrients and space (exclusive competition), lysing cell and/or blocking specific functions related to pathogen growth (antibiosis) or inducing host plant resistance (Zhao *et al*., 2013). A considerable number of plant pathogens have been studied for their possible use as bio-herbicides for the control of weeds Because of the diversity and complexity of reactions and numerous metabolic pathways, microorganisms form an amazing resource for the biological management of crop weeds (Pilgeram *et al*., 2010). The objective of any biological weed control strategy is not to achieve absolute eradication of the weed but rather reduce establishment of the weed population to a level below the economic threshold (Teka, 2014). Despite the existence of various *S. hermonthica* control strategies, increased yield loss due to this type of parasitism remains a daunting task in cereals production (Rodenburg *et al*., 2011; Jamil *et al*., 2012). Vinale *et al.,* (2008) reported that an effective bio-inoculant should penetrate the roots not only to directly antagonize root pathogens, but also to stimulate plant growth and vigor through various mechanisms such as nutrient mobilization, nitrogen use efficiency in crops, induction of host defense as well as the involvement of growth phytohormones from both plant and fungal origins.

The present study was therefore set to investigate the efficacy of *Trichoderma harzianum* fungi, bacterial strains, each alone and in combinations on *S. hermonthica* germination, and incidence and sorghum growth under laboratory and greenhouse conditions.

Materials and Methods

A series of laboratory and green house experiments were conducted at the Bio-pesticides and Bio-fertilizers Department, Environment, Natural Resources and Desertification Research Institute (ENDRI), National Centre for Research (NCR), and College of Agriculture Studies, Sudan University of Sciences and Technology, Sudan.

Laboratory experiments

A series of laboratory experiments were conducted to study the effects of *Trichoderma harzianum* fungi, bacterial strains, *Flavobacterium, Bacillus megatherium var. phosphaticum* (BMP) and *Azomonas,* alone or in combination on *Striga* early developmental stage.

Preparation of *Trichoderma harzianum* culture

T. harzianum fungus was obtained from the microbial collection of the Faculty of Agriculture, Omdurman Islamic University. The fungus was cultured in the dark at 25 ^{o}C for 10 days on potato dextrose agar (PDA) supplemented with 250 mg chloramphenicol in 1L sterile distilled water (Yahia *et al*., 2020).

Three agar plugs (7mm diameter) obtained from the edges of actively growing *T. harzianum* (Hassan *et al*., 2019) were transferred to 500 ml conical flasks containing 250 ml potato dextrose broth (PDB). The fungal broth culture was incubated at 25 ^{o}C for 10 days. Subsequently, the fungal spores were determined with a hemocytometer.

Preparation of the bacterial inoclum

The bacterial *Flavobacterium, Bacillus megatherium var. phosphaticum* (BMP) and *Azomonas* (selected on basis of their ability to suppress *S. hermonthica* germination) were obtained from ENDRI (Yahia *et al*., 2020). The bacterial cells were inoculated in Meat Peptone Broth Medium (MPB) (sterilized in autoclave for 20 min at 15 bars), then incubated overnight at 28**°C**. Counts of the developing colonies were expressed as Colonies Forming Units (CFU) per ml. The sixth dilution (10^{-6}) from each bacterial stock solution culture was used (Yahia *et al*., 2020).

Effects of *T. harzianum* and bacterial filtrate on *S. hermonthica* germination

Striga seeds were collected from parasitized sorghum plants in Gadarif state, Sudan in 2010. The seeds were surface disinfected as described by Hassan *et al.,* (2015). For conditioning, seeds were sprinkled on moist glass fiber filter paper discs (8 mm diameter), placed in Petri-dishes, lined with GFFP filter paper and moistened with 5 ml distilled water, PD broth medium or respective microbial filtrate as described by Hassan *et al.,* (2019). The petri-dishes were sealed with Parafilm, wrapped in aluminum foil and incubated at 30 °C ± 2, in the dark, for 10 days. The seeds were subsequently treated with 20 µl (aliquots) of GR24 at 0.001, 0.01 and 0.1 ppm per disc (as described by Hassan *et al*., 2019). The synthetic germination stimulant GR24 was kindly provided by Professor Zwanenburg (Department of Organic Chemistry, Radboud University, Nijmegen, The Netherlands). The seeds were re-incubated in the dark at 30 °C, subsequently examined for germination 24h later using a stereomicroscope. Seeds treated with water or un-inoculated broth medium were included as control.

Effects of *T. harzianum* and bacterial filtrate on *S. hermonthica* haustorium initiation

Striga seeds were conditioned (in *Trichoderma* or bacteria) and induced to germinate (germilings) with GR24 as previously described above. *Striga* germilings were blotted dry on filter paper and placed top down on GFFPD without seeds. Each pair of discs was treated with 40 µl of 2, 6-dimethoxybenzoquinone (DMBQ) (10 and 20 µM). The DMBQ was kindly provided by Professor. Y. Sugimoto, (Kobe University, Japan). The Petri dishes were sealed with Parafilm, wrapped in aluminum foil and incubated at 30 ± 2 °C in the dark for 48 h then examined under a stereomicroscope. A seed was considered to have a haustorium when the radicle tips were swollen and formed hairs. *Striga* germilings resulting from seeds conditioned in nutrient broth medium or in distilled water similarly treated with DMBQ were included as controls for comparison.

Green house experiment

A green-house experiment was conducted at the College of Agricultural Studies, Sudan University of Science and Technology, to study the effects of *Trichoderma harzianum* and *Flavobacterium* and BMP bacteria on *Striga* incidence and sorghum (cv. Wad Ahmed) growth. Treatments were arranged in a Randomized Complete Block Design (RCBD) with four replicates. Sorghum was sown in a soil mix of river silt and sand (2:1 v\v), placed in pots (19 cm. diameter). *Striga hermonthica* (10 mg) were mixed with soil in each pot. Surface sterilized sorghum seeds (4) were sown in each pot. Bacteria and *Trichoderma* fungi were applied at sowing as described by Yahia et al., (2018). Sorghum seeds inoculated with *bacteria* and *Trichoderma* each alone and in combination, were planted. Seeds were inoculated with (15ml each) each of the respective bacterial suspensions (10^{-4} dilution) were injected, within the root zone, at each pot as described by Elabaied *et al.,* (2017). Five grams of *T. harzianum* inoculum carried on wheat rice grains were added to the hole when applicable as described by Hasaan *et al.,* (2019). Sorghum seedlings were thinned to two plants/pot 15 days after sowing (DAS).

Striga infested and un-infested treatments were included as controls for comparison. Emerged *Striga* plants and sorghum height were measured 30, 60 and 90 days after sowing (DAS). Number of leaves and Leaf area were determined at 30 and 90 DAS. At harvest, sorghum shoot and root dry weight were measured while only *Striga* shoot dry weight was recorded. Data collected from laboratory and greenhouse experiments were subjected to

statistical analysis using SPSS statistical package and means were separated for signify-csance using the LSD at 5%. Data on percentage germination and haustorial initiation were calculated and transformed to arcsine while *Striga* emergence was transformed to square root and subjected to analysis of variance (ANOVA) (Gomez and Gomez. 1984).

Results and Discussion

Effects of bacterial strains on *Striga hermonthica* germination

Results in table (1) show the effect of bacterial strains BMP and *Flavobacterium* each alone or in combination in response to GR24 (0.1, 0.01 and 0.001ppm) on *S. hermonthica* seeds germination (during and after conditioning) as compared to water and medium controls. Application of the combination of both bacteria significantly ($p \leq 0.05$) inhibited *S. hermonthica* seeds germination during and after conditioning in response to GR24 concentrations as compared to medium control. *Flavobacterium* strain significantly ($p \leq 0.05$) reduced *S. hermonthica* seeds germination during and after conditioning in response to GR24 (0.001ppm) as compared to medium control.

Effects of *T. harzianum* and bacterial filtrate on *S. hermonthica* seeds germination and haustorium initiation

Application of the filtrate of *T. harzianum* alone or in combination with bacteria significantly ($p \leq 0.05$) inhibited *S. hermonthica* seed germination in response to GR24 as compared to the corresponding control (table 2). *T. harzianum* alone and in combination with BMP in response to GR24 (0.01ppm) reduced germination by 79 and 76%, respectively. *T. harzianum* alone or in combination with bacteria significantly ($p \leq 0.05$) inhibited haustorium initiation in response to DMBQ as compared to corresponding control. The combination of *T. harzianum* + *Flavobacterium* + *Azomonas* in response to DMBQ (10 and 20µM) reduced haustorium by 90 and 89%, respectively.

Table 1. Effects of bacteria on *Striga hermonthica* seed germination

Bacteria	GR24	Germination%	
		After conditioning	During conditioning
Water	0.1	60.49* (46.48)**	76.70 (93.04)
	0.01	57.34 (44.34)	71.20 (89.53)
	0.001	56.52 (43.75)	69.76 (87.84)
Meat Extract Medium	0.1	61.22 (46.93)	71.93 (90.31)
	0.01	52.30 (40.48)	69.93 (88.11)
	0.001	58.95 (45.46)	70.88 (88.64)
Flavobacterium	0.1	55.35 (42.91)	68.68 (86.65)
	0.01	49.88 (38.47)	60.01 (74.39)
	0.001	42.00 (31.48)	53.24 (64.07)
BMP	0.1	48.78 (37.55)	57.56 (70.82)
	0.01	55.96 (43.34)	65.89 (82.78)
	0.001	51.72 (40.00)	64.53 (81.41)
***Flavobacterium* +BMP**	0.1	48.36 (37.19)	50.31 (59.15)
	0.01	42.96 (32.36)	54.31 (65.90)
	0.001	47.03 (36.00)	45.27 (50.43)
LSD	**Bacteria**	**4.67**	**4.25**
	GR24	**3.62**	**3.30**
	Interaction	**7.63**	**6.33**

*Data out of brackets are arcsine transformed for analysis.

**Data between brackets are original data.

Table 2. Effects of *T. harzianum* and bacterial filtrate on *S. hermonthica* seeds germination and haustorium initiation

Fungi	Bacteria	GR24(ppm)	Germination (%)	DMBQ(µM)	Haustorium (%)
Distilled Water		0.1	65.49* (82.74)**	10	60.31 (74.36)
		0.01	56.08 (68.61)	20	53.25 (63.92)
Medium (Meat extract)		0.1	65.63 (82.31)	10	50.23 (59.03)
		0.01	58.91 (72.88)	20	61.17 (75.40)
Medium (PDA)		0.1	60.88 (75.52)	10	58.61 (72.09)
		0.01	65.94 (83.09)	20	61.08 (76.21)
Without fungi	**BMP**	0.1	27.61 (22.13)	10	16.19 (10.18)
		0.01	49.79 (57.47)	20	43.56 (47.33)
	Flavobacterium	0.1	55.40 (67.61)	10	52.28 (62.49)
		0.01	48.70 (56.41)	20	50.87 (59.44)
	Azomonas	0.1	49.69 (58.04)	10	45.96 (51.67)
		0.01	43.94 (48.22)	20	45.21 (50.44)
	BMP+Flavo	0.1	35.81 (34.50)	10	41.00 (43.37)
		0.01	50.39 (59.18)	20	49.71 (58.10)
	BMP+Azo	0.1	45.79 (51.34)	10	30.57 (26.81)
		0.01	38.62 (39.00)	20	28.69 (23.65)
	BMP+Flavo+Azo	0.1	46.14 (51.97)	10	28.47 (22.89)
		0.01	37.41 (37.62)	20	36.93 (36.60)
T. harzianum	**Without bacteria**	0.1	53.56 (63.42)	10	26.34 (20.50)
		0.01	24.13 (17.04)	20	33.17 (30.15)
	BMP	0.1	30.42 (26.38)	10	37.38 (37.04)
		0.01	26.20 (20.03)	20	34.05 (31.41)
	Flavobacterium	0.1	29.54 (24.44)	10	32.80 (29.54)
		0.01	30.07 (25.36)	20	30.26 (26.08)
	Azomonas	0.1	28.30 (22.76)	10	35.37 (33.70)
		0.01	26.87 (21.07)	20	34.69 (32.49)
	BMP+Flavo	0.1	28.60 (22.94)	10	31.68 (27.92)
		0.01	28.49 (23.14)	20	29.72 (25.17)
	BMP+Azo	0.1	32.27 (28.63)	10	27.19 (21.46)
		0.01	29.76 (25.05)	20	30.44 (25.96)
	BMP+Flavo+Azo	0.1	36.15 (35.02)	10	14.87 (06.88)
		0.01	30.92(26.81)	20	16.31 (08.05)
LSD	**Fungi**		**2.34**		**2.43**
	Bacteria		**3.51**		**3.64**
	GR24/DMBQ		**1.65**		**1.72**
	Interaction		**11.65**		**12.55**

*Data out of brackets are arcsine transformed for analysis.
**Data between brackets are original data.

Hassan *et al.,* (2019) reported that application of *T. harzianum* aqueous and ethyl acetate extracts, irrespective to their concentrations, significantly (p≤0.05) reduced *S. hermonthica* germination as compared to the corresponding control, and they added that *T. harzianum* culture filtrate gave the highest reduction as compared to other treatments and the control. Vinale *et al.,* (2008) indicated that secondary metabolites of *Trichoderma* spp. may have a role in both plant growth regulation and activation of plant defense responses. The combination of compost 100% + *T. harzianum* + BMP + *Flavobacterium* reduced germination by 68% (Hassan *et al.*, 2019). Moreover, Boari *et al.,* (2016) revealed that when fungal strains (i.e. *Fusarium oxysporum*, *F. solani*, *Botrytis cinerea*, *Trichoderma harzianum*) were grown in liquid

culture, the germination stimulants strigol, 5-deoxystrigol, 4-deoxyorobanchol, and the synthetic analogue GR24 were significantly degraded. Sugimoto *et al.,* (2002) reported that metabolites of the fungus *Fusarium solani* (Sud 96) inhibited *Striga hermonthica* germination induced by the germination stimulant GR24. Also, they added that 8-acetylneosolaniol completely inhibited *Striga* germination at 24 µM.

Green house experiment

Effects of *T. harzianum* and bacteria on *S. hermonthica* incidence

Results displayed that all treatments reduced the emergence of *S. hermonthica* insignificantly at the early sampling intervals (Table 3). Application of *T. harzianum* significantly ($p \leq 0.05$) reduced *S. hermonthica* incidence at 90 days after sowing (DAS) and insignificantly at 60 DAS as compared to the control. The combination of *Flavobacterium* + BMP + *T. harzianum* inhibited *Striga* emergence albeit not significantly at 60 and 90 DAS as compared to the control.

These findings might be due to changes in the composition of phenolic compounds in the plant root exudates (Ali *et al*., 2013). Elzein *et al.,* (2006) showed that coating sorghum seeds with *Fusarium oxysporum* was an effective way to control *Striga*. Induced resistance of specific strains of fungi in the genus *Trichoderma* colonize and penetrate plant root tissues and initiate a series of morphological and biochemical changes in the plant, considered to be part of the plant defense response, which subsequently leads to induced systemic resistance (Kapulnik and Chet, 2000). Avedi *et al.,* (2014) reported that application of *Fusarium oxysporium* f. sp. *Strigae* (Foxy2) reduce *S. hermonthica* emergence. Bhale (2016) reported that the mechanisms of *Trichoderma* include direct competition with the target organism, antibiosis and parasitism of the target organism and induce resistance of the host plant.

Table 3. Effects of *T. harzianum* and bacteria on *S. hermonthica* incidence

Treatments	*S. hermonthica* count			
	30 DAS	45 DAS	60 DAS	90 DAS
Control	0.84* (0.25)**	1.22 (1.00)	2.12 (4.25)	2.53 (6.50)
***Flavobacterium* + BMP**	0.71 (0.00)	0.84 (0.25)	1.92 (3.25)	2.71 (7.25)
T. harzianum	0.71 (0.00)	0.84 (0.25)	1.17 (1.50)	1.22 (1.25)
Flavobacterium*+BMP+*T. harzianum	0.71 (0.00)	1.43 (2.50)	1.25 (1.50)	1.53 (2.50)
LSD	**0.20**	**0.91**	**1.06**	**1.25**

*Data out of brackets are square root transformed for analysis

**Data between brackets are original data.

Effects of *T. harzianum* and bacterial strains on sorghum plant height

Application of bacteria and fungi each alone and their combination increased sorghum plant height insignificantly at all sampling times as compared to infested and not infested controls (Table 4). From overall mean, *T. harzianum* followed by the bacterial combination *Flavobacterium* + BMP gave the highest increment in plant height.

The positive effects of the co-inoculation with the *Trichoderma* fungi and the bacteria on growth of sorghum may be attributed to interactive effects between the bacterium and the fungus. Hassan *et al.,* (2019) reported that the combination of compost plus BMP+ *Flavobacterium* gave lowest number of *S. hermonthica* emergence and the highest sorghum plant height. *Trichoderma*-based biocontrol agents possess better ability to promote plant growth and soil remediation

activity. *Trichoderma* can promote the plant growth by increasing phosphate solubility and availability of micronutrient in the soil (Alori *et al*., 2017).

Table 4. Effects of *T. harzianum* and bacteria on sorghum height

Treatments	Plant height (cm)			
	30 DAS	45 DAS	60 DAS	90 DAS
Control (without *Striga*)	7.2	11.4	14.9	14.6
Control (with *Striga*)	7.2	11.9	12.2	14.8
***Flavobacterium* + BMP**	8.4	13.4	16.9	15.8
T. harzianum	7.7	14.6	16.4	17.9
Flavobacterium* + BMP + *T. harzianum	5.9	12.2	14.7	13.9
LSD	**2.21**	**3.74**	**6.82**	**3.48**

Effects of *T. harzianum* and bacterial strains on number of sorghum leaves and leaf area

All treatments increased number of sorghum leaves as compared to corresponding control (Table 5). At 30 DAS, *T. harzianum* significantly ($p \leq 0.05$) increased number of sorghum leaves as compared to infested control. The increment on number of leaves occurred by other treatments were not significant. At 90 DAS, the combination of fungi and bacteria gave the highest insignificant number of leaves.

All treatments increased sorghum leaf area at both times of sampling. At 30 DAS, the combination of *Flavobacterium* + BMP + *T. harzianum* significantly ($p \leq 0.05$) increased sorghum leaf area as compared to the infested control. At 90 DAS, the highest leaf area was obtained by the combination fungi and bacteria followed by bacteria. Alori *et al.*, (2017) reported that application of *T. harzianum* to plants resulted in augments of leaf area.

Table 5. Effects of *T. harzianum* and bacteria on number of sorghum leaves and leaf area

Treatments	Number of Leaves		Leaf area (cm^2)	
	30 DAS	90 DAS	30 DAS	90 DAS
Control (without *Striga*)	7.25	9.63	33.71	64.66
Control (with *Striga*)	5.63	8.00	26.75	59.26
***Flavobacterium* + BMP**	6.63	8.75	27.56	70.13
T. harzianum	7.25	8.63	32.78	61.30
Flavobacterium* + BMP + *T. harzianum	6.75	9.38	37.28	72.13
LSD	**1.46**	**2.42**	**9.75**	**19.67**

Effects of *T. harzianum* and bacterial strains on sorghum dry weight

All treatments increased sorghum shoot and root dry weight (Table 6). The bacterial combination *Flavobacterium* + BMP gave the highest sorghum shoot dry weight, followed by *T. harzianum*. While the highest sorghum root dry weight was obtained from the combination of *Flavobacterium* + BMP + *T. harzianum*, followed by *T. harzianum* alone.

Hassan *et al.*, (2019) reported that the combinations of compost plus *T. harzianum* + BMP + *Flavobacterium* increased sorghum shoot and root dry weight insignificantly as compared to the control. The combination of microorganisms could also increase the dry matter yield and nutrient uptake by wheat grown in a sandy soil (Singh and Kapoor, 1999). Integrated treatment comprising of soil application of *T. harzianum*, *P. fluorescens*, Jas mycorrhiza (AMF) + seedling treatment with *T. harzianum* and *P. fluorescens* + three foliar sprays of Mancozeb were found very effective in promoting the plant growth at experimental

field as well as at farmers' fields (Kabdwal *et al*., 2019). In addition, some *Trichoderma* spp. are able to colonize root surfaces, interact with the plant, and exchange compounds that can cause substantial changes in plant metabolism (Yedidia *et al*., 2000).

Table 6. Effects of *T. harzianum* and bacterial strains on sorghum dry weight

Treatments	Dry weight (g)	
	Shoot	Root
Control (without *Striga*)	5.07	10.45
Control (with *Striga*)	4.90	9.16
***Flavobacterium* + BMP**	20.62	10.36
T. harzianum	7.87	10.99
Flavobacterium* + BMP + *T. harzianum	5.82	12.48
LSD	**20.65**	**4.36**

Conclusion

In conclusion, the combinations of *T. harzianum* fungi with *Flavobacterium* plus phosphorus solubilizing bacteria (BMP) significantly reduced *S. hermonthica* infestation and enhanced sorghum growth in comparison to the infested control.

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Cite this article as:

Mona A. Azarig, Mohammed M. Hassan, Ahmed M. E. Rugheim, Magdoline M. Ahmed, Rania A. Abakeer, Rashida M. A. Abusin, Migdam E. Abdelgani. Impact of *Trichoderma harzianum* and bacterial strains against *Striga hermonthica* in sorghum. *Annals of Plant Sciences.* 9.10 (2020) pp. 4049 - 4058.

http://dx.doi.org/10.21746/aps.2020.9.10.2

Subject Editor: Mahendra Nath Mitta, Sri Venkateswara University, Tirupati

Source of support: Nil; **Conflict of interest:** Nil.