International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2020): 7.803

Biocontrol using *Trichoderma harzianum*; A Critical Component of Integrated *Striga* Management

Mona A. Azarig[1], Mohammed M. Hassan[2], Ahmed M. E. Rugheim[3], Magdoline M. Ahmed[4], Rania A Abakeer[5], Rashida M. A. Abusin[4], Migdam E Abdelgani[7]

[1] Ph. D. student, Sudan Academy of Sciences (SAS), Sudan

[2, 4, 5, 7]Environment, Natural Resources and Desertification Research Institute, National Center for Research, Sudan

[3]Landscaping and Arid Land Agriculture, Faculty of Agriculture, Omdurman Islamic University, Sudan

[4] Pests and Plant Health, College of Agriculture, Bahri University, Khartoum, Sudan

Corresponding Author: *rashidaabusin333[at]gmail.com*

Abstract: ***Series of laboratory, greenhouse and field experiments were conducted to study the effects of Trichoderma harzianum, bacterial strains, herbicide chlorsulfuron and nitrogen fertilizer (urea) on germination and parasitism of Striga hermonthica in sorghum. Results of laboratory experiments revealed that all concentrations of T. harzianum ethyl acetate extract significantly inhibited S. hermonthica germination using double discs technique and on soil as compared to water and ethyl acetate controls. The green house results showed that application of nitrogen followed by T. harzianum each alone or in combination reduced S. hermonthica incidence as compared to the control. Nitrogen significantly increased sorghum plant height as compared to the infested control. The combination of Nitrogen + T. harzianum significantly increased sorghum leaf area as compared to infested control. Field experiment results showed that the combination of Trichoderma fungi, bacterial strains, chlorsulfuron reduced Striga emergence as compared to the control. Among all treatments, the combinations of 2N + chlorsulfuron, 1N + chlorsulfuron, 2N + BMP + Azospirillumbrasiliense significantly decreased number of S. hermonthica as compared to control. The combination of 2N + chlorsulfuron, 1N + chlorsulfuron, 2N + BMP + Azospirillumbrasiliense significantly increase sorghum growth attributes.***

Keywords: bacteria, chlorsulfuron, nitrogen, *Striga*, *Trichoderma*

1. Introduction

Striga spp. is a root parasitic flowering plant in sub Saharan Africa causing severe constraints to crop production. It survives by diverting essential nutrients from the host crop such as *Sorghumbicolor (*L.), *Pennisetumglaucum* (L.), *Eleusinecoracana* (L. Gaertn), *Zea mays* (L.), *Oryzaglaberrima (*Steudel) and *O. sativa* (L.) (Atera *et al.*, 2011). *Striga* infection lowers the photosynthetic rate of the cereal host. This could be due to transfer of nitrogen to the parasite or to changes in host photosynthetic metabolism. *Striga* therefore not only parasitizes its cereal host, but also poisons it (Watling and Press, 2001). An integrated management approach using a combination of control measures has the potential to provide a solution of Striga problem (Hassan et al., 2009). Microorganisms can affect plant growth and development; change nutrients dynamics, susceptibility to disease, as well as it produce diverse active metabolites including herbicidal metabolites (Saxena and Pandey, 2001). Hanson (2003) reported that natural herbicides are eco-friendly, biodegradable, and less toxic to plants and beneficial microorganisms. They are biosynthesized through specialized pathways and exhibit a wide range of biocontrol activities. The use of soil-born microorganisms as biocontrol agents against *Striga* such as fungi (Yonli et al., 2006) and bacteria (Ahonsi et al., 2002) has been also suggested for an IPM strategy. *Trichoderma* may act as symbionts of plants and have been studied as bio-pesticides and bio-fertilizers due to their abilities to protect crops from weeds and promote vegetative growth (Harman, 2004). *Trichoderma* fungi could differently be used for bio-controlling *Striga* acting as a 'physiological' barrier, by preventing the germination of their seeds through the ability to bio-transform the stimulatory signals (Boari et al., 2016). Previous studies showed that *Striga* infestation is correlated with low soil fertility and elevating soil fertility would lead to reduce the infestation (Ransom, 1999). Yoneyama *et al.* (2007) reported that host plants grown under nutrient deficient conditions are more active in producing and exuding strigolactones and inducing germination of root parasitic plant seeds. Therefore, the use of fertilizers will improve soil fertility and plant fitness and crop yield besides reducing strigolactone production by the host plant and reduce the infection by parasitic weeds. The objective of this study was to investigate the effects of *Trichoderma harzianum*, bacteria, the herbicide chlorsulfuron and chemical fertilizer (urea) on germination and parasitism of *Striga hermonthica* in sorghum.

2. Materials and Methods

Laboratory experiments

Series of laboratory experiments were conducted at the Bio-pesticides and Bio-fertilizers Department, Environment, Natural Resources and Desertification

Research Institute (ENDRI), National Centre for Research (NCR), Khartoum, Sudan.

Effect of T. harzianum ethyl acetate extracts (TEAE) on germination of S. hermonthica

T. harzianum was obtained from the microbial collection of the Faculty of Agriculture, Omdurman Islamic University. Seven mm mycelial discs obtained from 7 days old cultures of *Trichoderma* were inoculated into 500 ml flasks containing 250 ml potato dextrose broth (PDB) then incubated for 15 days at 25^{o}C. Subsequently, *T. harzianum* spores and mycelia were removed from broth culture through filtration (Yahia *et al.,* 2018). The fungal metabolites were extracted from culture filtrates by ethyl acetate solvent. The extract was dried at 40^{o}C using a rotary evaporator (Vinale *et al.,* 2006) for further examination.

S. hermonthica seeds were collected in 2015 from infested sorghum plants at the Gezira Research Station, Sudan. *S. hermonthica* seeds were conditioned as described by ElKhair *et al.* (2017). Briefly, the sterilized discs, placed in 9 cm petri dishes lined with glass fiber filter papers (GF/C), were moistened with 5 ml distilled water. 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 para film, placed in black polythene bags were incubated at 30 ºC in the dark for 10 days.

First laboratory experiment

The dried extract was dissolved in a stone solvent. Then serial dilutions were prepared (5, 10, 15 and 25µM). Subsequently, the empty disc (without seeds) placed in petri dishes previously treated with TEAE, left to dry in laminar flow cabinet for 2h. then the empty disc was put on top of conditioned seeds, using double discs technique, and treated with 40ml of GR24 (0, 1 ppm). The seeds were re-incubated in the dark at 30 ºC, then examined for germination 24 h later using a stereomicroscope. Ethyl acetate and water were used as a control for comparisons.

Second laboratory experiment

The dried extract was dissolved in a stone solvent. Aliquot (1ml) of *T. harzianum*spores was homogeneously mixed with 10 g soil placed in 9cm petri dishes. Then serial dilutions were prepared (10, 100 and 1000µM). Subsequently, the soil placed in petri dishes were left to dry in laminar flow cabinet for 24h. Water and ethyl acetate were used as a control for comparisons. Discs contain conditioned *S. hermonthica* seeds were placed on the surface of the treated soil with (TEAE**)** for 24h, each disc was treated with 20µl of GR24 at 1, 0.1, 0.01 and 0.001ppm. The seeds were re-incubated in the dark at 30 ºC, then examined for germination 24 h later using a stereomicroscope as described by Gafar *et al.* (2015). Treatments were arranged in a Randomized Complete Design (RCD) with four replicates.

Greenhouse experiment

This experiment was conducted to study the effects of *T. harzianum* and nitrogen each alone or in combination on *S. hermonthica* incidence and sorghum growth. Treatments were laid out in a Randomized Complete Block Design (RCBD) with four replicates. Plastic pots (19cm diameter), with drainage holes at the bottom, were filled with soil mixture (7Kg/pot) of river silt and sand (1: 1v/v). *S. hermonthica* infestation was accomplished by mixing 10 mg of disinfected *S. hermonthica* seeds in the top soil surface in each pot. Surface disinfected sorghum seeds {7/bag (19cm diameter)} were planted and immediately watered. *S. hermonthica* infested and un-infested controls were included for comparison. Sorghum seedlings were thinned to 2 plants per pot after 10 days of sowing.

Five gram of *T. harzianum* inoculum carried on rice was added in each pot at sowing where applicable. Nitrogen as urea at 47.6 kg ha^{-1} was applied immediately after sowing.

Treatments effects were assessed by counting emerged *S. hermonthica* plants at 45, 60, 75 and 90 DAS. Data collected for sorghum growth attributes included sorghum height; number of leaves and chlorophyll content were measured at 45, 60, 75 and 90DAS. At harvest, sorghum shoot and root dry matters were recorded.

Field experiment

Field experiment was conducted during two seasons June – October 2013 and June – October 2014 at the demonstration farm of the College of Agricultural Studies, Sudan University of Science and Technology, Shambat, Khartoum, Sudan.

This experiment was conducted to study the effects of *T. harzianum*, bacterial strains, nitrogenchemical fertilizer (43.8 kg N ha^{-1} and 87.6 kg N ha^{-1}) and the herbicide chlorsulfuron (3.57g a. i. ha^{-1}) each alone or in combination on *S. hermonthica* incidence and sorghum growth.

The field was disc ploughed, harrowed, leveled, ridged and divided into sub-plots (3 x 3m). *S. hermonthica (*10 mg) were mixed with soil in each hole. The bacterial strains (*Bacillusmegatherium* var. *phosphaticum* (BMP), *Azospirillumbrasiliense* (Ab) and *Flavobacterium* spp. (F)) were obtained from ENDRI.

Sorghum (cv. Wad-Ahmed) seeds were coated with the combinations of BMP + *Flavobacterium* or BMP + *A. brasiliense* carried on charcoal powder when applicable. Five gram of *T. harzianum* inoculum carried on rice was added in each hole at sowing where applicable. The nitrogen (urea) at rates of 43.8 kg N ha^{-1} and 87.6 kg N ha^{-1}were applied at sowing where applicable. The chlorsulfuron at rate of 1.25g/ fed^{-1} was applied at sowing where applicable.

Weeds other than *S. hermonthica* were removed. Treatments were laid out in a Randomized Complete Block Design (RCBD) with four replicates. Data collected for sorghum growth attributes included plant height, chlorophyll contents; sorghum column diameter/cm, dry matter/g and 100 seeds weight were measured as described by (Yahia et al., 2020 and Rezig et al., 2016).

Data for *S. hermonthica* included number of *Striga* emergence, *Striga* height and dry weight.

Statistical analysis

Data collected from laboratory, greenhouse and field experiments were subjected to statistical analysis using SPSS 22 statistical package and means were separated for significance using the LSD at 5%. Data on percentage germination was calculated and transformed to arcsine while Striga emergence were transformed to square root (Gomez and Gomez.1984) and subjected to analysis of variance (ANOVA).

3. Results and Discussion

Effects of T. harzianumethyl acetate extract (TEAE) on S. hermonthica seeds germination

Results in table (1) show the effects of different concentrations of *T. harzianum* ethyl acetate extract (TEAE) on *S. hermonthica* germination using double discs technique. All TEAE concentrations (5, 10, 15 and 25µM) significantly ($p \leq 0.05$) inhibited germination as compared to water and ethyl acetate controls. TEAE concentrations 15 and 25µM reduced germination by 96.5 and 96.9%, respectively.

Table 1: Effects of T. harzianumethyl acetate extract (TEAE) on S. hermonthica seeds germination

Treatments	Germination (%)
Water	72.31* (90.22)**
Ethyl acetate	68.12 (86.05)
TEAE# 5µM	9.43 (5.27)
TEAE 10µM	12.52 (6.24)
TEAE 15µM	8.77 (3.13)
TEAE25µM	6.70 (2.84)
LSD	11.02

*Data out of brackets are arcsine transformed for analysis.
**Data between brackets are original data.
T. harzianum ethyl acetate extract

Effects of T. harzianumethyl acetate extract (TEAE) on S. hermonthica seeds germination on soil

All treatments significantly ($p \leq 0.05$) reduced *S. hermonthica* germination in response to GR24 concentrations as compared to the control (Table 2). The highest significant ($p \leq 0.05$) reduction in germination were obtained by TEAE 10µM in response to GR24at0.01 and 0.1ppm, followed by TEAE 1000µM in response to GR24 0.01ppm and TEAE 100µM in response to GR24 0.01 and 0.1ppm as compared to corresponding controls. TEAE, 10µM, in response to GR24 at 0.1 and 0.01ppm reduced germination by 71.4 and 66.7%, respectively.

Table 2: Effects of TEAE on S. hermonthica seeds germination on Soil

Extract	GR24	Germination%
Water	**1**	79.50* (95.55)**
	0.1	82.91 (96.82)
	0.01	53.86 (65.02)
Ethyl acetate	**1**	69.22 (87.18)
	0.1	68.36 (85.70)
	0.01	50.69 (58.75)
TEAE 1000µM	**1**	44.94 (55.92)
	0.1	45.58 (56.96)
	0.01	26.08 (30.71)
TEAE 100µM	**1**	46.81 (58.61)
	0.1	37.57 (43.97)
	0.01	27.32 (33.18)
TEAE 10µM	**1**	39.07 (46.47)
	0.1	24.45 (27.68)
	0.01	20.58 (21.67)
LSD Extract		**6.43**
LSD GR24		**4.98**
LSD Interaction		**11.14**

*Data out of brackets are arcsine transformed for analysis.
**Data between brackets are original data.
T. harzianum ethyl acetate extract

Previous reports indicated that germination of *S. hermonthica* and *S. asiatica* needs both ethylene biosynthesis and action and that GR24 reduces abscisic acid (ABA) and increased gibberellins (GAs) and cytokinins (CKs) levels in germinating *S. hermonthica* seeds (Parker and Riches, 1993). The observed inhibition of germination by *T. harzianum*, consistent with previous reports on inhibition of *S. hermonthica* germination by *Fusariumsolani* (Sud), could be attributed to direct effects on embryo growth resulting from seed invasion by the fungus and/or to fungal toxins (Sugimoto et al., 2002). Similarly Yahia *et al.* (2018) and Dor *et al.* (2011) reported a reduction in germination and loss of seed viability in several *Orobanche* and *Striga* species due to toxins produced by *Trichoderma* and *Fusariumoxysporuf. sp. orthocrars*.

Pot experiment

Effects of nitrogen and T. harzianum on S. hermonthica incidence

Results displayed in table (3) show the effects of nitrogen and *T. harzianum* and their combination on *S. hermonthica* emergence. At 30 days after sowing (DAS), all treatments delayed *S. Hermonthica* emergence. At 45, 60 and 75 DAS, application of nitrogen followed by *T. harzianum* each alone significantly ($p \leq 0.05$) reduced number of *S. hermonthica* incidence as compared to the control. Over all means, results showed that all treatments reduced *S. hermonthica* emergence, irrespective to interval time.

Seed germination is a key phase of the parasitic plant life cycle that is stimulated by the secondary metabolites, mainly strigolactones (SLs), secreted by the host roots. Jamil *et al.* (2010) explained that host plants reduce the secretion of underground signaling molecules in response to the quick supply of mineral nutrients, and consequently resulting in lower *Striga* seed germination and infection. Previous studies have correlated SLs production with levels of *Striga* parasitism especially in susceptible lines, and have also shown that SLs production is enhanced under mineral deficiency conditions while in presence of P micro-dosing significantly down regulated SLs production. Induced resistance of specific strains of *Trichoderma* fungi 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). Suitable N and P fertilizer use in combine with potential microorganisms might be a promising and affordable strategy to control Striga infestation in sorghum. Suppression of *Striga* emergence by nitrogen is consistent with several reports (Hassan et al., 2009; Abusin et al., 2017) and may be attributed to a decrease in stimulant production and/or to direct toxicity to the parasite at early developmental stages (Parker and Riches, 1993).

Table 3: Effects of nitrogen and T. harzianum on S. hermonthica incidence

Treatment	*S. hermonthica* count			
	30 DAS#	45 DAS	60 DAS	75 DAS
Control (with*Striga*)	0.75* (0.25) **	1.50 (1.00)	2.48 (4.25)	2.91 (6.50)
Nitrogen	0.50 (0.00)	0.50 (0.00)	0.85 (0.50)	1.18 (1.00)
T. harzianum	0.50 (0.00)	1.00 (0.50)	1.11 (1.50)	1.29 (1.25)
Nitrogen+ *T. harzianum*	0.50 (0.00)	1.81 (2.50)	2.00 (2.50)	2.12 (2.75)
LSD	**0.39**	**0.91**	**1.28**	**1.26**

#Days After Sowing
*Data out of brackets aresquareroot transformed data (√x+0.5 x: variable)
**Data between brackets are original data.

Effects of nitrogen and T. harzianum on sorghum plant height

Application of nitrogen, *T. harzianum* and combination increased sorghum plant height insignificantly at 30, 45 and 60 DAS (Table 4). At 75 DAS, nitrogen significantly ($p \leq 0.05$) increased sorghum plant height as compared to the infested control. Generally, application of nitrogen alone and in combination with *T. harzianum* gave the highest plant growth.

Hashemabadi et al., (2018) reported that the N-fertilizer and biological treatments significantly improved most growth attributes and nutrient uptake and increased the concentrations of secondary metabolites as compared to the control. Enhancement of plant growth by *Trichoderma* might be due to production of secondary metabolites which may act as auxin like compound (Vinale et al., 2006).

Table 4: Effects of nitrogen and T. harzianum on sorghum plant height

Treatment	Plant height (cm)			
	30 DAS#	45 DAS	60 DAS	75 DAS
Control (without *Striga*)	7.20	11.38	14.93	14.60
Control (with *Striga*)	7.20	11.88	12.20	14.78
Nitrogen	8.13	14.13	17.88	19.48
T. harzianum	7.70	14.55	16.43	17.88
Nitrogen +*T. harzianum*	8.78	14.25	17.20	17.13
LSD	2.61	3.54	7.05	3.43

#Days After Sowing

Effects of nitrogen and T. harzianum on number of leaves

The combination of nitrogen + *T. harzianum* increased sorghum number of leaves insignificantly at 30 and 60 DAS (Table 5). While application of nitrogen gave the highest increment in number of leaves at 60 DAS.

Effects of nitrogen and T. harzianum on leaf area

Application of nitrogen + *T. harzianum* significantly ($p \leq 0.05$) increased sorghum leaf area as compared to infested control at 30 DAS (Table 5). Generally, all treatments increased leaf area, the highest increment was obtained by the combination followed by nitrogen alone.

Table 5: Effects of nitrogen and T. harzianum on number ofleaves and leaf area

Treatment	number of leaves		Leaf area (cm^2)	
	30 DAS#	60 DAS	30 DAS#	60 DAS
Control (without *Striga*)	5.63	9.63	33.71	64.66
Control (with *Striga*)	7.25	8.00	26.75	59.26
Nitrogen	7.00	9.50	35.93	65.84
T. harzianum	7.25	8.63	32.78	61.30
Nitrogen+*T. harzianum*	8.00	9.25	41.13	67.08
LSD	**1.49**	**2.37**	**10.06**	**21.61**

#Days After Sowing

Effects of nitrogen and T. harzianum on sorghum dry weight

All treatments increased shoot and root sorghum dry weight albeit not significantly (Table 6). Application of *T. harzianum* followed by nitrogen gave the highest rootdry weight as compared to both controls. Application of nitrogen followed by *T. harzianum* gave the highest shootdry weight as compared to both controls

Haque et al. (2010) reported that certain doses of NPK and 50% *Trichoderma*/compost show better performance on the growth, dry matter accumulation and yield of mustard. Also they added that dry matter production was significantly ($P \leq 0.05$) influenced by the combination of biofertilizer and chemical fertilizer. *Trichoderma* sp. also increased nutrient uptake through enhancement of root growth (Harman et al., 2004). Integrated *Striga* control approaches are most attractive when they reduce *Striga* infestation on long term and increase crop yield in the current season of application.

Table 6: Effects of nitrogen and T. harzianum on sorghum dry weight

Treatment	Dry weight (g)	
	Root	Shoot
Control (without *Striga*)	5.07	10.45
Control (with *Striga*)	4.56	7.75
Nitrogen	6.84	12.39
T. harzianum	7.87	10.99
Nitrogen+*T. harzianum*	4.90	9.16
LSD	**5.03**	**4.73**

Field experiment

Effects of nitrogen, bacteria, T. harzianum and chlorsulfuron on S. hermonthica emergence

At 30 DAS, none of the treatments affect *S. hermonthica* emergence as compared to the control (Table 7). At 45 DAS, the combination of 87.6 kg N ha^{-1}+ chlorsulfuron significantly ($p \leq 0.05$) decreased number of *S. hermonthica* as compared to control. At 60 and 75 DAS, application of 87.6 kg N ha-1+ chlorsulfuron followed by 43.8 kg N ha^{-1} + chlorsulfuron, 87.6 kg N ha-1+ BMP + *Azospirillumbrasiliense* and 2N alone significantly ($p \leq 0.05$) decreased *S. hermonthica* emergence as

compared to the control. At 90 DAS, the lowest significant ($p \leq 0.05$) number of *S. hermonthica* was obtained by 87.6 kg N ha-1+ chlorsulfuron. Generally, the combinations of 87.6 kg N ha-1+ chlorsulfuron, 43.8 kg N ha-1+ chlorsulfuron and 87.6 kg N ha-1+ BMP + *Azospirillumbrasiliense* gave the highest inhibition in *S. hermonthica* emergence.

Inhibition of *Striga* germination and emergence by *Fusarium* species with the increase of soil fertility supports report on increased nitrogen effect on *Striga* when combined with biological control and other means (Yonli et al.2006). Nitrogen is reported to decrease germination stimulants production of Striga and exerts a direct toxicity on the parasite during early growth stages. These results are in line with that of Yahia et al. (2019) who noted that the combination of T. *harzianum*+ BMP+TAL1399 + fertilizers significantly reduced *O. crenata* emergence as compared to the control. Nevertheless, the significant reduction in *Striga* emergence as a result of combining mycoherbicides, chemical fertilizers and herbicides and host plant resistance is an important feature to prevent further *Striga* distribution and infestation in the fields (Parker and Riches, 1993).

Table 7: Effects of nitrogen, bacteria, T. harzianum and chlorsulfuron on S. hermonthica emergence

Nitrogen	Treatments	*Striga* count				
		Days after sowing				
		30	45	60	75	90
0N	Control	1.00* (0.67) **	6.80 (49.00)	10.50 (110.67)	13.02 (170.33)	6.36 (40.33)
	BMP# + F##	1.05 (0.67)	7.00 (50.67)	10.51 (110.33)	12.59 (159.33)	8.27 (68.00)
	BMP + Ab###	1.72 (3.00)	6.19 (40.33)	7.45 (56.00)	11.35 (129.67)	6.03 (36.67)
	T. harzianum	1.48 (2.00)	7.09 (50.00)	9.45 (93.00)	11.40 (130.33)	6.99 (52.00)
	Chlorsulfuron	1.10 (1.00)	4.38 (20.00)	6.42 (47.33)	11.00 (124.00)	6.81 (47.33)
43.8 kg N ha^{-1}	Control	1.05 (0.67)	6.66 (54.00)	7.46 (57.33)	8.64 (77.67)	6.49 (42.00)
	BMP + F	0.88 (0.33)	2.77 (8.33)	8.90 (84.00)	10.47 (112.00)	7.17 (51.00)
	BMP + Ab	1.38 (2.33)	3.95 (17.33)	6.67 (45.00)	8.47 (71.67)	6.74 (46.00)
	T. harzianum	0.71 (0.00)	8.05 (65.00)	8.73 (78.33)	9.94 (103.67)	6.13 (41.67)
	Chlorsulfuron	1.00 (0.67)	3.33 (15.67)	4.04 (19.33)	5.51 (33.33)	4.91 (24.00)
87.6 kg N ha^{-1}	Control	1.43 (2.00)	4.92 (30.00)	6.38 (41.33)	8.48 (71.67)	5.93 (34.67)
	BMP + F	1.05 (0.67)	6.61 (46.67)	8.86 (79.00)	10.55 (128.33)	5.72 (35.33)
	BMP + Ab	0.88 (0.33)	6.29 (53.67)	5.83 (35.33)	6.35 (41.00)	4.68 (21.67)
	T. harzianum	1.05 (0.67)	6.20 (44.67)	8.99 (92.00)	10.12 (111.33)	7.65 (62.33)
	Chlorsulfuron	0.88 (0.33)	1.47 (2.00)	1.47 (2.00)	3.07 (13.33)	3.71 (16.67)
LSD Nitrogen		**0.43**	**1.83**	**1.56**	**1.78**	**1.15**
LSD Treatments		**0.55**	**2.37**	**2.02**	**2.29**	**1.48**
LSD Interaction		**0.95**	**4.10**	**3.49**	**3.97**	**2.57**

*Data out of brackets aresquareroot transformed data ($\sqrt{x+0.5}$ x: variable)
**Data between brackets are original data.
Bacillusmegatherium var. *phosphaticum*
##*Flavobacterium* spp.
Azospirillumbrasiliense

Effects of nitrogen, bacteria, T. harzianum and chlorsulfuron on sorghum plant height

At 30 DAS, the combinations of 2N + BMP + *Azospirillumbrasiliense* and 1N + BMP + *Flavobacterium* spp. gave the highest sorghum plant height (Table 8). At 60 DAS, 2N alone or in combination with chlorsulfuron, *T. harzianum* and BMP + *Azospirillumbrasiliense* obtained the highest sorghum plant height. At 75 DAS, application of 2N +chlorsulfuron followed by 87.6 kg N ha^{-1}+ BMP + *Azospirillumbrasiliense* and 2N alone significantly ($p \leq 0.05$) increased sorghum plant height as compared to the control. Generally, the highest sorghum growth was obtained by 2N + BMP + *Azospirillumbrasiliense*, 87.6 kg N ha^{-1}+chlorsulfuron and 87.6 kg N ha^{-1}, respectively.

Many rhizosphere bacteria can produce plant growth regulators, such as auxins, cytokinins, GAs, and ABA. Phytohormone production by microbes can modulate the endogenous plant hormone levels and consequently can have an enormous influence on plant growth and development (Zahir et al., 2001). The present study suggest that nitrogen, bacteria, *T. harzianum* and the herbicide afforded the most consistent performance and resulted in the highest suppression of the *Striga*. These results are in line with that of Yahia et al. (2019) who found that *T. harzianum* alone or in combination with nitrogen plus phosphorusor BMP+TAL1399+nitrogen and phosphorus and the combination with BMP+TAL1399+nitrogen plus phosphorus significantly increased plant height as compared to the infested control.

Table 8: Effects of nitrogen, bacteria, T. harzianum and chlorsulfuron on sorghum plant height

Nitrogen	Treatments	Plant height (cm)			
		Days after sowing			
		30	45	60	75
0N	Control	23.33	43.97	65.13	60.57
	BMP# + F##	16.60	43.50	66.20	58.53
	BMP + Ab###	21.80	34.83	63.73	73.97
	T. harzianum	18.17	31.90	55.00	67.43
	Chlorsulfuron	19.80	30.43	48.97	54.87
43.8 kg N ha^{-1}	Control	17.47	33.80	66.53	76.60
	BMP + F	24.77	43.07	61.70	72.43
	BMP + Ab	20.03	29.63	63.53	68.53
	T. harzianum	16.10	26.53	61.50	57.77

	Chlorsulfuron	18.93	31.97	53.30	81.60
87.6 kg N ha^{-1}	**Control**	18.47	41.47	69.00	86.67
	BMP + F	20.80	37.40	65.83	72.63
	BMP + Ab	28.23	36.03	68.43	91.87
	T. harzianum	19.43	40.07	69.43	64.17
	Chlorsulfuron	20.33	35.93	70.87	92.30
LSD Nitrogen		**6.05**	**5.59**	**9.19**	**10.10**
LSD Treatments		**7.81**	**7.21**	**11.86**	**13.05**
LSD Interaction		**13.53**	**12.49**	**20.54**	**22.60**

 # Bacillusmegatherium var. *phosphaticum*
##Flavobacterium spp.
Azospirillumbrasiliense

Effects of nitrogen, bacteria, T. harzianum and chlorsulfuron on sorghumchlorophyll content
Application of 43.8 kg N ha^{-1}+*T. harzianum* and 43.8 kg N ha^{-1}+ chlorsulfuron significantly (p≤0.05) increased sorghum chlorophyll content at 30 DAS as compared to the control (Table 9). At 60 DAS, 87.6 kg N ha^{-1}+ chlorsulfuron and 43.8 kg N ha^{-1}+ BMP + *Azospirillumbrasiliense* significantly (p≤0.05) increased chlorophyll content as compared to the control. Generally, the highest chlorophyll content was obtained by 43.8 kg N ha^{-1}+*T. harzianum*, 43.8 kg N ha^{-1}+ chlorsulfuron, 43.8 kg N ha-1alone and 87.6 kg N ha^{-1}+ chlorsulfuron, respectively.

Effects of nitrogen, bacteria, T. harzianum and chlorsulfuron on sorghumleaf area
Application of 1N + BMP + *Flavobacterium* spp. gave the highest sorghum leaf area at 45 DAS (Table 9). At 75 DAS, the combinations of 87.6 kg N ha^{-1}+ BMP + *Azospirillumbrasiliense* and 87.6 kg N ha^{-1}+ chlorsulfuron significantly (p≤0.05) increased leaf area as compared to the control. Generally, the highest leaf area was obtained by 87.6 kg N ha^{-1}+ BMP + *Azospirillumbrasiliense* and43.8 kg N ha^{-1}+ BMP + *Flavobacterium* spp., respectively.

Table 9: Effects of nitrogen, bacteria, *T. harzianum* and *chlorsulfuron* on *sorghumchlorophyll* content and leaf area

Nitrogen	**Treatments**	**Chlorophyll (SP)**		**Leaf area (cm^2)**	
		Days after sowing			
		30	**60**	**45**	**75**
0N	**Control**	28.20	19.53	352.22	176.92
	BMP$^{\#}$ + F$^{\#\#}$	28.40	25.80	257.20	226.39
	BMP + Ab$^{\#\#\#}$	26.13	28.93	289.75	250.92
	T. harzianum	31.67	24.07	289.75	200.53
	Chlorsulfuron	28.50	30.67	337.61	231.65
43.8 kg N ha^{-1}	**Control**	35.10	30.77	289.75	207.22
	BMP + F	35.20	23.73	354.61	218.91
	BMP + Ab	29.47	31.67	296.32	229.47
	T. harzianum	37.73	30.93	265.27	177.49
	Chlorsulfuron	37.33	29.17	239.95	231.50
87.6 kg N ha^{-1}	**Control**	32.07	24.20	337.85	216.28
	BMP + F	34.77	26.90	241.31	246.88
	BMP + Ab	31.87	28.97	338.70	261.88
	T. harzianum	32.00	26.87	341.21	195.70
	Chlorsulfuron	31.63	33.90	313.71	251.76
LSD Nitrogen		**3.85**	**5.16**	**54.57**	**31.52**
LSD Treatments		**4.97**	**6.66**	**70.45**	**40.69**
LSD Interaction		**8.61**	**11.54**	**122.02**	**70.48**

 # Bacillusmegatherium var. *phosphaticum* *##Flavobacterium* spp. *### Azospirillumbrasiliense*

Effects of nitrogen, bacteria, T. harzianum and chlorsulfuron on sorghum column diameter
Application of 2N + BMP + *Azospirillumbrasiliense* significantly (p≤0.05) increased column diameter as compared to the control (Table 10). While the combinations of 2N + chlorsulfuron and1N + BMP + *Azospirillumbrasiliense* increased column diameter albeit not significantly.

Effects of nitrogen, bacteria, T. harzianum and chlorsulfuron on sorghumshoot dry weight
Application of 2N + chlorsulfuron, 2N and 1N gave the highest insignificant sorghum shoot dry weight (Table 10). Wu et al. (2005) performed a thorough greenhouse study to evaluate the effect of a mixture of four biofertilizers, namely an AMF (*Glomusmossae* or *Glomusintraradices*), an N-fixer (*Azobacterchroococcum*), a P-solubilizer (*B. megaterium*), and a K-solubilizer (Bacillus mucilaginous) on growth of *Zea mays* and soil properties. The mixture of the four microbes significantly increased the growth of *Z. mays* and resulted in the highest biomass and seedling height. The presence of the bacteria in the inoculum resulted in an at least five fold higher root infection rate by AMF.

Table 10: Effects of nitrogen, bacteria, T. harzianum and chlorsulfuron on sorghum column diameter and dry weight

Nitrogen	Treatments	Column diameter (cm)	Shoot dry weight (g)
0N	**Control**	6.34	264.2
	BMP# + F##	4.37	261.4
	BMP + Ab###	6.04	245.3
	T. harzianum	6.29	299.8
	Chlorsulfuron	4.97	205.8
43.8 kg N ha^{-1}	**Control**	6.46	347.8
	BMP + F	5.81	278.2
	BMP + Ab	7.09	248.0
	T. harzianum	6.65	174.9
	Chlorsulfuron	6.47	299.5
87.6 kg N ha^{-1}	**Control**	6.25	385.8
	BMP + F	6.22	290.3
	BMP + Ab	8.69	286.2
	T. harzianum	6.51	286.2
	Chlorsulfuron	7.70	404.0
LSD Nitrogen		**0.85**	**72.3**
LSD Treatments		**1.10**	**93.4**
LSD Interaction		**1.91**	**161.7**

Bacillusmegatherium var. *phosphaticum*
##*Flavobacterium* spp.
Azospirillumbrasiliense

4. Conclusions

- Biological control of *Striga hermonthica* appears to be an effective and ecofriendly approach being practiced world over.
- Further, biological control strategy is highly compatible with sustainable agriculture and has a major role to play as a component of integrated pest management.
- The use of suitable levels of organic N-fertilizers, *Trichoderma* fungi and bacteria can decrease *Striga* infestation in addition to improve plant growth.
- *Trichoderma* fungi, nitrogen alone and their combination suppresses *Striga* emergence and alleviates its damage to sorghum.
- The combination of 87.6 kg N ha^{-1}+ chlorsulfuron, 43.8 kg N ha^{-1}+ chlorsulfuron, 87.6 kg N ha^{-1}+ BMP + *Azospirillumbrasiliense* significantly decrease *Striga* infestation in addition to improve sorghum growth attributes.
- Application of *Trichoderma* fungi, bacterial strains, nitrogen and herbicide, at low level, is a promising approach to the combat *Striga* infestation and increase sorghum growth as well.

References

[1] Abusin R. M. A, Eltayeb A. H, Hassan M. M. and Babiker A. G. T. (2017). Integrated Management of *Striga hermonthica* on Sorghum, Asian Journal of Advances in Agricultural Research 4 (2): 1-8.

[2] Ahonsi, M. O.; Berner, D. K.; Emechebe, A. M.; Lagoke, S. T.; (2002). Selection of rhizobacterial strains for suppression of germination of *Striga hermonthica* (Del.) Benth. Seeds. *Biological Control*, 24 (2): 143-152.

[3] Altomare, C. W. A.; Norvell, T.; Björkman, G. E. and Harman (1999). Solubilization of phosphates and micronutrients by the plant-growth promoting and biocontrol fungus *Trichoderma harzianum*Rifai strain 1295-22. *Applied Environmental Microbiology*, 65: 2926-2933.

[4] Atera, E. andItoh, K. (2011). Evaluation of ecologies and severity of *Striga* weed on rice in sub-Saharan Africa. *Agric. Biol. J. N. Am.*, 2: 752-760.

[5] Boari, A.; Ciasca, B.; Pineda-Martos, R.; Lattanzio, V. M.; Yoneyama, K. and Vurrom M. (2016). Parasitic weed management by using strigolactone-degrading fungi. *PestManag. Sci.*, 72 (11): 2043-2047.

[6] Dor, E. et al. (2011) The synthetic strigolactone GR24 influences the growth pattern of phytopathogenic fungi. *Planta*, 234: 419– 427.

[7] ElKhair, K. H.; Hassan, M. M.; Rugheim, A. M. E; Ahmed, M. M.; Abakeer, R. A.; Abusin, R. M. A.; Osman, A. G.; Abdelgani, M. E. and Babiker, A. G. T. (2017). Potential role of intercropping, bacterial strains and inorganic fertilizers in integrated *Striga hermonthica* management on sorghum. *Advances in Environmental Biology*, 11 (8): 1-10.

[8] Gafar, N. Y.; Hassan, M. M.; Rugheim, A. M. E.; Osman, A. G.; Mohamed, I. S.; Abdelgani, M. E. and Babiker, A. G. T. (2015). Evaluation of endophytic bacterial isolates on germination and haustorium initiation of *Striga hermonthica* (del.) Benth. *International Journal of Farming and Allied Sciences*, 4 (4): 302-308.

[9] Hanson, J. R. (2003). Natural Products: The Secondary Metabolites. UK: Royal Society of Chemistry: Cambridge. pp.1-154.

[10] Haque1, M.; Haque A; Ilias G. N. M. and Molla1 A. (2010). Trichoderma-Enriched Biofertilizer: A Prospective Substitute of Inorganic Fertilizer for Mustard (*Brassica campestris*) Production. *The Agriculturists* 8 (2): 66-73.

[11] Harman, G. E.; Howell, C. R.; Viterbo, A.; Chet, I. andLorito, M. (2004). *Trichoderma* species –

opportunistic, avirulent plant symbionts. *Nat. Rev. Microbiol*., 2: 43–56.

[12] Hashemabadi D; Sabzevari F; , Kaviani B; , Ansari MH (2018).organic N-fertilizer, rhizobacterial inoculation and fungal compost improve nutrient uptake, plant growth and the levels of vindoline, ajmalicine, vinblastine, catharanthine and total alkaloids in Catharanthusroseus L. Folia Hort.30 (2): 21-31.

[13] Hassan, M. M.; Abdelgani, M. E. and Babiker, A. G. T. (2009). Potential of bacteria strains and nitrogen in reduction of *Striga hermonthica (*Del.) Benth. InfestingSorghum. *Advances in Environmental Biology*, 3: 1-9.

[14] Jamil, M.; Charnikhova, T.; Verstappen, F. andBouwmeester, H. (2010). Carotenoid inhibitors reduce strigolactone reduction and *Striga hermonthica* infection in rice. *Arch. Biochem. Biophys.*, 504: 123–131.

[15] Kapulnik, Y. and Chet, I. (2000). Induction and accumulation ofPR proteins activity during early stages of root colonizationby the mycoparasite*T. harzianum* strain T-203. *PlantPhysiol. andBiochem*., 38: 863-873.

[16] Marley, P. S.; Ahmed, S. M.; Shebayan, J. A. Y. andLagoke, S. T. O. (1999). Isolation of *Fusariumoxysporum* with potential for biocontrol of the witchweed (*Striga hermonthica*) in the Nigerian Savanna. *Biocontrol Science and Technology*, 9: 159-163.

[17] Parker, C. and Riches, C. R. (1993). Parasitic Weeds of the World: Biology and Control. CAB International, Wallingford, Oxon, UK. pp 332.

[18] Ransom, J. K. (1999). The status quo of *Striga* Control: Cultural, chemical and integrated aspects. In Advances in Parasitic Weed Control at On-farm Level, Vol I, Joint Action to Control Striga in Africa, 133-144 (Eds: Kroschel, J., Mercer-Quarshie, H., Sauerborn, J.) Weikersheim, Germany: MargrafVerlag.

[19] Rezig A. M., Abdelhalim T. S., Hassan M. M., Abusin R. M., Eltayeb A. H., Samejima H. and Babiker A. G. T. (2016). Influence of cowpea root powder and exudates on germination and radicle length in Striga hermothica, sorghum and pearl millet strains. African Journal of Agricultural Research, 11 (24): 2082-2091.

[20] Saxena, S. andPandey, A. K. (2001). Microbial metabolites as eco-friendly agrochemicals for the next millennium. *Appl. Microbiol. Biotechnol.,* 55: 395-403.

[21] Sugimoto, Y.; Ahmed, N. E.; Yasuda, N. (2002). Trichothecene inhibitors of *Striga hermonthica* germination produced by *Fusariumsolani*. *Weed Science*, 50: 658–661.

[22] Vinale, F.; Marra, R.; Scala, F.; Ghisalberti, E. L.; Lorito, M. and Sivasithamparam, K. (2006). Major secondary metabolites produced by two commercial *Trichoderma* strains active against different phytopathogens. *Letters in Applied Microbiology*, 43: 143-148.

[23] Watling, J. R., and Press, M. C. (2001). Impacts of infection by parasitic angiosperms on host photosynthesis. *Plant Biology*, 3: 244–250.

[24] Wu, S. C.; Cao, Z. H.; Li, Z. G.; Cheung, K. C. and Wong, M. H. (2005). Effect of biofertiliser containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. *Geoderma*, 125: 155-166.

[25] Yahia M. Y. A., Hassan, M. M., Elamin M. A., Abdalla N. K., Rugheim A. M. E., Osman A. G., AbdelganiM. E. andBabiker A. G. T. (2020). *Trichoderma harzianum* and bacterial strains as bioagents for suppressing *Orobanche crenata* growth and parasitism in faba bean. International Journal of Agriculture, Environment and Bioresearch, 5 (3): 10-20.

[26] Yahia, M. A.; Hassan, M. M.; Elamien, M. A. M.; Abdalla, N. K.; Rugheim, A. M. E.; Elsalahi, R. H.; Abusin, R. M. A.; Abakeer, R. A.; Ahmed, M. M.; Osman, A. G.; Abdelgani, M. E. and Babiker, A. G. T. (2018). Laboratory and field studies of *Trichoderma harzianum*, bacterial strains and imazethapyr on *Orobanche crenata*Forsk. infesting*Vicia faba*. *Asian Journal of Agricultural and Food Sciences*, 6 (6): 209-217.

[27] Yahia, M. Y. A.; Hassan, M. M.; Elamien, M. A. M.; Abdalla, N. K.; Rugheim, A. M. E.; Abusin, R. M. A.; Abakeer, R. A.; Ahmed, M. M.; Osman, A. G.; Abdelgani, M. E. and Babiker, A. G. T. (2019). Counteracting the effect of *Orobanche crenata* infestation on faba bean (*Vicia faba* l.) by soil microorganisms and chemical fertilizers. *International Journal of Agriculture and Environmental Research*, 5 (4): 469-484.

[28] Yoneyama, K.; Yoneyama, K.; Takeuchi, Y. and Sekimoto, H. (2007). Phosphorus deficiency in red clover promotes exudation of orobanchol, the signal for mycorrhizalsymbionts and germination stimulant for root parasites. *Planta*, 225: 1031–1038.

[29] Yonli, D.; Traoré, H.; Hess, D. E.; Sankara, P. and Sérémé, P. (2006). Effect of growth medium, burial distance and depth of *Striga* seeds on efficacy of *Fusarium* isolates to control *Striga hermonthica* in Burkina Faso. *Weed Research*, 46: 73-81.

[30] Zahir, Z. A.; Asghar, H. N. andArshad, M. (2001). Cytokinin and its precursors for improving growth and yield of rice. *Soil Biol. Biochem*., 33: 405-408.