

EFFECTS OF BACTERIA, CITRIC ACID AND POTASSIUM PHOSPHATE DIHYDRATE ON STRIGA HERMONTHICA INCIDENCE AND SORGHUM GROWTH

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ABSTRACT

Series laboratory and greenhouse experiments were conducted to investigate the effects of bacteria, Potassium Phosphate Dihydrate and citric acid on early developmental stages of *Striga hermonthica* and explore their potential for deployment as a component of an integrated management strategy. In vitro experiments, results revealed that germination of *S. hermonthica* significantly decreased after inoculation with bacterial cultures alone or in combination with Potassium Phosphate Dihydrate and citric acid. *Striga* seeds treated with KH_2PO_4 alone or in combination with ISO10, BMP, Flavobacterium or Flavobacterium +BMP reduced germination by 50-83% as compared to the corresponding control. Moreover, the application of KH_2PO_4 (30 and 50 μ M) alone or in combination with Iso10 and ISO10 and BMP+Flavobacterium significantly ($P \leq 0.5$) increased shoot and root dry weights compared to the corresponding control. With respect to *Striga* dry weight result showed that all treatment reduced *Striga* dry weight. In among treatments, KH_2PO_4 (30 and 50 μ M) and bacterial isolate ISO10 each alone and their combinations gave the best results in reducing *Striga* dry weight.

Keywords: *Striga*, Sorghum, Germination, Bacteria, Citric Acid, Potassium Phosphate Dihydrate

1. INTRODUCTION

Soil microorganisms interfering with early developmental stages were thought of as possible alternatives and/or viable supplements to other control methods (Sauerborn *et al.*, 2007). The symbiotic relationship formed between legumes and rhizobia plays an integral role in agriculture as bacteria fix atmospheric nitrogen (N_2). Rhizobia symbiosis with legumes produces 50% of 175 million tons of total biological N_2 fixation annually worldwide (Yadov and Verma, 2014). Therefore, inoculation of legumes with efficient rhizobia is one of the most important and ergonomically eco-friendly practices used for improvement of N fixation (Denton *et al.*, 2013). Most microorganisms possess an enzymatic system which enables them to mineralize phosphorus-containing organic compounds (Kannaiyan *et al.*, 2004). The transformation of insoluble phosphate into soluble form is carried out by a number of microbes present in the soil.

Paul and Choudhury (1991) observed that seed soaking with 0.5 to 1% solutions of KCl or potassium sulfate (K_2SO_4) significantly increased plant height, yield attributes, and grain yield in wheat. Likewise, Bejandi *et al.* (2009) also found highest shoot length in soybean following priming. Seed priming is effective in all crops to improve seedling growth, seedling vigor and yield. It includes imbibition of seed up to radical emergence followed by retrying to original state. Pre-germinated seeds allow rapid seed emergence and metabolic repair of seeds occur during imbibition in priming process as hydration level of seeds is controlled to allow necessary metabolic activity for seed germination (Islam *et al.*, 2012).

Sorghum [*Sorghum bicolor* (L.) Moench] is a viable food grain for many of the world's most food insecure people who live in marginal areas with poor and erratic rains and often poor soils. Worldwide, it is the fifth major cereal crop in terms of production, after maize, wheat, rice and barely. It is a staple food crop for millions of people in Africa, South Asia and Central America. In terms of tonnage, sorghum is Africa's second most important cereal (AATF, 2011). It is also an important feed grain and fodder crop in the Americas and Australia. In the simplest food preparations, the whole grain is boiled or roasted. More often, the grain is ground or pounded into flour, often after hulling. Sorghum flour is used to make thick or thin porridge, pancake, and dumplings, germinated, dried and ground to form malt, which is used as a substratum for fermentation in local brewery industry

Sorghum is a potential crop for moderately saline areas (Almodares and Sharif, 2007) and shown to contain intraspecific variability for salinity (Igartua *et al.*, 1995). However, Salinity reduced sorghum growth and biomass production (Ibrahim, 2004). Nevertheless, the development of high-yielding salinitytolerant sorghums is the best option to increase the productivity in such soils (Igartua *et al.* 1994). Krishnamurthy *et al.* (2007) reported that there are large genotypic variations for tolerance to salinity in sorghum. Ibrahim (2004) reported that in sorghum, total soluble sugar increased with increasing salinity level. Sucrose content of sorghum could be an indicator for its salt tolerance (Juan *et al.*, 2005). In sorghum, the fructose level was always higher than that of the glucose in response to various salinity treatments (Gill *et al.*, 2001).

The objective of this study was to examine the effects of bacterial strains, citric acid and potassium phosphate dihydrate on *Striga hermonthica* infesting sorghum.

2. MATERIALS AND METHODS

2.1. Laboratory experiments

Series of laboratory experiments were undertaken to investigate the effects of bacterial isolate and strains, potassium phosphate dehydrate and citric acid concentrations on germination of *S. hermonsica*. Treatments were arranged in a Complete Randomized Design (CRD) with 4 replicates. All treatments were repeated four times. The experiments were conducted at the Environment, Natural Recourses and Desertification Research Institute (ENDRRI) Khartoum, Sudan.

2.2 *S. hermonthica* seeds surface disinfection

S. hermonthica seeds were collected in 2012 from infected sorghum fields at the Gedaref Research Station Farm, Sudan. Seeds were surface sterilized as described by (Hsiao *et al.*, 1981). Briefly, the seeds were soaked in 70% for 2 min in 70% ethanol and rinsed three times with distilled water. Subsequently, the seeds were immersed in 1%NaOCl solution for 3 min with continuous agitation, thoroughly washed with sterilized distilled water; air dried and kept in sterilized vials, at ambient temperature till used.

2.3 Samples preparation

Matured grains of sorghum (*S.bicolor* (L.)Moench.) Variety were collected from Agriculture Research Corporation- Sudan. The grains were sorted by removing broken kernels and others unwanted materials and were immediately washed with water.

2.4 Bacterial isolate and strains inoculums

*Bacillus megatherium*var. *phosphaticum*(BMP) and *Flavobacterium* were obtained from the Biological Nitrogen Fixation Laboratory, isolate ISO10 was obtained from the parasitic weeds laboratory, Department of Biopesticides and Biofertilizers, Environment, Natural Resources and Desertification Research Institute (ENDRI), National Centre for Research (NCR), Khartoum, Sudan.

2.5 Potassium phosphate dehydrate and citric acid preparation

Potassium phosphate dihydrate (KH_2PO_4)and citric acid(C_3H_4 (OH) (COOH) $_3$.H $_2$ O) were prepared in five concentrations (10, 20, 30, 40 and 50 μM).

2.6 GR24

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

2.7 Effects of bacteria, KH_2PO_4 and citric acid on *S. hermonthica* germination

Striga seeds were conditioned as described by (Babikeret *al.*, 1993).Glass fiber filter papers (GFFP) discs (8mm diameter) were cut, wetted thoroughly with water and placed in an oven (100 °C for 1h.) to be sterilized just before use (Hassanet *al.*,2010a). For pre-conditioning, sterilized discs, placed in 9 cm Petri dishes lined with glass fiber filter papers, were moistened with 4ml distilled water, media (meat peptone broth and nutrient broth) or the respective bacterial isolate culture (ISO10) and strains(BMP and/or*Flavobacterium*)) alone or in combination with citric acid or potassium phosphate dihydrate concentrations (10, 30, 50, 75, 100%). About 25-50, surface disinfected *S. hermonthica*seeds were sprinkled on each of the glass fiber discs. The Petri-dishes, sealed with par film and wrapped in black polythene, were incubated in the dark at 30°C for 11days. Then each disc was subsequently treated with the synthetic germination stimulantGR24 (30 μl /disc) at 0.1 and 0.01ppm, were re-incubated, and examined for germination after 24h.using stereomicroscope.

3. GREENHOUSE EXPERIMENT

This experiment was conducted to study the effects of bacterial isolate and strains with KH_2PO_4 and citric acid on *S. hermonthica* seed incidence and sorghum performance. The experiment was conducted in the Agricultural College, Sudan University of Science and Technology, during May, to August 2016.

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). Artificial infestation of soil was accomplished by mixing *S. hermonthica* seeds (1g) with 1kg soil, *S. hermonthica* infested and uninfested controls were included for comparison. Bacterial combination (BMP+*Flavobacterium*) and ISO10 with three concentrations (0, 30 and 50 μM) of KH_2PO_4 and citric acid were used. *Sorghum bicolor* seeds (5/pot) were sown at 2cm soil depth. The pots were subsequently irrigated every 2 days. *S. bicolor* seedlings were thinned to 2 plants per pot after 2 weeks of sowing.

Treatments were arranged in a Randomized Complete Block Design (RCBD) with four replicates. Data collected for *S. hermonthica* emergence was measured at 2,4,6,8 and 12 weeks after sowing (WAS). Data collected for Sorghum growth attributes were plant height, leaf area, chlorophyll content, number of leaves and dry weight.

4. STATISTICAL ANALYSIS

Prior to analysis data on percentage (germination) were arcsine transformed, data on *S. hermonthica* emergence and dry weight were square root transformed to fulfill ANOVA requirements. The analyses were performed across experiments using Microsoft Excel. Means separations were made by the LSD at 5%.

5. RESULTS

5.1 Laboratory experiments

5.2 Effects of ISO10, citric acid and KH_2PO_4 on *S. hermonthica* germination

Results showed that ISO10 applied alone significantly ($P \leq 0.5$) inhibited *S. hermonthica* germination by 43% as compared to the medium control (Table 1). Application of the high concentration of citric acid or KH_2PO_4 in combination with ISO10 significantly ($P \leq 0.5$) inhibited *S. hermonthica* germination by 83 and 49% respectively, as compared to the medium control.

Table 1 Effects of ISO10, citric acid and KH_2PO_4 on *S. hermonthica* germination

Treatments			Germination
Bacteria	Chemical	Chemical conc.	
DW		0	64.90* (81.25)**
Medium		0	66.38 (83.81)
ISO10		0	43.48 (47.53)
ISO 10	Citric acid	100	31.39 (27.38)
		75	45.22 (49.98)
		50	49.20 (57.04)

KH₂PO₄	30	48.65 (56.01)
	10	48.80 (56.53)
	100	40.81 (42.79)
	75	52.44 (62.23)
	50	63.68 (80.07)
	30	69.38 (87.53)
	10	63.75 (80.00)

LSD Bacteria **3.04**
LSD Chemical **3.04**
LSD Chemicalconc. **4.29**
LSD Interaction **18.22**

*Data out of brackets are arcsine transformed for analysis.

**Data between brackets are origin data.

5.2 Effects of BMP, citric acid and KH₂PO₄ on *S. hermonthica* germination

Application of BMP + citric acid (concentrations 100, 75 and 10µM) significantly (P≤0.5) reduced *Striga* germination by 100, 50 and 48% respectively, as compared to the medium control (Table 2). The combination of BMP with the high concentration of KH₂PO₄(100µM) significantly (P≤0.5) inhibited germination by 70% as to the medium control.

5.3 Effects of *Flavobacterium*, citric acid and KH₂PO₄ on *S. hermonthica* germination

From results in table (3), *Flavobacterium* alone significantly (P≤0.5) inhibited germination by 94% as compared to the medium control. Application of *Flavobacterium* in combination with 100, 75 and 50µM of KH₂PO₄ significantly (P≤0.5) inhibited germination by 88, 87 and 72% respectively, as compared to the medium control.

5.4 Effects of BMP+*Flavobacterium*, citric acid and KH₂PO₄ on *S. hermonthica* germination

Application of 100µM citric acid alone completely inhibited germination (100%)m while 75µM of citric acid alone significantly (P≤0.5) inhibited *S. hermonthica* germination by 68% as compared to corresponding control (Table 4). The combination of *Flavobacterium*+BMP significantly (P≤0.5) inhibited germination as compared to the medium control.

Table 2 Effects of BMP, citric acid and KH₂PO₄ on *S. hermonthica* germination

Treatments			Germination
Bacteria	Chemical	Chemicalconc.	
DW		0	57.51* (70.00)**
Medium		0	53.95 (64.92)
BMP		0	54.58 (65.68)
BMP	Citric acid	100	00.00 (00.00)
		75	34.56 (32.39)

	50	60.28 (74.70)
	30	45.24 (50.42)
	10	35.24 (33.80)
	100	25.53 (19.24)
	75	39.54 (41.44)
KH₂PO₄	50	48.03 (55.11)
	30	52.45 (62.64)
	10	40.69 (42.75)

LSD Bacteria	3.02
LSD Chemical	3.02
LSD Chemicalconc.	4.26
LSD Interaction	18.09

*Data out of brackets are arcsine transformed for analysis.

**Data between brackets are origin data.

Table 3 Effects of *Flavobacterium*, citric acid and KH₂PO₄ on *S. hermonthica* germination

Treatments			Germination
Bacteria	Chemical	Chemicalconc.	
DW		0	68.93* (87.04)**
Medium		0	63.49 (79.62)
<i>Flavobacterium</i>		0	12.28 (04.60)
<i>Flavobacterium</i>	Citric acid	100	52.19 (62.15)
		75	59.02 (73.11)
		50	61.87 (77.03)
		30	57.36 (70.70)
		10	60.26 (75.24)
	KH₂PO₄	100	17.99 (09.54)
		75	18.39 (10.00)
		50	27.50 (22.50)
		30	53.07 (63.24)
		10	58.11 (72.00)

LSD Bacteria	2.89
LSD Chemical	2.89
LSD Chemicalconc.	4.08
LSD Interaction	17.32

*Data out of brackets are arcsine transformed for analysis.

**Data between brackets are origin data.

Table 4 Effects of BMP+*Flavobacterium*, citric acid and KH_2PO_4 on *S. hermonthica* germination

Bacteria	Treatments		Germination
	Chemical	Chemical conc.	
Medium		0	40.26* (42.00)**
		0	54.67 (66.06)
DW	Citric acid	75	26.79 (21.06)
		100	00.00 (00.00)
	KH_2PO_4	75	49.85 (58.30)
		100	54.01 (65.42)
<i>Flavobacterium</i> + BMP		0	31.67 (28.25)
	Citric acid	75	33.07 (30.28)
		100	46.20 (52.06)
	KH_2PO_4	75	45.98 (51.53)
100		46.20 (52.06)	

LSD Bacteria	1.93
LSD Chemical	2.23
LSD Chemical conc.	1.93
LSD Interaction	9.47

*Data out of brackets are arcsine transformed for analysis.

**Data between brackets are origin data.

6. GREENHOUSE EXPERIMENT

6.1 Effects of bacteria, citric acid and KH_2PO_4 on *S. hermonthica* emergence

Results showed that application of both concentrations (30 and 50 μM) of citric acid and KH_2PO_4 alone or in combinations with ISO10 or BMP+*Flavobacterium* significantly ($P \leq 0.5$) reduced *Striga* emergence at 45, 60 and 75 days after sowing (DAS), except KH_2PO_4 (50 μM) alone and the combinations of ISO10+citric acid (50 μM) and ISO10+ KH_2PO_4 (50 μM) which significantly ($P \leq 0.5$) increased the number of *Striga* emergence as compared to corresponding control (Table 5). From overall mean, ISO10 alone, KH_2PO_4 (30 μM) alone, ISO10+citric acid (30 μM) and ISO10+ KH_2PO_4 (30 μM) completely inhibited *Striga* emergence.

6.2 Effects of bacteria, citric acid and KH_2PO_4 on sorghum height

At 30 DAS, application of KH_2PO_4 (50 μM), ISO10 each alone and KH_2PO_4 (30 μM) + ISO10 significantly ($P \leq 0.5$) increased sorghum plant height as compared to corresponding control (Table 6). At 45 DAS, KH_2PO_4 (30 and 50 μM) alone significantly ($P \leq 0.5$) increased plant height as compared to the corresponding control. At 60 DAS, KH_2PO_4 (30 and 50 μM), citric acid (30 μM), ISO10, BMP+*Flavobacterium*, combination of KH_2PO_4 (30 μM) with BMP+*Flavobacterium* and combinations of KH_2PO_4 (30 and 50 μM) with ISO10 significantly

($P \leq 0.5$) increased plant height as compared to corresponding control. At 75 DAS, all treatments significantly ($P \leq 0.5$) increased plant height as compared to corresponding control, except the combinations of citric acid (30 and $50 \mu\text{M}$) with BMP+*Flavobacterium*. At 90 DAS, all single treatments and combinations significantly ($P \leq 0.5$) increased plant height as compared to corresponding control. From over all means, application of $\text{KH}_2\text{PO}_4(50 \mu\text{M})$ alone and the combination of $\text{KH}_2\text{PO}_4(50 \mu\text{M}) + \text{ISO10}$ gave the highest plant height.

Table5 Effect of bacteria,citric acid and KH_2PO_4 on *Striga* emergence

Treatments			<i>Striga</i> Count(number)			Mean
Bacteria	Chemical	Conc.	45 days	60 days	75 days	
Control	Control		12.01*(4.50)**	14.80(6.75)	14.44(6.50)	5.92
	B [#]	30	0.00(0.00)	5.74(1.00)	8.13(2.00)	1.00
		50	9.90(3.00)	11.35(4.00)	11.35(4.00)	3.67
	C ^{##}	30	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00
		50	18.43(10.00)	18.43(10.00)	17.46(9.00)	9.67
	ISO10	Control		0.00(0.00)	0.00(0.00)	0.00(0.00)
B		30	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00
		50	5.74(1.00)	5.74(1.00)	5.74(1.00)	1.00
C		30	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00
		50	5.74(1.00)	8.13(2.00)	8.13(2.00)	1.67
BMP+ Flavo.		Control		13.17(5.50)	17.46(9.00)	17.46(9.00)
	B	30	11.04(4.00)	0.00(0.00)	0.00(0.00)	1.33
		50	5.74(1.00)	5.74(1.00)	5.74(1.00)	1.00
	C	30	14.18(6.00)	14.18(6.00)	14.18(6.00)	6.00
		50	12.57(4.75)	8.45(2.25)	8.45(2.25)	3.08

LSD Bacteria

0.13

0.09

0.10

LSD Chemical

0.13

0.09

0.10

LSD Conc.

0.13

0.09

0.10

LSD Interaction

0.38

0.27

0.29

* indicates square root transformed data ($\sqrt{x+0.5}$ x: variable)

**Data between brackets are origin data

B=Citric acid

C= KH_2PO_4

Table 6 Effects of bacteria, citric acid and KH_2PO_4 on sorghum height

Treatments			Plant height (cm)					Mean
Bacteria	Chemical	Con	30da	45da	60da	75da	90da	
Control without <i>Striga</i>	0		47.28	72.50	94.00	83.75	112.5	82.01
Control	Control		52.28	64.25	54.50	55.50	24.50	50.21
	B*	30	52.50	67.00	93.75	98.75	147.5	91.90
		50	44.00	45.25	65.25	75.00	61.25	
	C**	30	51.53	105.7	110.0	112.7	118.7	99.76
		50	71.63	81.25	119.0	120.0	145.0	107.3
ISO10	Control		59.00	53.00	74.00	82.50	108.7	75.45
	B	30	55.13	67.50	74.00	87.50	99.50	76.73
		50	55.38	57.00	90.00	81.25	87.50	74.23
	C	30	73.50	85.00	103.2	101.2	130.0	98.60
		50	57.50	81.75	100.7	82.50	107.7	86.05

BMP+ Flavo.	Control		57.00	66.50	79.00	78.75	86.25	73.50
	B	30	46.63	68.25	74.75	79.25	98.75	73.53
		50	42.25	52.50	83.75	70.00	105.0	70.70
	C	30	65.00	80.00	102.5	95.00	111.7	90.85
		50	59.13	75.50	89.75	85.00	107.5	83.38

LSD Bacteria **5.50 12.74 13.34 8.05 11.68**

LSD Chemical **5.50 12.74 13.34 8.05 11.68**

LSD Conc. **5.50 12.74 13.34 8.05 11.68**

LSD Interaction **16.50 38.23 40.02 24.16 35.03**

* B=Citric acid

**C= KH₂PO₄

6.3Effects of bacteria, citric acid and KH₂PO₄onsorghumleaves number

At 30 DAS, BMP+*Flavobacterium* and KH₂PO₄(50μM) each alone significantly (P≤0.5) increased number of leaves per plantas compared to corresponding control (Table 7). At 60 DAS, KH₂PO₄(30 and 50μM), citric acid (30μM), ISO10, BMP+*Flavobacterium*, combinations of KH₂PO₄(30 and 50μM) with ISO10 and citric acid (30μM) with BMP+*Flavobacterium*significantly (P≤0.5) increased number of leaves per plantas compared to corresponding control. At 90 DAS, citric acid (30 and 50μM), KH₂PO₄(30 and 50μM), ISO10 and BMP+*Flavobacterium* each alone significantly (P≤0.5) increased number of leaves per plantas compared to corresponding control, while the increments occurred by the combinations were insignificant. From overall means, the highest number of leaves was obtained by the

combination of citric acid ($30\mu\text{M}$) with BMP+*Flavobacterium* followed by $\text{KH}_2\text{PO}_4(50\mu\text{M})$ alone.

Table 7. Effects of bacteria, citric acid and KH_2PO on leaves number

Treatments			Number of leaves			Mean
Bacteria	Chemical	Conc.	30 days	60 days	90 days	
Control without <i>Striga</i>	0		5.13	7.00	4.33	5.48
Control	Control		5.50	4.38	3.78	4.55
	B*	30	5.00	7.00	4.75	5.58
		50	4.50	5.00	6.00	5.17
	C**	30	5.13	7.75	5.00	5.96
50		5.88	7.50	5.00	6.13	
ISO10	Control		5.63	6.25	5.25	5.71
	B	30	5.75	5.00	4.75	5.17
		50	5.63	6.25	4.75	5.54
	C	30	5.88	7.00	5.25	6.04
50		6.00	6.50	5.25	5.92	
BMP+ <i>Flavobacterium</i>	Control		6.00	6.00	4.50	5.50
	B	30	5.75	7.00	5.75	6.17
		50	4.75	5.75	4.50	5.00
	C	30	5.50	6.75	4.00	5.42
50		5.88	5.50	4.00	5.13	

LSD Bacteria **0.34 0.51 0.67**

LSD Chemical **0.34 0.51 0.67**

LSD Conc. **0.34 0.51 0.67**

LSD Interaction **1.02 1.53 2.00**

* B=Citric acid

**C= KH_2PO_4

6.4 Effects of bacteria, citric acid and KH_2PO_4 on sorghum leaf area

At 30 DAS, application of $\text{KH}_2\text{PO}_4(30 \text{ and } 50\mu\text{M})$ significantly ($P \leq 0.5$) increased sorghum leaf area as compared to corresponding control (Table 8). At 60 DAS, the highest insignificant increment of leaf area was obtained by the combination of $\text{KH}_2\text{PO}_4(30\mu\text{M})$ + BMP+*Flavobacterium*. At 90 DAS, citric acid ($30\mu\text{M}$) and $\text{KH}_2\text{PO}_4(30 \text{ and } 50\mu\text{M})$ each alone significantly ($P \leq 0.5$) increased sorghum leaf area as compared to corresponding control. From overall means, the highest leaf area was obtained by citric acid ($30\mu\text{M}$) followed by the combination of $\text{KH}_2\text{PO}_4(30\mu\text{M})$ with BMP+*Flavobacterium* and $\text{KH}_2\text{PO}_4(50\mu\text{M})$ alone.

Table 8. Effects of bacteria, citric acid and KH_2PO on sorghum leaf area

Treatments			Leaf area (cm^2)			Mean
Bacteria	Chemical	Conc.	30 days	60 days	90 days	

Control without <i>Striga</i>	0	41.81	148.88	258.75	149.81	
Control	Control	74.06	109.50	155.63	113.06	
	B*	30	54.00	150.19	312.19	172.13
		50	26.63	71.72	172.50	90.28
	C**	30	124.13	128.81	235.31	162.75
		50	100.46	111.38	289.69	167.18
ISO10	Control	52.31	97.31	189.38	113.00	
	B	30	53.44	53.25	158.81	88.50
		50	46.31	71.81	229.69	115.94
	C	30	112.31	132.00	247.50	163.94
		50	58.22	138.38	242.81	146.47
BMP+ <i>Flavobacterium</i>	Control	53.81	67.31	174.19	98.44	
	B	30	32.06	108.09	210.00	116.72
		50	27.28	44.25	147.00	72.84
	C	30	68.91	164.44	272.81	168.72
		50	65.63	115.13	200.63	127.13
LSD Bacteria		17.10	28.87	40.44		
LSD Chemical		17.10	28.87	40.44		
LSD Conc.		17.10	28.87	40.44		
LSD Interaction		51.30	86.62	121.31		

6.5 Effect of bacteria, citric acid and KH_2PO_4 on sorghum chlorophyll content

At 30 and 90 DAS, application of ISO10, KH_2PO_4 (30 and 50 μM) and citric acid (30 μM) each alone significantly ($P \leq 0.5$) increased sorghum leaf chlorophyll content as compared to corresponding control (Table 9). At 60 DAS, all treatments and combinations significantly ($P \leq 0.5$) increased chlorophyll content as compared to corresponding control, except the combination of ISO10+citric acid (30 μM). From overall means, the highest leaf chlorophyll content was obtained by citric acid (30 μM) and KH_2PO_4 (30 μM) followed by the combination of KH_2PO_4 (30 μM) with ISO10.

6.6 Effects of bacteria, citric acid and KH_2PO_4 on sorghum and *Striga* Dry weight

Results of sorghum shoot dry weight showed that application of KH_2PO_4 (30 and 50 μM), ISO10+ KH_2PO_4 (30 μM), citric acid (30 μM), ISO10 and BMP+*Flavobacterium* significantly ($P \leq 0.5$) increased shoot dry weight as compared to the corresponding control (Table 10).

The combination of KH_2PO_4 (50 μM) + BMP+*Flavobacterium* followed by KH_2PO_4 (30 and 50 μM) and ISO10 each alone significantly ($P \leq 0.5$) increased root dry weight as compared to the corresponding control.

From the results of *S. hermonthica* dry weight, all single treatments and combination significantly ($P \leq 0.5$) decreased *S. hermonthica* dry weight as compared to the corresponding

control, except BMP+*Flavobacterium*. The highest declining were obtained by KH_2PO_4 (30 and $50\mu\text{M}$) and ISO10 each alone and their combinations.

Table 9 Effect of bacteria, citric acid and KH_2PO_4 on sorghum chlorophyll content

Treatments			chlorophyll content			Mean
Bacteria	Chemical	Conc.	30 days	60 days	90 days	
Control without <i>Striga</i>	0		37.78	34.25	34.30	32.95
Control	Control		30.30	18.10	28.8	18.89
	B*	30	35.50	38.98	40.55	38.34
		50	29.15	24.70	25.75	26.53
	C**	30	34.25	38.23	42.55	38.34
50		36.18	39.68	37.15	37.67	
ISO10	Control		33.33	33.65	39.18	35.38
	B	30	26.15	28.40	30.15	28.23
		50	25.40	31.20	29.00	28.53
	C	30	32.63	40.80	40.60	38.01
50		29.60	36.20	34.18	33.33	
BMP+ <i>Flavobacterium</i>	Control		31.10	37.13	35.63	34.62
	B	30	33.48	40.08	39.68	37.74
		50	27.43	29.98	33.33	30.24
	C	30	36.10	39.60	36.25	37.32
50		32.48	32.65	36.03	33.72	

LSD Bacteria **2.70** **3.77** **3.95**

LSD Chemical **2.70** **3.77** **3.95**

LSD Conc. **2.70** **3.77** **3.95**

LSD Interaction **8.11** **11.31** **11.84**

* B=Citric acid

**C= KH_2PO_4

Table 10. Effects of bacteria, citric acid and KH_2PO_4 on sorghum and *Striga* dry weight

Treatments			Dry weight (g)			
Bacteria	Chemical	Conc.	Shoot	Root	R:Sh ratio	<i>Striga</i>
Control without <i>Striga</i>	0		10.00	6.35	0.64	//
Control	Control		4.25	5.35	1.26	14.40* (6.32)**

	B*	30	17.00	6.65	0.39	2.75 (0.23)
		50	4.00	3.35	0.84	2.07 (0.13)
	C**	30	22.00	10.68	0.49	0.00 (0.00)
		50	25.00	13.43	0.54	2.75 (0.23)
ISO10	Control		15.00	9.45	0.63	0.00 (0.00)
	B	30	6.50	3.90	0.60	8.68 (2.28)
		50	7.50	8.68	1.16	0.00 (0.00)
	C	30	19.00	10.10	0.53	0.00 (0.00)
		50	12.00	9.90	0.83	0.00 (0.00)
	BMP+ Flavobacterium	Control		10.25	6.25	0.61
B		30	13.00	6.08	0.47	4.97 (0.75)
		50	4.50	4.60	1.02	13.28 (5.28)
C		30	16.00	8.30	0.52	8.21 (2.04)
		50	11.25	13.63	1.21	9.16 (2.63)
LSD Bacteria			4.25	2.65	0.07	
LSD Chemical			4.25	2.65	0.07	
LSD Conc.			4.25	2.65	0.07	
LSD Interaction			12.74	7.95	0.20	

*indicates square root transformed data ($\sqrt{x+0.5}$ x: variable)

**Data between brackets are origin data

*B=Citric acid

**C= KH_2PO_4

7.DISCUSSION

Parasitic weeds of the genus *Striga*, pose a severe problem to agriculture. They inflict significant losses in yields of staple food crops in sub-Saharan Africa where low soil fertility and low-input farming are predominant. Generally, *Striga* germination was increased with increasing of citric acid or KH_2PO_4 concentrations when combined with ISO10. While ISO10 alone or in combination with citric acid or KH_2PO_4 at 100% reduced germination by 83 and 49% respectively as compared to the control. Moreover, BMP plus citric acid at 100% was completely inhibited *Striga* germination. In general bacterial strains combined with the chemicals at the highest concentration sustained the lowest germination. Daffalla *et al.* (2014) reported that several factors influence germination of *Striga* in the soil including temperature, moisture, pH, nutrients, soil type, and stimulants produced by host plants. A negative relationship was observed between salt levels and germination percentage of *Striga* seeds during or after conditioning and haustorium. Hassan *et al.* (2010b) reported that *Striga* and *Orobanche* spp. seeds primed in NaCl significantly reduced germination in response to GR24. Daffalla *et al.*, (2014) reported that *Striga* seeds primed in $\text{C}_2\text{H}_4\text{O}_2 \cdot \text{NH}_3$ displayed 15-20% germination compared to the corresponding control (75%). Osmotic potential may significantly affect germination of the parasitic weed.

Regarding sorghum growth, results revealed that citric acid alone or in combination with ISO10 gave the highest growth followed by the combination of BMP+*Flavobacterium* and citric acid as

compared to other treatments and the control. Basra *et al.* (1989) found that priming of corn seed using polythelene glycol or potassium salts (K_2HPO_4 or KNO_3) resulted in accelerated germination.

Results revealed that all treatment reduced *Striga* emergence as compared to the corresponding control. Hassan *et al.* (2010b) reported that *Striga* and *Orobanche* spp. seeds rarely germinated when incubated in NaCl solution. That soil saturated with 75 \square M NaCl resulted in complete absence of *Striga* emergence.

Results showed that citric acid, bacterial ISO10 alone or in combination together sustained the highest leave number, leave area and chlorophyll content. Sorghum shoot dry weight was increased when treated with citric acid alone or in combination with ISO10, BMP+*Flavobacterium*. However with respect to *Striga* dry weight, results showed that all treatments reduced *Striga* dry weight as compared to the control. Basra *et al.* (1989) found that priming of corn seeds using polythelene glycol or potassium salts (K_2HPO_4 or KNO_3) resulted in accelerated germination. Seyyedi *et al.* (2015) reported that seed priming in KH_2PO_4 showed an excessive effect of biologic fertilizer of *Nigella sativa* dry weight. Ashraf and Foolad (2005) also reported that seedlings grown from primed seeds of different field crops showed higher vigor than unprimed seeds. Seed priming exerts stimulating effects on the germination process by mediating cell division and helps the repairing process of damaged membranes of seeds (Arif *et al.*, 2008; Hassanpouraghdam *et al.*, 2009). Fastened germination rate and uniformity of seed emergence reduces metabolic phase (Islam *et al.*, 2012). Seed priming increases speed and uniformity of germination rate (Khalil *et al.*, 2010), break seed dormancy, imbibition, mobilization of reserve food materials and activation of several enzymes (Asgedom and Becker, 2001). Rapid seed germination leads to increased germination percentage, seedling establishment and final yield. The success of seed priming is influenced by the complex interaction of factors including plant species, water potential of the priming agent, duration of priming, temperature, seed vigor and dehydration, and storage conditions of the primed seed (Ghassemi-Golezani *et al.*, 2011). So, effect of salts priming on salt tolerance of sorghum seeds still requires more investigations at biochemical level before applying the method at field.

In conclusion, application of citric acid alone or in combination with bacterial strains, irrespective to the concentrations, improved sorghum growth. All treatments used in this study reduced *S. hermonthica* incidence in sorghum and mitigated its negative effects on sorghum plants. In among all treatments, the citric acid alone or in combination with bacterial strains reduced *Striga* incidence, as well as increased sorghum growth, leave number, leave area and dry weight. The results need to be verified in field experiments and the cost effectiveness of the treatments needs to be considered.

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