

*Full Length Research Paper*

# **Mycorrhiza co-association with *Aspilia plurisetata* Schweif. and phosphorus uptake effects on growth of gadam sorghum in the semi-arid lower Eastern Kenya**

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**Mycorrhiza fungi are important components of soil microbiota in the rhizosphere and greatly influence the uptake of mineral elements by plants. A greenhouse experiment was conducted at the University of Embu to evaluate effect of *Aspilia plurisetata* rhizosphere mycorrhiza on phosphorus uptake by gadam sorghum. Pots were filled with soil from a predetermined source in the semi-arid Gakurungu, Tunyai and Kanyuambora regions of Kenya. A completely randomized block design was used with each treatment replicated four times giving n=144. Regular watering was maintained for thirty-five days. Data were analyzed using three-way ANOVA. Seed emergence, hypocotyl development and stand count were enhanced at  $P \leq 0.05$  in both mycorrhiza fungi inoculated gadam sorghum seeds and in pots whose soils were taken from the rhizosphere of *A. plurisetata* plants. The growth attributes had a positive correlation with yield at 95% confidence. Soil phosphate level was enhanced where seed inoculation with mycorrhiza was done and in soils previously grown *A. plurisetata* vegetation. *A. plurisetata* bush fallows can be used for phosphate bio-remediation and cover crop in arid and semi-arid environments.**

**Key words:** Rhizosphere, Mycorrhiza, Phosphorus, *Aspilia plurisetata*, inoculation.

## **INTRODUCTION**

The use of fertilizers to supplement soil nutrients in promoting plant growth is prevalent in modern agriculture

(Cordell et al., 2009). Phosphorus (P) is the most important nutrient element (after nitrogen) limiting

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agricultural production in most regions of the world (Holford, 1997). In East Africa, phosphorus deficiency occurs in many soils, not only due to P depletion through crop harvest and erosion, but also due to P-fixing soils in the region (Nziguheba, 2007). Soil degradation caused by conventional agriculture in these fragile ecosystems poses an added threat to agricultural production (Conacher, 2009; Stavi and Lal, 2015). Much attention has to be given in restoring and maintaining soil health, particularly in arid and semi-arid environments. A suitable cover crop, with multiple benefits (Poeplau and Don, 2015; Thapa et al., 2018; Daryanto et al., 2018; Sturm et al., 2018) would be ideal for these environments.

It is hypothesized that a complex association occurs between mycorrhiza, soils and plants, leading to the availability of phosphorus in the soils in usable forms to plants. Virtually all crop plants (except Brassicaceae, Amaranthaceae and Polygonaceae) worldwide are host to some form of mycorrhizal association (Cooke and Lefor, 1998; Stanescu and Maherali, 2017). Among the three major nutrients (nitrogen, phosphorus and potassium) required by plants, phosphorus constitutes a particularly critical component because on one hand it is limiting for crop yield on a large proportion of global arable land and, on the other hand, it is a non-renewable resource (Plenchette et al., 2005; Cordell et al., 2009; Vance, 2008). Agricultural experts point to a phosphate crisis in the foreseeable future (Gilbert, 2009). Different approaches have to be explored to ensure a continued supply of phosphates in the soil and hence sustained food production. Soil biology has emerged over the last decade as a critical part of the knowledge base for successful and sustainable agricultural production (Shen et al., 2011). A key component of this subject is the plant-mycorrhizal fungi relationship, which has enormous potential for improved management of contemporary farming systems (Shen et al., 2011; Harrier and Watson, 2003).

The majority of terrestrial plant species are capable of interacting with mycorrhiza fungi (MF) in nature to provide an effective pathway by which phosphorus is scavenged and rapidly delivered to cortical cells within the root (Smith et al., 2011, 2001). MF acts as a catalyst to concentrate P in a manner that makes it available to plants (Ferrol et al., 2016; Schubert and Lubraco, 2000; Calvet et al., 2004). The plant supplies the fungi with sugars produced by photosynthesis, while the hyphae network improves the plant capacity to absorb water and nutrients (Plenchette et al., 2005; Smith et al., 2003).

Mycorrhizal symbioses contribute significantly to plant nutrition and particularly to phosphorus uptake (Smith et al., 2011). The large surface area of the mycorrhiza fungi hyphal network is very efficient in nutrient uptake (Plenchette et al., 2005; Barea et al., 2008). Furthermore, phosphorus is a highly immobile element because it is easily adsorbed by soil particles and a phosphate-free zone rapidly occurs around plant roots (Roy-Bolduc and

Hijri, 2011). Extra radical hyphae extend beyond this depletion zone, absorbing bio-available phosphate that would otherwise not be accessible to the plant (Fellowes, 2007; Raverkar and Bhattacharya, 2014). Phosphate ions in the soil also become rapidly bound with cations, forming insoluble complexes that are unavailable to plants (Fellowes, 2007; Raverkar and Bhattacharya, 2014).

Recent physiological and molecular research has revealed that the MF pathway plays a major role in P uptake, regardless of the extent to which a mycorrhiza fungi plant benefits in terms of increased growth or P uptake (Smith et al., 2010). This experiment investigated the *Aspilia pluriseta* Schweif plant in mediating phosphorus mining from the soil aided by mycorrhiza and eventual phosphates availability in soil colloids for use by other plants. Performance trials were conducted with Gadam sorghum (*Sorghum bicolor* L.) grown on rhizosphere soils of *A. pluriseta* and growth attributes observed over time.

## MATERIALS AND METHODS

### Study area

This experiment was set up at the University of Embu located on latitude 00° 31' 52.03" N and longitude 37° 27' 2.20" E at an elevation of 1480 m above sea level. The average annual rainfall around the university greenhouses is 1252 mm and is received in two distinct rainy seasons; the long rains (mid-March to June) with an average rainfall of 650 mm and the short rains (mid- October to December) with an average of 450 mm (Jaetzold et al., 2006). The area has a mean annual temperature of 19.5°C, a mean maximum of 25°C, and a mean minimum of 14°C. Over 65% of the rains occur in the long rain season (Jaetzold et al., 2006). The average temperature inside the greenhouse was 24°C while average humidity was 65%. The soils are mainly humic nitisols derived from basic volcanic rocks (Jaetzold et al., 2006) and are deep, well-weathered with moderate to high inherent fertility but over time soil fertility has declined due to continuous mining of nutrients without adequate replenishment.

### Approach

Mature plots of *A. pluriseta* vegetation were identified in preselected sites in Tharaka Nithi (Gakurungu at 00°12'00" S, 37°51'00" E and Tunyai at 00°10'00" S, 37°50'00" E) and Embu (Kanyuambora at 0°21'0" S, 37°28'30" E) counties. The specific sites where the soil was extracted were purposively selected and each sub-site had colonies of *A. pluriseta* vegetation, silty clay, silt loam and sandy loam soils. Soil categorization was based on USDA soil taxonomy. A five-meter length tape measure was used to delineate the clearance area of 1 m<sup>2</sup> of the selected sub-site. The underneath vegetation was cleared using a panga. Using a mattock, a forked jembe and a spade about 20 kg of soil from each depth of interest were dug in each of the subsites at the depths of 0-20 cm, 21-40 cm and 41-60 cm. A three-meter tape measure was used to take the vertical distances. The soil was put in separate, sterilized hessian bags that were clearly labeled using a felt pen for each depth of soil. A similar procedure was used to collect soil in the

Cs	Cs	Sl	Sl	Sl	Ls	Sl	Ls	Cs	Sl	Sl	Cs
<b>D2Oa</b>	<b>D1wpa</b>	<b>D2Oa</b>	<b>D2Oi</b>	<b>D3Oa</b>	<b>D2wpa</b>	<b>D1wpa</b>	<b>D3Oa</b>	<b>D2wpi</b>	<b>D2wpi</b>	<b>D3wpa</b>	<b>D3wpi</b>
Cs	Ls	Cs	Cs	Sl	Cs	Cs	Cs	Sl	Sl	Cs	Sl
<b>D1Oa</b>	<b>D3wpa</b>	<b>D3Oa</b>	<b>D2Oi</b>	<b>D1Oa</b>	<b>D3Oi</b>	<b>D2wpa</b>	<b>D1wpi</b>	<b>D1wpi</b>	<b>D3wpi</b>	<b>D3wpa</b>	<b>D2wpa</b>
Ls	Ls	Ls	Ls	Sl	Ls	Ls	Ls	Sl	Ls	Cs	Ls
<b>D1Oa</b>	<b>D1Oi</b>	<b>D2Oa</b>	<b>D2Oi</b>	<b>D1Oi</b>	<b>D3Oi</b>	<b>D1wpa</b>	<b>D1wpi</b>	<b>D3Oi</b>	<b>D2wpi</b>	<b>D1Oi</b>	<b>D3wpi</b>

**Figure 1.** Block 1 experimental layout in the greenhouse. Sl-sandy loam soil; Cs-silty clay soil; Ls-silt loam soil; O-soils without *A. plurisetia* vegetation; wp-soils with *A. plurisetia* vegetation; a-Gadam seeds not inoculated and i-Gadam seeds inoculated; D1-soil depth level 1 (0-20 cm); D2-soil depth level 2 (21-40 cm); D3-soil depth level 3 (41-60 cm).

sites and subsites for the control experiment but in adjacent areas where *Aspilia* was not growing. All other parameters remained the same, except the lack of *A. plurisetia* vegetation in the patches selected. The soil collected was transported in clearly labeled hessian bags to the University of Embu greenhouse using a three-tone truck. At the greenhouse, the soil was thoroughly mixed for each respective soil type, depth and presence or absence of *Aspilia* vegetation. A sample of the initial homogenous soil was tested for phosphorus (Olsen, 1954 protocol), phosphates (using colorimetric method) and pH (using McLean, 1983 procedure). Sterilized pots (30 cm x 40 cm) were filled with this homogenous soil to about one-third full. Two seeds of mycorrhiza fungi-inoculated Gadam sorghum (inoculation protocol according to (Habte and Osorio, 2001) were planted into pots and a similar number planted with uninoculated sorghum seeds as a control. Two kilograms of *A. plurisetia* rhizosphere soil earlier tested and found to contain mycorrhiza fungi of the order Glomerales was mixed with 1kg gadam sorghum seeds. Each of the 4 treatments (Figure 1) as mentioned was replicated four times, giving n=144. Each pot was watered after every two days using a two-liter watering can for the first one week. Thereafter, the watering regime was reduced to once a week but ensuring the pots remained moist. Watering was done uniformly to all the pots. This was maintained for thirty-five days. Data on plant growth attributes were taken every week and corroborated with treatments given in the pots.

#### Data analysis methods

Data obtained from various treatments were subjected to SAS Edition 8.2. Differences between treatment means were examined using least significant difference (LSD) at  $P \leq 0.05$ . Plant growth attributes obtained on the soil types, rhizosphere depths and locations with *A. plurisetia* were compared to their controls and conclusions made.

## RESULTS

### Effect of soils on gadam sorghum seed emergence and stand county

Soil type influenced gadam sorghum seeds emergence. Greater seed emergence was experienced in silt loams at

97% within the first seven days. Seeds planted in silty clay soils had the lowest seed emergence in the first week and significantly differed from the other soils at  $P \leq 0.05$  (Table 1).

Silt loam soils differed significantly from silty clay and sandy loam on germination and stand count in the first 35 days of growth at 95% confidence level.

### *A. plurisetia* effect on gadam sorghum seeds early growth

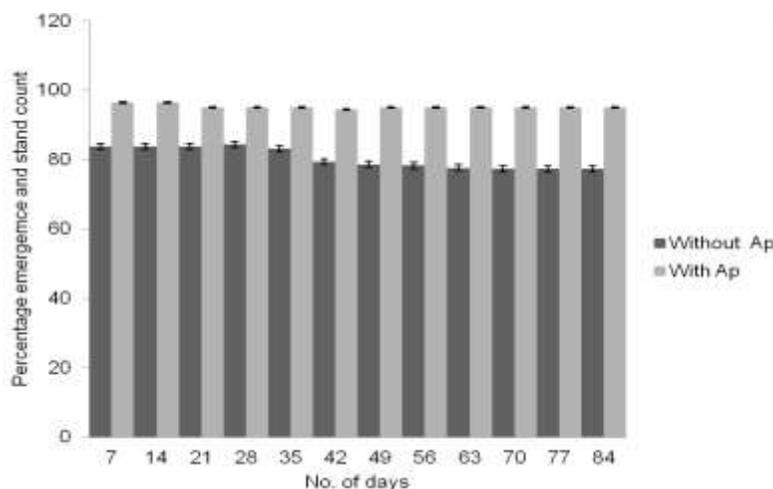
Gadam seed emergence and early growth were highest in soils that had *A. plurisetia* vegetation (Figure 2) at 96.5% in the first week and differed significantly with seeds planted in soils that were not previously growing *A. plurisetia* at  $P \leq 0.05$  (Table 2). Similarly, stand count was almost maintained in soils with *A. plurisetia* vegetation than those without (Figure 2).

### Effect of gadam sorghum seeds inoculation with mycorrhizal fungi on seed emergence, stand count and plant height

Gadam seed emergence and stand count gave similar results when the sorghum seeds were inoculated with mycorrhizal fungi (Figure 3). The inoculated seeds had higher percent seed emergence and stand count compared to the uninoculated. Besides, aggregate gadam crop stability was higher in inoculated sorghum seeds. The average sorghum plant height was 124 cm at physiological maturity (Figure 4). Although both growth curves were normal, the curve with soils where *A. plurisetia* previously grew peaked higher. Similar results were obtained when gadam sorghum seeds were inoculated with mycorrhizal fungi from the rhizosphere of *A. plurisetia*.

**Table 1.** Effect of soil type on germination and stand count on gadam sorghum in the first 35 days of growth (% values).

Soil type	Duration of crop stand count (days)				
	7	14	21	28	35
Silty clay	83.3 <sup>b</sup>	83.3 <sup>b</sup>	83.3 <sup>b</sup>	84.4 <sup>b</sup>	82.3 <sup>b</sup>
Silt loam	96.9 <sup>a</sup>	96.9 <sup>a</sup>	94.8 <sup>a</sup>	94.8 <sup>a</sup>	94.8 <sup>a</sup>
Sandy loam	90.2 <sup>ab</sup>	90.2 <sup>ab</sup>	90.2 <sup>ab</sup>	90.2 <sup>ab</sup>	90.2 <sup>a</sup>
LSD	7.2	7.2	7.6	7.6	7.8



**Figure 2.** Percentage gadam seed emergence and stand count over the growing period.

**Table 2.** Effect of using *Aspilia* sourced soils on gadam sorghum seedlings (% survival).

Factor	Duration of crop stand count (days)											
	7	14	21	28	35	42	49	56	63	70	77	84
Without Ap	83.8 <sup>b</sup>	83.8 <sup>b</sup>	83.8 <sup>b</sup>	84.4 <sup>b</sup>	83.1 <sup>b</sup>	79.4 <sup>b</sup>	78.6 <sup>b</sup>	78.3 <sup>b</sup>	77.6 <sup>b</sup>	77.3 <sup>b</sup>	77.3 <sup>b</sup>	77.3 <sup>b</sup>
With Ap	96.5 <sup>a</sup>	96.5 <sup>a</sup>	95.1 <sup>a</sup>	95.1 <sup>a</sup>	95.1 <sup>a</sup>	94.4 <sup>a</sup>	95.1 <sup>a</sup>					
LSD	5.9	5.9	6.2	6.2	6.3	7.2	7.2	7.3	7.3	7.4	7.4	7.4

**Changes in pH, phosphorus and phosphate levels before and after gadam sorghum harvest**

The initial rhizosphere reading for pH and phosphorus was higher but the opposite was true of phosphates after the experiment (Table 3). Phosphate level in soils grown with *A. plurisetata* vegetation had a higher content compared to soils without the vegetation (Table 3)

**Correlation of gadam sorghum growth attributes with yield**

All growth attributes for gadam sorghum had a positive

correlation with yield at 95% confidence level (Table 4). Soil depth, however, exhibited a negative correlation with yield.

**DISCUSSION**

Seed germination and eventual crop stand is a process that influences crop yield and quality (Tuan et al., 2019). Gadam sorghum germination and subsequent plant survival was significantly affected by the type of soil in which the plant was grown. Soils that had *A. plurisetata* previously growing produced higher sorghum crop yields. We found out that the water retention capacity of the

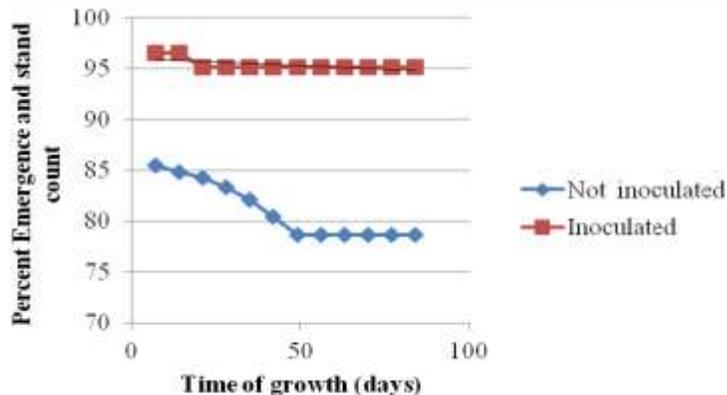


Figure 3. Sorghum emergence and stand count for inoculated and uninoculated seedlings.

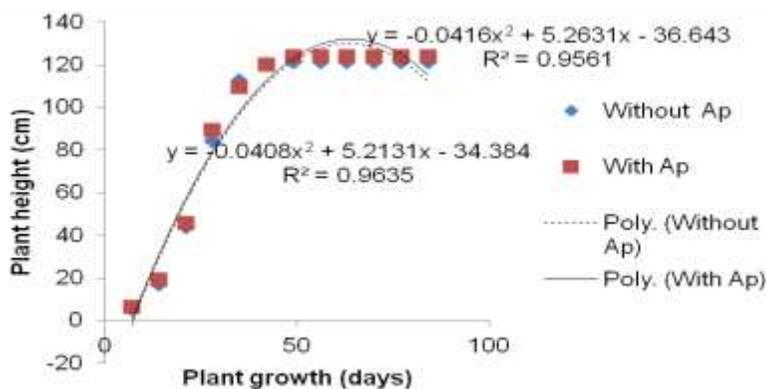


Figure 4. Height of gadam sorghum crop in *Aspilina* soils compared to non *Aspilina* soils.

Table 3. pH, phosphates (ppm) and phosphorus (ppm) content levels in the soil.

Factor	Before planting	One week after harvesting	Before planting	One week after harvesting	Before planting	One week after harvesting
	pH1	pH2	Phos1	Phos2	Phr1	Phr2
Silty clay	6.3 <sup>a</sup>	6.2 <sup>a</sup>	96.2 <sup>a</sup>	96.8 <sup>a</sup>	25.7 <sup>a</sup>	24.2 <sup>a</sup>
Silt loam	6.0 <sup>b</sup>	5.9 <sup>b</sup>	76.9 <sup>a</sup>	78.9 <sup>a</sup>	34.5 <sup>a</sup>	32.8 <sup>a</sup>
Sandy loam	6.3 <sup>a</sup>	6.1 <sup>a</sup>	80.8 <sup>a</sup>	80.8 <sup>a</sup>	35.3 <sup>a</sup>	34.5 <sup>a</sup>
LSD	0.2	0.2	39.2	39.6	15.8	15.9
With Ap	6.0 <sup>b</sup>	5.9 <sup>b</sup>	103.6 <sup>a</sup>	107.7 <sup>a</sup>	31.2 <sup>a</sup>	30.0 <sup>a</sup>
Without Ap	6.3 <sup>a</sup>	6.2 <sup>a</sup>	65.6 <sup>b</sup>	63.3 <sup>b</sup>	31.8 <sup>a</sup>	31.0 <sup>a</sup>
LSD	0.2	0.2	32	32.3	12.9	13
0-20 cm	6.4 <sup>a</sup>	6.3 <sup>a</sup>	108.6 <sup>a</sup>	108.4 <sup>a</sup>	40.3 <sup>a</sup>	39 <sup>a</sup>
21-40 cm	6.1 <sup>b</sup>	5.9 <sup>b</sup>	65.3 <sup>b</sup>	67.3 <sup>b</sup>	28.5 <sup>a</sup>	27.8 <sup>a</sup>
41-60 cm	6.1 <sup>b</sup>	6.0 <sup>b</sup>	80.0 <sup>ab</sup>	80.8 <sup>ba</sup>	25.7 <sup>a</sup>	24.7 <sup>a</sup>
LSD	0.2	0.2	39.2	39.6	15.8	15.9
Mean	6.2	6.1	84.6	85.5	31.5	30.5

pH1-Initial soil pH; pH2-pH one week after harvesting; Phos1-Initial soil phosphates in ppm; Phos2-Soil phosphates in ppm one week after harvesting; Phr1-Initial soil phosphorus in ppm; Phr2-Soil phosphorus in ppm one week after harvesting.

**Table 4.** Correlation coefficient table in relation to the yield of gadam sorghum.

Parameter	Correlation value to yield (Days after seed germination)											
	7	14	21	28	35	42	49	56	63	70	77	84
Site <sup>1</sup>	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Inoculation <sup>2</sup>	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Block	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Soil	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Depth	-0.35	-0.35	-0.35	-0.35	-0.35	-0.35	-0.35	-0.35	-0.35	-0.35	-0.35	-0.35
Stand count	0.39	0.39	0.39	0.37	0.39	0.4	0.39	0.39	0.38	0.38	0.38	0.38
Plant height	0.48	0.38	0.38	0.34	0.37	0.49	0.51	0.51	0.51	0.51	0.51	0.51
No. of Leaves	0.27	0.52	0.32	0.08	0.4	0.39	0.31	0.39	0.45	0.59	0.63	0.63
Length of Leaves	0.16	0.14	0.11	0.21	0.25	0.39	0.45	0.45	0.45	0.45	0.45	0.45

<sup>1</sup>Whether or not *A. pluriset*a had grown in the soils <sup>2</sup>whether or not gadam sorghum seeds were inoculated with Arbuscular mycorrhiza fungi (AMF).

different soil types affected stand count with time. The smaller the particles were, the more the water holding capacity and with time, seeds that germinated dried up. Sladonja et al. (2014) obtained similar results on pyrethrum seed emergence tested for different soil types while Idu et al. (2003) observed that germination and emergence of *Helianthus annuus* L. were low in clay treatments compared to other soil treatments with bigger particle size. Anderson et al. (2004) reported that varied rainfall, sowing time, soil type, and cultivar influence on plant population for wheat in Western Australia and obtained similar results.

Early growth of the gadam sorghum plant was influenced by the use of soils that had *A. pluriset*a initially growing in it. Varga (2015) noted that mycorrhiza fungi negatively influenced seed germination but at the same time improved plant growth. This partly explains the behavior of sorghum seedlings stand county stability compared to seedlings in soils that had not been growing *A. pluriset*a.

Inoculation with mycorrhizal fungi affected seed emergence, stand count and plant height in this experiment. Results obtained closely mirrored the observation made by Caravaca et al. (2002) that rhizosphere aggregate stability of afforested semiarid plant species was significantly improved upon mycorrhiza fungi inoculation. Not only does mycorrhizal association with plants improve drought tolerance (Fitter, 1988) but also seedling survival (Janos, 1980). Gadam sorghum is a short stature crop (Chimoita et al., 2019) and this is confirmed in this experiment showing the plant as having an average height of 124 cm at physiological maturity. Having a small height is one of the drought escaping mechanism of plants in dry land areas as assimilate partitioning is done early during the plant's life (Basu et al., 2016).

Kavanová et al. (2006) revealed that phosphorus is critical for cell division and elongation on the grass plants at the early stages of growth. This phenomenon could have contributed towards greater plant height for seeds that were inoculated with mycorrhizal fungi in this

current experiment and further corroborates research by Bhuiyan et al. (2008) that phosphorus inoculation was found to be positive and significant on mungbean plant height. Besides, Bam et al. (2006) showed that germination and vigor in rice improved significantly when seeds were soaked in potassium and phosphorus salts. Soil that had *A. pluriset*a previously growing had mycorrhiza that acted as a source of inoculation and therefore continued with phosphates synthesis with gadam sorghum as the host plant. This finding agrees with Tiamtanong et al. (2015) that mycorrhiza fungi inoculation enhances the development of soil phosphates by increasing phosphates enzyme activity. The phosphorus in the soil was converted to phosphates through the action of mycorrhiza.

### Conclusion

Gadam sorghum plant emergence, stand count and aggregate plant stability were improved by

inoculating seeds with *A. pluriseta* rhizosphere soils or growing sorghum as follower crop in *A. pluriseta* bush fallows. These attributes enhance overall crop yield, grain quality and land productivity. *A. pluriseta* bush fallows can be used for phosphate bio-remediation and cover crop in arid and semi-arid environments.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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