

## Effect of mixed organic-inorganic fertilizer on growth and phosphorus uptake of setaria grass (*Setaria splendida*)

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### Abstract

Increasing phosphorus (P) availability in tropical P deficient soils is a challenging task. Vermicomposting of organic wastes in the presence of phosphate rock facilitates the release of P and this has the potential to address crop P needs. A study was conducted to assess and compare the effects of application of gafsa phosphate rock (GPR) alone and GPR in combination with empty oil palm fruit bunches, earthworms (*Pontoscolex corethrurus*), arbuscular mycorrhiza fungi (*Glomus mosseae*), and P-enriched vermicompost, in fulfilling the P requirements of the setaria (*Setaria splendida* L.) grass. Application of mixed organic fertilizer combined with GPR was effective in increasing dry matter yield of grass, with 19% higher dry matter production as compared to the use of GPR alone. Among the organic fertilizers, application of P-enriched vermicompost was the best to support the grass growth. Nitrogen, P, Ca, and Mg uptake of the grass treated with P-enriched vermicompost was higher. Nitrogen and P utilization efficiency of the setaria grass treated with P-enriched vermicompost was also high. Residual P in soil treated with GPR was higher as compared to that treated with P-enriched vermicompost. However, plant available P was higher than that for the other GPR application techniques. The different types of earthworms had no effect on the quantum of nutrient uptake and the dry matter yield of the setaria grass. Application of a mixture of GPR and empty oil palm fruit bunch to the soil increased the dry matter of setaria compared to the use of inorganic fertilizer alone. We conclude that soil treated with P-enriched vermicompost was an efficient treatment for increasing availability of P (24.28 mg kg<sup>-1</sup>), N, P, Ca and Mg uptake (53.76, 41.83, 13.58 and 15.16 mg pot<sup>-1</sup>, respectively); which ultimately enhanced root volume (163 cm<sup>3</sup>) and dry matter yield (5.75 to 6.46 g pot<sup>-1</sup>).

**Keywords:** Phosphate rock, vermicompost, earthworm, arbuscular mycorrhizae, dry matter yield, *Setaria splendida*.

**Abbreviations:** GPR- gafsa phosphate rock; - P- Without application of P fertilizer, soil only; +P- application of gafsa phosphate only; EFB – GPR- application of empty fruit bunch without gafsa phosphate rock; EFB + GPR- application of empty fruit bunch and gafsa phosphate rock; PEV- phosphorus enriched vermicompost; W + AM + EFB – GPR- application of earthworm, empty fruit bunch and inoculating with arbuscular mycorrhizae but without adding gafsa phosphate rock; W + AM + EFB + GPR- application of earthworm, empty fruit bunch, gafsa phosphate rock and inoculation with arbuscular mycorrhizae

### Introduction

Phosphorus (P), a major macronutrient, can limit normal plant growth if not applied at the proper time and right amount. It must be applied as either organic or inorganic forms for optimal crop production in deficit soils low in available P (Chien et al., 2011). Phosphorus availability to plants is controlled mainly by soil pH, soluble Al, Fe, Ca, and organic matter content. Most soils in the tropical region including Malaysia are acidic, predominantly Ultisols and Oxisols. These soils have a greater ability to fix phosphate because of their characteristically high Fe and Al content, which causes considerable immobilization of any fertilizer P applied to these soils. Therefore, regular P fertilizer applications are required to maintain an adequate supply of plant-available P. In these circumstances, the use of phosphate rock (PR) as a direct-application fertilizer could be

the most cost effective method as compared to the use of water-soluble P fertilizer source. Moreover, the escalation in costs of manufacturing and distribution of soluble P fertilizers makes PR the cheapest and an important alternative source of P.

The addition of plant residues to soil acts both as a source of nutrients and also influences the availability of soil nutrients. The addition of plant residues increases the availability of soil P to plants. The incorporation of organic matter in highly weathered soils enhances the dissolution of PR. This may be due to competition between the decomposition products of the organic matter and P for soil sorption sites, the release of H<sup>+</sup> protons or organic acids and the release of phosphatase enzyme from the microorganisms (Guppy et al., 2005). In addition,

organic matter contains organic P, which can be mineralized into inorganic P forms.

The use of earthworms in manure management has increased enormously in recent years. Earthworms utilize a broad array of organic wastes, such as crop residues, animal manure, biosolids, and industrial waste (Chan and Griffiths, 1988; Hartenstein and Bisesi, 1989; Edwards, 1998). When earthworms feed, they fragment the waste substrate, hasten decomposition of the organic matter and change the physico-chemical properties of the material, leading to a composting effect, through which the unstable organic matter is oxidized and stabilized (Orozco et al., 1996; Vincelas-Akpa and Loquet, 1997). Earthworms improve P availability to plants from soils treated with low-soluble P fertilizers, such as PR (Ouédraogo et al., 2005), promote arbuscular mycorrhizal populations and increase nutrient availability to plants (Gormsen et al., 2004).

Vermicompost is a unique organic manure source due to its plentiful amounts of nutrients, growth enhancing substances, and numerous favorable microbes which include P solubilizing and cellulose decomposing organisms (Sultan, 1997). Utilization or recycling of organic wastes is made possible by vermicomposting technology. It is a better disposal mechanism compared to conventional methods like incineration which may be hazardous (Preetha et al., 2005).

Plants P uptake is enhanced considerably through inoculation of arbuscular mycorrhiza which can also improve P uptake from water-insoluble P sources, such as PR (Bolan, 1991). The arbuscular mycorrhiza fungi are known to work more efficiently at low soil solution P levels (Sharma et al., 1999). Compared to water-soluble P fertilizers, the application of PR is less likely to increase soil solution P concentration to the levels that are detrimental to arbuscular mycorrhiza colonization and effectiveness (Manjunath et al., 1989). Enhanced uptake of other essential elements in soils colonized by arbuscular mycorrhiza has also been reported (Marschner and Dell, 1994).

In this study we investigated the effectiveness of GPR with and without earthworms, empty oil palm fruit bunch, arbuscular mycorrhiza and P-enriched vermicompost on the growth and P uptake of setaria grass (*Setaria splendida* L.). In addition, we also compared the ability of ecologically different earthworms for increasing growth and P uptake by the grass.

## Results and discussion

### Effects of mixed organic-inorganic fertilizers

#### Dry matter yield

Two-way ANOVA showed significant differences ( $P < 0.05$ ) in dry matter (DM) yield of *Setaria splendida* due to interactive effect of phosphate sources and harvesting months (Table 3). Highest dry matter yield ( $6.46 \text{ g pot}^{-1}$ ) was obtained from P-enriched vermicompost treated pots at 5<sup>th</sup> month of harvest followed by  $6.20 \text{ g pot}^{-1}$  in the same treatment in the 2<sup>nd</sup> month of harvest. Inclusion of W+AM+EFB+GPR also increased dry matter of *Setaria splendida* during 5<sup>th</sup> and 6<sup>th</sup> month of harvest. Treatments without phosphate rock (-GPR) applied as sole or in combination produced lower dry matter yield.

Arancon et al. (2005) found that the growth of pepper plants treated with vermicompost is much better than plants grown in plots treated solely with inorganic fertilizers. This is attributed to the presence of plant growth hormones and humic acids in the vermicompost (Arancon et al., 2006). Humic acids are molecules that adjust several processes of

plant growth together with major and trace elements adsorption (Atiyeh et al., 2002). The characteristics of vermicompost and compost summarized in Table 1 shows that vermicompost contains higher values of humic acid ( $0.08 \text{ g g}^{-1}$ ) than compost ( $0.04 \text{ g g}^{-1}$ ). Vermicompost can influence plant growth in various means. Its elevated nutrient and humic acid contents encourage plant growth, whereas its elevated organic matter get better soil physical and biological characteristics favorable for plant development (León-Anzueto et al., 2011). Humic substances are known stimulators of plant growth (Dell'Amico et al., 1994). They amplify membrane permeability, ease the nutrient transport within roots, and favor respiration. Favorable results were obtained by several researchers due to application of vermicompost which include higher seed germination of green gram (Karmegam et al., 1999), tomato (Zaller, 2007), petunia (Arancon et al., 2008) and pine trees (Lazcano et al., 2010), vegetative growth, stimulating shoot and root development (Edwards et al., 2004), elevated plant flowering, number and biomass of the flowers produced (Arancon et al., 2008), as well as increasing fruit yield (Singh et al., 2008). Additionally, vermicompost enhanced the quality of vegetables like tomatoes (Gutiérrez-Miceli et al., 2007), spinach (Peyvast et al., 2008), lettuce (Coria-Cayupán et al., 2009), Chinese cabbage (Wang et al., 2010) and sweet corn (Lazcano et al., 2011).

Composting techniques such as vermicomposting, phospho-composting etc. are used to improve the nutrient quality potential of composts (Kanitkar, 2006). In our study, P-enriched vermicompost had higher contents of N ( $16700 \text{ mg kg}^{-1}$ ), P ( $14800 \text{ mg kg}^{-1}$ ), and K ( $164100 \text{ mg kg}^{-1}$ ) (Table 1) which might have contributed to higher dry matter yield for the setaria grass, in comparison to the other treatments.

### Phosphorus uptake

The P uptake under the influence of organic-inorganic fertilizer and harvest time showed statistically ( $P < 0.05$ ) different values for P uptake (Table 4). Among various treatments, highest P uptake ( $41.83 \text{ mg P pot}^{-1}$ ) in *Setaria splendida* grass was found with the application of P-enriched vermicompost during 1<sup>st</sup> month of harvest. However, further increase in harvest time significantly decreased P uptake in plants. The pots receiving (-GPR) treatment recorded lower P uptake in the plants. The concentration of P in soluble form in P-enriched vermicompost was high and could be easily taken up by the grass. Application of inorganic P fertilizer (GPR) in combination with empty oil palm fruit bunch as an organic matter source and beneficial organisms (earthworm and arbuscular mycorrhiza) was the most effective method of providing P. In addition, the utilization efficiency of the mixed inorganic fertilizer, organisms and organic matter was  $0.49 \text{ g dry matter mg P}^{-1}$  higher than the P utilization efficiency of the grass treated with P-enriched vermicompost ( $0.37 \text{ g dry matter mg P}^{-1}$ ). The uptake of P in P-enriched vermicompost is often significantly greater than in other treatments. This may be attributed to enhanced microbial activity in P-enriched vermicompost, which resulted in an increase in the concentration of P in the enriched vermicompost. Beneficial microbes like P solubilizers in the vermicompost induced solubilization of rock phosphate in enriched vermicompost and helped in P uptake.

### Nitrogen, potassium, calcium, and magnesium uptake

The interactive effect of mixed organic-inorganic fertilizers and harvesting months showed that N, K, Ca and Mg uptake

**Table 1.** Chemical characteristics of P-enriched vermicompost and compost.

Variable	P-enriched vermicompost	Compost
C (mg kg <sup>-1</sup> )	239600	238800
N (mg kg <sup>-1</sup> )	16700	15400
P (mg kg <sup>-1</sup> )	14800	9500
K (mg kg <sup>-1</sup> )	164100	87300
C:N ratio	-	-
pH (1:10 in water)	8.46	8.54
Humic acid (g g <sup>-1</sup> )	0.08	0.04

in setaria grass was significantly different at  $P < 0.05$  (Table 5). The N uptake was greater (53.76 and 45.99 mg N pot<sup>-1</sup>) with the application of P-enriched vermicompost treated plants during 1<sup>st</sup> and 2<sup>nd</sup> month of harvest. This treatment showed statistically non-significant differences with W+AM+EFB+GPR by recording 53.87 and 45.37 mg N pot<sup>-1</sup> during 3<sup>rd</sup> and 4<sup>th</sup> month of harvest.

Maximum K uptake (216.76 mg K pot<sup>-1</sup>) in setaria grass was noted at 4<sup>th</sup> harvest from the pots receiving W+EFB+GPR+AM, followed by, 186.12 mg K pot<sup>-1</sup> in the same treatment during 2<sup>nd</sup> month of harvest. The decreasing trend of K uptake was observed in 4<sup>th</sup> and 5<sup>th</sup> months of harvest.

In case of Ca uptake, P-enriched vermicompost had significantly higher Ca uptake (13.58 mg Ca pot<sup>-1</sup>) in plants during 2<sup>nd</sup> harvesting month followed by EFB+GPR which recorded 10.57 mg Ca pot<sup>-1</sup> of Ca uptake during 2<sup>nd</sup> month of harvest (Table 5). The lowest Ca uptake (0.51 mg Ca pot<sup>-1</sup>) was obtained from -GPR treatment during 5<sup>th</sup> month of harvest. Overall, results indicated that Ca in setaria plants rose during 2<sup>nd</sup> harvest and further harvestings decreased plant uptake.

The results for Mg uptake showed statistically significant differences for treatments and their interactive effect with harvesting months. Maximum Mg uptake (15.16 and 13.21 mg Mg pot<sup>-1</sup>) was found in P-enriched vermicompost and EFB + GPR treatments, respectively, during 4<sup>th</sup> month of crop harvest. Both of these treatments had non-significant differences in their mean values. However, minimum Mg uptake (0.26 mg Mg pot<sup>-1</sup>) was obtained from -GPR treatment during 5<sup>th</sup> month harvest (Table 5). Vermicompost holds nutrients (N, P, soluble K and Mg and exchangeable P and Ca) in forms that are readily available to plants (Edwards and Burrows, 1988; Edwards et al., 2004). Vermicompost has a large surface area which provides several micro-sites for microbial activities and for the strong retention of nutrients (Arancon et al., 2004; 2006).

The uptake of nutrients by the setaria grass increased considerably when P-enriched vermicompost was applied, which shows its superiority over other treatments for the uptake of N, P, Ca and Mg, particularly when compared to the grass treated with inorganic fertilizers and W+AM+EFB+GPR. There were differences in nutrient utilization efficiency among the different treatments (Table 6). Nitrogen utilization efficiency of EFB-GPR treated grass was 0.174 g dry matter mg<sup>-1</sup> N which was significantly higher than N utilization efficiency for the W+AM+EFB-GPR, W+AM+EFB+GPR, -GPR and +GPR treatments but was not significantly different from the P-enriched vermicompost treatments. Phosphorus utilization efficiency of W+AM+EFB+GPR treated plants was 0.491 g DM mg<sup>-1</sup> P, which was significantly higher compared to P-enriched vermicompost (0.367 DM mg<sup>-1</sup> P) and EFB+GPR (0.359 g DM mg<sup>-1</sup> P) treatments. Similarly, K utilization efficiency of EFB-GPR treated plants was 0.065 g DM mg<sup>-1</sup> K which was significantly higher than the other treatments, including the P-enriched vermicompost treatment. Calcium utilization efficiency of

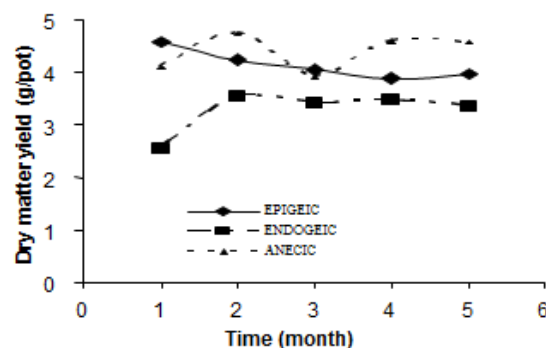


Figure 1. Effect of earthworm type on dry matter yield of *Setaria splendida*.

W+AM+EFB+GPR treated plants was 0.829 g DM mg<sup>-1</sup> Ca, which was significantly higher than Ca utilization for +GPR (0.756 g DM mg<sup>-1</sup> Ca), P-enriched vermicompost (0.741 g DM mg<sup>-1</sup> Ca), -GPR (0.712 g DM mg<sup>-1</sup> Ca), and EFB+GPR (0.691 g DM mg<sup>-1</sup> Ca) treatments. Magnesium utilization efficiency of W+AM+EFB-GPR treated grass was 1.843 g DM mg<sup>-1</sup> Mg, which was significantly higher than P-enriched vermicompost, EFB-GPR and EFB+GPR, but not significantly different from W+AM+EFB+GPR (1.740 g DM mg<sup>-1</sup> Mg) treated plants as shown in Table 6.

Among the nutrients measured, Mg was most influential in terms of increasing the dry matter of the setaria grass whilst Ca played a less significant role (Table 6). In a previous study, it was found that the nutrients uptake by grass treated with P-enriched vermicompost was generally higher (Sabrina et al., 2011). However, the nutrient utilization efficiency results showed that nutrients uptake by plants treated P-enriched vermicompost was low. This might be due to utilization of nutrients for root development instead of shoot production.

Root growth in soil is influenced by soil nutrients supply and availability of sufficient soil water. Vegh (1991) reported that an increase in soil nutrient supply results in higher root elongation, which leads to changes in nutrient availability to plants (Barber, 1984). The root volume of the grass treated with P-enriched vermicompost (163 cm<sup>3</sup>) was significantly higher than root volume of the plants treated with -GPR (23 cm<sup>3</sup>), +GPR (28 cm<sup>3</sup>), and EFB -GPR (67 cm<sup>3</sup>) as shown in Table 7. A well developed root system expectedly produces many clusters of grass.

#### Available and residual P

The maximum available P (26.80 mg P kg<sup>-1</sup>) resulted from the W+AM+EFB+GPR treatment, which was significantly higher as compared to available P for the -GPR, EFB -GPR, and W+AM+EFB-GPR treatments as shown in Table 8. However, the amount of available P for the W+AM+EFB+GPR treatment was not significantly different

**Table 2.** Organic and inorganic treatments applied in the study.

Treatments	Description
- GPR	Without adding P fertilizer, soil only
+ GPR	Adding gafsa phosphate rock only
EFB – GPR	Adding empty fruit bunch without GPR
EFB + GPR	Adding empty fruit bunch and gafsa phosphate rock
PEV	P-enriched vermicompost
W + AM + EFB – GPR	Adding earthworm, empty fruit bunch and inoculating with AM but without adding gafsa phosphate rock
W + AM + EFB + GPR	Adding earthworm, empty fruit bunch, gafsa phosphate rock and inoculating with AM

Abbreviations: AM= arbuscular mycorrhizae; EFB= oil palm empty fruit bunch; GPR= gafsa phosphate rock; PEV = P-enriched vermicompost; W= earthworm.

**Table 3.** Effect of combination of phosphate rock, oil palm empty fruit bunch, earthworm, arbuscular mycorrhizae and P-enriched vermicompost on dry matter yield of *Setaria splendida*.

Treatment	Dry matter yield (g pot <sup>-1</sup> ) at month				
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>
- GPR	3.05 f-k	2.67 g-k	2.08 ijk	1.74 jk	1.67 k
+ GPR	4.27 d-j	3.24 f-j	2.66 h-k	2.78 g-k	3.30 f-i
EFB – GPR	3.05 f-k	3.08 f-k	3.38 e-i	3.31 f-i	3.74 e-h
EFB + GPR	4.16 d-h	3.68 e-h	3.48 e-i	3.82 e-h	4.20 d-h
PEV	5.76 abc	6.20 ab	5.75 abc	4.90 b-e	6.46 a
W+AM+EFB-GPR	3.39 e-i	4.02 d-h	3.80 e-h	4.19 d-h	4.42 c-f
W+AM+EFB+GPR	4.02 d-h	4.39 c-f	5.48 a-d	6.19 ab	5.94 ab
SE	0.4506				

Abbreviations: AM= arbuscular mycorrhizae; EFB= oil palm empty fruit bunch; GPR= gafsa phosphate rock; PEV = P-enriched vermicompost; W= earthworm. Means with same letters are not significantly different at P= 0.05 level (Duncan's multiple range test).

from the P-enriched vermicompost (24.28 mg P kg<sup>-1</sup>) treatment. Even though, P uptake by grass, treated with P-enriched vermicompost was higher than other treatments with the exception of W+AM+EFB+GPR treatment. The amount of plant available P in soil at harvest was 24.28 mg P kg<sup>-1</sup> which was higher than control (-GPR), inorganic fertilizer only (+GPR), and W+AM+EFB-GPR treatments.

Some researchers attributed larger P release from vermicompost to enhance microbial activity (Lee, 1985; Scheu, 1987). However, others attributed it to the increase of phosphatase activity (Lavelle and Martin, 1992). Our results clearly suggest that integrating earthworms, arbuscular mycorrhiza and phosphate rock with decomposing empty oil palm fruit bunch may enhance P availability to plants and contribute to solving the problem of P availability in P deficient tropical soils.

Residual P measured based on changes in 0.5M NaOH extractable-P ( $\Delta P$ ) of soil applied with W+AM+EFB+GPR was 129.67 mg P kg<sup>-1</sup>, which was significantly higher compared to  $\Delta P$  of P-enriched vermicompost (45.67 mg P kg<sup>-1</sup>) (Table 9). The amounts of  $\Delta P$  in soil treated with W+AM+EFB+GPR were twice the amount of  $\Delta P$  from other treatments. The  $\Delta P$  of P-enriched vermicompost was not significantly different from  $\Delta P$  of EFB+GPR (63.33 mg P kg<sup>-1</sup>) and +GPR (inorganic fertilizer only) (49.67 mg P kg<sup>-1</sup>) treatments. Generally, more a plant absorbs nutrients from soil the less nutrients that are left in the soil. However, in this study the amount  $\Delta P$  in soil was not significantly related to  $\Delta P_s$  and  $\Delta P_b$ .

#### **Effect of earthworm types on growth and P uptake by *Setaria splendida***

The results of this study indicated that neither dry matter yield nor root volume of the *Setaria splendida* grass was significantly affected by the different types of earthworms.

The trends of the dry matter yield for the different types of earthworms used in this study are shown in Fig 1. There was no clear pattern in dry matter yield for the anecic worm's treatment. Grass dry matter yield for the epigeic worms treatment decreased with harvest time, while dry matter yield for the endogeic worm's treatment increased initially and remained constant after the 2<sup>nd</sup> harvest. The effects of different types of earthworms on the N, P, K, Ca and Mg uptake were not significantly different (data not shown). Earthworm type did not affect total N, P, K, Ca, Mg, available and residual P and pH significantly at the end of the experiment (data not shown). There are several reasons why different earthworm types did not show a significant effect on plant growth and nutrients uptake. The first reason might be unfavorable conditions for earthworm activities. Under unfavorable conditions such as dry or hot temperatures, earthworm activity decreases and they become inactive (Edwards and Bohlen, 1996). This is supported by the evidence of high mortality of earthworms during the research period, and no fecundity. The second reason might be that the population of earthworms was not enough to have a significant effect on plant growth. The contributions of earthworms to plant growth in some agro-ecosystems are significant when their populations are sufficiently high (> 300 individuals m<sup>-2</sup>) and when favorable weather conditions exist (Eriksen-Hamel and Whalen, 2007). They assumed that the 0-15 cm depth soil with a high earthworm population (300 individuals m<sup>-2</sup>) could contain 14 kg N ha<sup>-1</sup> more compared to soils with low earthworm population (30 individuals m<sup>-2</sup>). Most of this mineral N was generated by the activities of the endogeic *Aporrectodea caliginosa*. The results showed that in comparison to *E. fetida* and *A. caliginosa*, the endogeic *P. corethrurus* was the most tolerant to the high temperature conditions that existed in the glasshouse.

**Table 4.** Effect of combination of phosphate rock, oil palm empty fruit bunch, earthworm, arbuscular mycorrhizae and P-enriched vermicompost on P uptake of *Setaria splendida*.

Treatment	P uptake (mg P pot <sup>-1</sup> ) at month				
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>
- GPR	17.30 de	2.05 k	1.43 k	3.15 jk	1.65 k
+ GPR	22.43 bc	3.24 jk	1.92 k	6.59 ijk	3.20 jk
EFB -GPR	14.47 efg	5.61 ijk	3.59 jk	12.35 fgh	1.72 k
EFB + GPR	25.47 b	8.12 hij	3.92 jk	13.18 efg	3.11 jk
PEV	41.83 a	9.79 ghi	6.33 ijk	17.38 de	4.06 jk
W+AM+EFB-GPR	19.94 cd	2.05 k	3.06 jk	16.09 def	3.01 jk
W+AM+EFB+GPR	16.37 def	2.39 k	4.58 jk	23.58 bc	5.65 ijk
SE	1.531				

Abbreviations: AM= arbuscular mycorrhizae; EFB= oil palm empty fruit bunch; GPR= gafsa phosphate rock; PEV = P-enriched vermicompost; W= earthworm. Means with the same letters are not significantly different at P= 0.05 level (Duncan's multiple range test).

**Table 5.** Effect of combination of phosphate rock, oil palm empty fruit bunch, earthworm, arbuscular mycorrhizae and P-enriched vermicompost on nutrient uptake of *Setaria splendida*.

Treatment	Nutrients uptake (mg pot <sup>-1</sup> ) at month				
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>
	Nitrogen				
- GPR	27.07 c-f	23.91 def	35.70 b-e	22.34 def	7.48 gh
+ GPR	36.35 bcd	29.26 c-f	36.76 bcd	24.48 c-f	3.01h
EFB - GPR	18.06 fg	26.43 c-f	26.25c-f	21.72 ef	2.88 h
EFB + GPR	29.42c-f	28.44c-f	29.77 c-f	26.64c-f	2.67 h
PEV	45.99 ab	53.76 a	38.76bc	33.36b-e	5.43 h
W+AM+EFB-GPR	30.94c-f	29.55 c-f	35.92b-e	34.83b-e	3.82 h
W+AM+EFB+GPR	34.83 b-e	31.37c-f	53.87 a	45.37 ab	3.57 h
SE	4.170				
	Potassium				
- GPR	91.69 f-k	76.46 h-l	41.69 lmn	20.25n	36.26 lmn
+ GPR	110.61 e-i	91.07 f-k	60.73 j-n	36.09 lmn	92.01 f-k
EFB - GPR	68.73i-n	60.00 j-n	47.14 k-n	19.90 n	57.46 k-n
EFB + GPR	121.71 d-h	70.56 i-m	49.10 k-n	24.70 mn	64.87 i-n
PEV	174.54 abc	154.85 b-e	108.79 e-j	50.26 k-n	125.12 d-h
W+AM+EFB-GPR	110.16 e-i	160.14 bcd	132.41 c-g	84.91 g-l	107.97 e-j
W+AM+EFB+GPR	136.88 c-f	186.12 ab	216.76 a	119.17 d-h	136.32 c-f
SE	14.46				
	Calcium				
- GPR	4.07 d-l	7.57 b-g	2.13 i-l	2.01 i-l	0.51 l
+ GPR	5.98 c-i	7.98 b-e	2.55 h-l	3.90 e-l	1.08 kl
EFB - GPR	4.13 d-l	7.84 b-f	3.61 f-l	4.00 e-l	1.39 kl
EFB + GPR	5.68 c-j	10.57 ab	3.75 e-l	6.43 b-h	1.55 jkl
PEV	8.29 bcd	13.58 a	6.90 b-g	8.91 bc	2.64 h-l
W+AM+EFB-GPR	4.51 d-l	9.10 bc	3.36 g-l	5.72 c-j	1.48 jkl
W+AM+EFB+GPR	5.17 c-k	7.67 b-f	5.73 c-j	8.92 bc	2.05 i-l
SE	1.233				
	Magnesium				
- GPR	1.37 hi	2.27 ghi	0.89 hi	2.63 e-i	0.26 i
+ GPR	1.49 hi	2.94 d-h	1.09 hi	4.73 cde	0.51 hi
EFB - GPR	1.34 hi	4.66 c-f	2.12 ghi	9.62 b	0.79 hi
EFB + GPR	2.02 ghi	4.94 cd	2.24 ghi	13.21 a	0.97 hi
PEV	2.43 f-i	4.95 cd	3.91 c-g	15.16 a	1.61 ghi
W+AM+EFB-GPR	1.09 hi	2.08 ghi	1.19 hi	5.87 c	0.67 hi
W+AM+EFB+GPR	1.30 hi	2.23 ghi	1.73 ghi	8.80 b	0.97 hi
SE	0.7127				

Abbreviations: AM= arbuscular mycorrhizae; EFB= oil palm empty fruit bunch; GPR= gafsa phosphate rock; PEV = P-enriched vermicompost; W= earthworm. Means with the same letters are not significantly different at P=0.05 level (Duncan's multiple range test).

## Materials and methods

### Comparing organic and inorganic fertilizers preparation of compost, P-enriched compost and their characteristics

Samples of empty oil palm fruit bunches obtained from Experimental Farm of Universiti Putra Malaysia were air dried at ambient temperature (28-30°C) for 2 days to reduce the moisture content, ground to pass through a 2 mm sieve size and then used in the preparation of compost and P-enriched vermicompost materials. The GPR (from Tunisia, 280000 mg kg<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>) was added to the 5 kg of empty oil palm fruit bunches at the equal rate of 150 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> in the field, mixed and then incubated for 30 days in plastic cages (60 x 40 x 40 cm<sup>3</sup>) covered with plastic lids for aeration, in three replicates. After 30 days, adult *Pontoscolex corethrurus* earthworms, an exotic dominant earthworm found in oil palm plantation soils (Sabrina et al., 2009), were added to the mixtures at the ratio of 1:10 (population/media). Fresh cattle dung collected from the Animal Science Department's Experimental Farm, Faculty of Agriculture, UPM was air-dried (20% w/w), crushed and sieved (<2 mm size) to remove indigenous earthworms or cocoons before application to the media (0.15 mg/worm/day). The P-enriched vermicompost was harvested after 90 days. Conventional compost was also prepared as described above, but without addition of earthworms. The characteristics of the materials are shown in Table 1.

### Arbuscular mycorrhizal inoculum

The AM inoculum containing *Glomus mosseae* (Gerdemann and Nicholson, 1963) Gerdemann & Trappe UK 118 consisting spores, external hyphae, and infected root fragments was obtained from the International Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM). Whole inoculum was propagated on Sorghum (*Sorghum bicolor* L.) media in glasshouse pot cultures (Feldmann and Idczak, 1991) for 4 months. In order to identify the infective vesicular arbuscular mycorrhizal propagules of fungi, the most probable number of infective propagules method was carried out (Sieverding, 1991), giving 88.32 infective propagules 100 g<sup>-1</sup> inoculum. The spore number was 560 spores 100 g<sup>-1</sup> inoculum, extracted using the wet sieving method (Gerdemann and Nicolson, 1963) and 20 g of this mycorrhizal inoculum was thoroughly mixed in the pots.

### Location and organic-inorganic fertilizer treatments

The experiment was laid out using a randomized complete block design with three replications at the Experimental Farm Field No. 2, Glasshouse Complex, Faculty of Agriculture, Universiti Putra Malaysia. The treatments are shown in Table 2. *Setaria grass* (*Setaria splendida* L.) was obtained from the Grass Museum, Animal Science Department, UPM. Five grass cuttings (about 15 cm long) were planted in each pot (16 x 19 cm wide and height). Nitrogen (urea) and K (KCl) was applied in solution form to supply nutrients equivalent to 100 kg N ha<sup>-1</sup> and 60 kg K ha<sup>-1</sup>, respectively. This was done at 2 monthly intervals.

The grass was watered twice a day in the morning (08:00 h) and in the afternoon (18:00 h) for 5 minutes using a sprinkler irrigation system installed in the glasshouse to get the surface of the pot wet. Dry matter yield, N, P, K, Ca, and Mg uptake by *Setaria splendida* were determined at one month intervals for five months. Roots were washed thoroughly with tap

water and air dried and was placed into a 2000 mL cylinder containing 1000 mL water, the volume of water increased was indicated as a volume of root.

### Effect of earthworm type on growth and p uptake by *Setaria grass*

#### Plant growth media and earthworms

Air-dried, sieved (2 mm) Bungor (Typic Paleudult) soil with pH of 4.64, CEC 5.9 cmol (+) kg<sup>-1</sup>, fine sandy clay texture, fine structure, 9700 mg kg<sup>-1</sup> C and 700 mg kg<sup>-1</sup> N was used as the plant growth media in this study.

Three types of earthworms: epigeic (*Eisenia fetida*) obtained from commercial vermicompost, endogeic (*Pontoscolex corethrurus*) from oil palm plantation areas and anecic (*Amyntas gracilis*) from a sandy soil under an agroforestry planting system (tree, cover crop and grass) in Kelantan, Malaysia. All earthworms had different ecological functions based on Bouche's classification. Five adult earthworms were added into each pot at planting time. The fresh empty oil palm fruit bunch was added at the rate equal to the rate of 37.5 ton ha<sup>-1</sup> of the field in each pot. Nitrogen and K fertilizer application and irrigation practice were similar to that described earlier.

#### Treatments, experimental design and location

The experiment was conducted using a randomized complete block design with three treatments (*Eisenia fetida*, *Pontoscolex corethrurus*, and *Amyntas gracilis*) and three replications at the glasshouse complex, Universiti Putra Malaysia. Dry matter yield, N, P, K, Ca, and Mg uptake by *Setaria splendida* were measured at monthly intervals for five months.

#### Soil analysis

For both studies, soil sampling was performed at the end of the experiment. Soil pH was measured with a glass electrode pH meter (PHM210, Metrolab) in a 1:2.5 (soil/water ratio) suspension. Organic carbon was determined by the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> wet combustion method followed by titration (Walkley and Black 1934). The micro-Kjeldahl method was used to determine total nitrogen (Bremner, 1960). The extractable P, K, Ca, and Mg were measured using 15.8M HNO<sub>3</sub> and 12.1M HCl (1:3 ratio) (Mehlich, 1953). The dissolution of GPR was measured by changes in 0.5M NaOH/prewash with 1M NaCl ( $\Delta$ P) of GPR treated soil compared to the control (Mackay et al., 1986; Hanafi and Syers, 1994). Plant available P in soil was determined using 0.5M NaHCO<sub>3</sub> pH 8.5 at 1:20 (soil/reagent ratio) based on the Olsen method (Olsen et al., 1954). The exchangeable Na, K, Ca, and Mg were measured after leaching the sample with neutral 1M ammonium acetate (Blakemore et al., 1987). The concentration of N, P and K in solution were determined using an auto-analyzer (Technicon Ltd.), and that for Ca and Mg were measured using atomic absorption spectrometer (Perkin-Elmer, 5100 pc, Perkin Elmer) in the presence of 1000 mg SrCl<sub>2</sub> L<sup>-1</sup>, as an ionization suppressant.

#### Plant analysis

The plant samples obtained from both studies were digested using 5 mL concentrated 17.8M H<sub>2</sub>SO<sub>4</sub> and further oxidized with 9.79M H<sub>2</sub>O<sub>2</sub> (30% reagent grade) using the method of Thomas et al. (1967). The digested sample was mixed

**Table 6.** Effect of combination of phosphate rock, oil palm empty fruit bunch, earthworm, arbuscular mycorrhizae and P-enriched vermicompost on nutrients utilization efficiency of *Setaria splendida*.

Treatment	Nutrients utilization efficiency (g DM mg <sup>-1</sup> )				
	N	P	K	Ca	Mg
- GPR	0.099 c	0.452 a	0.043 c	0.712 b	1.622 a
+ GPR	0.125 d	0.440 a	0.042 c	0.756 b	1.515 a
EFB – GPR	0.174 a	0.441 a	0.065 a	0.790 ab	0.918 b
EFB + GPR	0.166 ab	0.359 a	0.059 b	0.691 b	0.828 b
PEV	0.164 ab	0.367 a	0.047 c	0.741 b	1.050 b
W+AM+EFB-GPR	0.147 c	0.453 a	0.033 d	0.827 ab	1.843 a
W+AM+EFB+GPR	0.153 cd	0.491 a	0.033 d	0.829 a	1.740 a

Abbreviations: AM= arbuscular mycorrhizae; EFB= oil palm empty fruit bunch; GPR= gafsa phosphate rock; PEV = P enriched vermicompost; W= earthworm. Means with the same letters in each column are not significantly different at P=0.05 level (Duncan's multiple range test).

**Table 7.** Effect of combination of phosphate rock, oil palm empty fruit bunch, earthworm, arbuscular mycorrhizae and P-enriched vermicompost on root volume of *Setaria splendida*.

Treatment	Root volume (cm <sup>3</sup> )
- GPR	23 c
+ GPR	28 c
EFB – GPR	67 bc
EFB + GPR	157 a
PEV	163 a
W+AM+EFB-GPR	107 ab
W+AM+EFB+GPR	150 a

Abbreviations: AM= arbuscular mycorrhizae; EFB= oil palm empty fruit bunch; GPR= gafsa phosphate rock; PEV = P enriched vermicompost; W= earthworm. Means with the same letters are not significantly different at P=0.05 level (Duncan's multiple range test).

**Table 8.** Effect of combination of phosphate rock, oil palm empty fruit bunch, earthworm, arbuscular mycorrhizae and P-enriched vermicompost on plant available P in soil.

Treatment	Available P (mg kg <sup>-1</sup> )
- GPR	21.33 b
+ GPR	20.87 b
EFB – GPR	21.73 b
EFB + GPR	22.28 ab
PEV	24.28 ab
W+AM+EFB-GPR	21.33 b
W+AM+EFB+GPR	26.80 a

Abbreviations: AM= arbuscular mycorrhizae; EFB= oil palm empty fruit bunch; GPR= gafsa phosphate rock; PEV = P enriched vermicompost; W= earthworm. Means with the same letters in each column are not significantly different at P=0.05 level (Duncan's multiple range test).

**Table 9.** Effect of combination of phosphate rock, oil palm empty fruit bunch, earthworm, arbuscular mycorrhizae and P-enriched vermicompost on changes in 0.5 M NaOH extractable P ( $\Delta P$ ), P uptake ( $\Delta P_s$ ), and plant available P ( $\Delta P_b$ ) in soil.

Treatment	$\Delta P$	$\Delta P_s$		$\Delta P_b$
		mg P kg <sup>-1</sup>		
GPR	49.67 b	11.6 b	1.8 a	
EFB+ GPR	63.33 b	28.2 b	3.2 a	
PEV	45.67 b	57.8 a	5.2 a	
W+AM+EFB+GPR	129.67 a	26.9 b	4.4 a	

Abbreviations: AM= arbuscular mycorrhizae; EFB= oil palm empty fruit bunch; GPR= gafsa phosphate rock; PEV = P-enriched vermicompost; W= earthworm. Means with the same letters in each column are not significantly different at P=0.05 level (Duncan's multiple range test).

thoroughly with distilled water and made up to 250 mL. The solution was allowed to stand overnight to permit the silica to settle. The solution was subsequently analyzed for N, P, K, Ca, and Mg. Nitrogen, P, and K were measured using Auto-Analyzer (Technicon Ltd.), while Ca and Mg were measured with an Atomic Absorption Spectrophotometer (Perkin-Elmer, 5100 pc, Perkin Elmer) after addition of 1000  $\mu\text{g L}^{-1}$  LiCl<sub>2</sub> to eliminate the interferences in Ca determination.

### Statistical Analysis

Experiments were established in a randomized complete block design with three replications. All the data obtained for the various treatments were statistically analyzed using two-way analysis of variance with the exception of data on the effect of earthworm types on growth and P uptake by *Setaria*

*splendida*, which were analyzed using one-way analysis of variance. Means separation was done using Duncan's Multiple Range Test (DMRT) at 5% level of probability. All statistical analyses were performed using SAS software program (version 8e) (SAS, 1999).

### Conclusion

Oil palm waste disposal is a severe problem in many countries in the world. Vermicomposting can be a substitute technology for the management of oil palm waste especially empty fruit bunch and frond. Of the treatments studied, P-enriched vermicompost provided best results for dry matter yield of setaria grass, nutrient uptake and P availability. Therefore, we recommend application of P-enriched vermicompost prepared from oil palm waste using

*Pontoscolex corethrurus* earthworms on high P-fixation soils to minimize environmental impacts and to enhance P availability. Further research efforts should be intended for multicrop testing of P-enriched vermicompost on different soils with varying P-fixing capacity.

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