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AVAILABILITY OF MAJOR NUTRIENTS IN AN ULTISOL IN SOUTHERN SRI LANKA TREATED WITH SELECTED ORGANIC AMENDMENTS INTERMIXED WITH HYDROPHOBIC ORGANIC MANURE

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ABSTRACT

The addition of a small amount of hydrophobic organic manure blended with commonly used organic amendments is found to induce slight water repellency, improve aggregate stability, and reduce organic matter decomposition without detrimentally affecting hydrophysical properties of tropical Ultisols. The present study was conducted to explore any negative impacts of this strategy on the availability of soil nutrients. A field experiment was conducted using Cattle (CM) and Goat manure (GM), and *Gliricidia sepium* (GL) leaves amended with soils in 5% rate. Separate plots were prepared by mixing 2% of *Casuarina equisetifolia* (CE) leaves which shows hydrophobic characteristics, with 5% of each manure (CM, GM, GL) separately to induce slight water repellency. Bush bean was established in the field and the major nutrients (N, P, and K) were tested at initial, vegetative, flowering, and harvesting stages. Data were statistically analyzed using SAS 9.1 statistical software. The results revealed that the highest and the least N contents were at the initial (40-130 mg/kg) and vegetative stages (8-20 mg/kg), respectively. The P contents were high at initial and vegetative stages (1-55 mg/kg) compared with other stages (<6 mg/kg). The highest and the least K contents were at flowering (370-600 mg/kg) and vegetative (40-210 mg/kg) stages, respectively. The nutrient contents of the samples with intermixed 2% CE were statistically the same or higher than those without 2% CE in most of the growth stages. The yields of the plots with GL and GM with 2% CE were significantly higher than those with only 5% GL and GM. The harvest index was significantly high in the treatment with 5% GM intermixed 2% CE. It can be concluded that the addition of extra 2% CE would not be unfavorable on nutrient availability and it would result in higher yields and harvest indices.

Key words: Availability, Dynamics, Hydrophobic, Manure, Nutrients, Organic

INTRODUCTION

Continuous application of chemical fertilizers is reported to deteriorate soil properties thereby demanding frequent applications of both major and minor nutrient elements to achieve an economical crop production (Javier and Tabien, 2003). In such situations, the next best alternative is organic amendments usage. Organic amendments are capable of improving soil physical, chemical, and biological properties to promote crop growth and to increase the yield (Makinde and Ayeni, 2013). When considering the effects caused by organic amendments in soil, the water repellent effects are less discussed. Water repellency reduces the spontaneous wetting of soil when water is placed on the surface (Letey *et al*., 2000), and it is caused by either the intermixing or coating of mineral soil particles with hydrophobic organic substances (De Bano, 1981). Generally, readily wettable soils become water repellent at least to some degree with the application of organic amendments. Sri Lankan red-yellow podzolic soils amended with cattle and goat manures are found to show increased persistence of water repellency (Leelamanie, 2014).

Formation of hydrophobic films on soil particles is responsible for the reduction of exces-

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sive microbial activity by limiting moisture availability. This minimizes the microbial utilization of soil nutrients eventually enhancing soil nutrient levels especially in topsoil (Elbl *et al*., 2014). Plant available N is found to be increased with the decreased microbial activity due to suppressed microbial mediated N losing processes such as immobilization, nitrification, and denitrification (Ketterings *et al*., 2003). Low moisture conditions generated within hydrophobic soil layers which inhibit water infiltration and soil wetting can limit water dependent cycling processes of nutrients especially, the immobilization and leaching (Eriksen *et al*., 1998; Fernelius *et al*., 2015). Generally, amending organic matter (OM) is considered as an effective mean of increasing the cation exchange capacity (CEC) of the soil (Peinemann *et al*., 2000). Hydrophobic soils contain high amount of OM due to physical stabilization because, the low water solubility of organic compounds in hydrophobic domains of humified OM enhances their physical protection against decomposition (Davidson and Janssens, 2006). The increased exchange sites created by the elevated OM content might result in increased nutrient retention by fixation of cations on exchange sites and increasing the concentration of them on the absorption sites (Buscher *et al*., 1990). Therefore, hydrophobic conditions can be expected to retain prominent levels of nutrients for longer periods of time than hydrophilic soils.

However, some contradictory findings reveal that the hydrophobic films on the soil particles support leaching of nutrients under high extent of water repellency (Elbl *et al*., 2014), due to formation of preferential flow paths, hindering their availability to the plants (Nguyen *et al*., 1999). The bio resistance of some hydrophobic organic materials makes them poor in supplying nutrients through mineralization (Tiessen and Stewart, 1983). Therefore, hydrophobic organic amendments might have negative impacts on the available nutrients as well.

With the development of organic farming as a concept of sustainable agriculture, use of organic amendments has been spreading extensively among global farming communities regardless of the tropical or temperate boundaries of the climate (Aveyard, 1988; Hole *et al*., 2005). Sri Lanka is a country which the organic manure usage is prioritized by most of the local farmers under organic farming concept. However, Sri Lankan soils are reported to be low in fertility due to extreme OM decomposition accelerated by the tropical environmental conditions (Leelamanie, 2014). Even though the recommendation of organic manure application rate is 10 t/ha, no subsequent improvement in soil OM level can be observed. Therefore, the addition of nearly 40 t/ha of organic manure is found to be essential to attain considerable levels of aggregate stability as well as increased OM status in Sri Lankan red-yellow podzolic soils (Leelamanie *et al*., 2013).

Even though the usage of organic amendments is important as a source of available nutrients, hydrophobic effects of them on nutrients availability have not been extensively studied. By inducing slight water repellency with the mixing of a small amount of hydrophobic organic manure [2% *Casuarina equisetifolia* leaves (CE)] with normal organic amendments application rates, it is possible to improve the aggregate stability in red-yellow podzolic soils in low country wet-zone agro ecological region of Sri Lanka (Leelamanie *et al*., 2013). This slight water repellent condition is not harmful on soil hydrophysical properties (Liyanage and Leelamanie, 2016). Slightly induced water repellency is found to reduce OM depletion subsequently resulting OM accumulation in the soil as well (Leelamanie and Liyanage, 2016). Aggregate stability, hydrophysical properties, and OM accumulation have pronounced effects on nutrient dynamics. Therefore, the objectives of the present study were to examine the nitrogen (N), phosphorous (P), and potassium (K) availability of an Ultisol which belongs to the red-yellow podzolic great soil group, amended with different organic amendments, and to explore the impacts of the mixing of an extra amount of hydrophobic organic manure (2% CE) on major soil nutrients availability.

MATERIALS AND METHOD

The nutrients availability of a soil incorporated with organic amendments was tested using a bush bean (Variety; Thai Bush, *Phaseolus vulgaris* L.) cultivation. An appropriate allotment (7x15 m^2) was selected at the farm of the Faculty of Agriculture, University of Ruhuna in Mapalana area. The soil in the experimental site belongs to red-yellow podzolic great soil group which falls under Rhodudults according to the USDA Soil Taxonomy (Soil survey staff, 2014). The area is in the low country wet zone (WL2) agro-ecological region (Panabokke, 1996). The mean air temperature of the area is 28°C and it is fairly uniform throughout the year. The average relative humidity is nearly 75% and annual pan evaporation is 1560 mm with mean annual rainfall around 2354 mm (Weerasinghe, 1989).

Cattle manure (CM) and goat manure (GM) were used as the animal manure types, and *Gliricidia sepium* (GL) leaves were used as the green manure. CE was used as a hydrophobic organic additive to induce water repellency of the manure samples. Table 2 refers the initial nutrient contents of the used organic amendments. The CM, GM, and GL were crushed to reduce the particle size to assure uniform mixing with the soil. The soil was treated with CM, GM, and GL at a rate of 5% to reach a level of 40 t/ha where non-treated soil represented the control. The amount of each type of organic manure which was required to attain the application rate was calculated using the average bulk density (1.3 g cm^{-3}) of the field considering a soil depth of 10 cm. Separate plots were prepared by mixing soils with 5% of CM, GM, and GL intermixed with 2% of CE to induce slight water repellency. An inorganic fertilizer application was used as the chemical treatment according to the recommendation of the Department of Agriculture, Sri Lanka [Basal dressing: N (87.4 kg/ha), P_2O_5 (128.25 kg/ha), K_2O (75 kg/ha), Top dressing: N (57.5 kg/ha)] (http://www.agridept.gov.lk). The organic amendments incorporation was done two weeks be-

Table 1: Basic properties of the field soil (means ± standard deviation)

Property	Value
Bulk density (g cm^{-3})	1.3±0.1
Particle density (g cm^{-3})	2.7±0.1
Porosity (%)	52.6±3.0
Sand (%)	86±0.5
Silt (%)	10.8±0.3
Clay (%)	3.2±0.3
Organic Matter (g/100 g)	4.3±0.05
pH	6.5±0.71
Electrical Conductivity (mS m^{-1})	0.03±0.01
Available Nitrogen (mg/kg)	41.2±1.7
Available Phosphorous (mg/kg)	1.3±0.3
Available Potassium (mg/kg)	4.1±1.3

Table 2: Available nutrients, organic matter contents, and the C/N ratio of the organic materials used in the study (means ± standard deviation)

Organic Material	Available Nutrient Contents (mg/kg)			Organic Matter Content (g/100 g)	C/N Ratio
	Nitrogen	Phosphorous	Potassium		
Gliricidia (GL)	488.9±0.1	184.9±1.7	16121.9±0.01	89.4±0.8	15.9±0.2
Casuarina (CE)	120.8±2.7	202.3±2.6	2188.3±11.2	93±0.4	53.6±0.6
Cattle manure (CM)	136.5±0.6	167.7±4.9	8151.6±19.5	79.1±0.3	28.4±2.4
Goat manure (GM)	218.45±14.9	206.3±0.6	11043.04±14.5	80.8±0.6	17.9±0.3

fore planting as recommended by Javier and Tabien, 2003 and Viharnaa and Seran, 2012. The planting beds were arranged according to the randomized complete block design (RCBD) with three blocks across the slope, each containing eight planting plots. The treatments were randomly allocated within the blocks.

The seeds used in the field establishment were commercially available bush bean. The germination induced seeds were placed in the planting holes within 1 cm depths with 30 cm spacing. For the nutrients analysis, moist soil samples were collected at a depth of 10 cm. Three soil samples were collected from each plot/replicate and mixed to obtain composite samples. The field soil samples were frozen to eliminate further microbial mineralization for the determination of available nitrogen until they were analyzed immediately. The air dried (<2 mm) soil samples were prepared for the P and K determination. The soil was analyzed for initially available N, P, and K at 2 weeks after manure incorporation, and thereafter 5 weeks after planting, 8 weeks after planting/at onset of flowering, and 12 weeks after planting/at the harvesting. The yield of the bush bean was measured at the harvesting stage as well.

The ammonium N and nitrate N contents of the samples were determined using a UV visible spectrophotometer (Shimadzu/ UV-160) (Yu *et al*., 1994; Amin and Flowers, 2004; Cataldo *et al*., 1975). A KCl extract was used where the Berthelot reaction of ammonium was used for the determination of ammonium N and the sodium salicylate reaction under a highly acidic condition was used for the determination of nitrate N (Ikerra *et al*., 1999; Iwagbue *et al*., 2011). Exchangeable K content was determined using a 1M ammonium acetate extract fed to a flame photometer (Sherwood/Model 360). The borax extractable phosphorus was determined by feeding a borax and sulphuric acid extractant (pH 1.5) (Manipura *et al*., 1969) to a UV visible spectrophotometer (Murphy and Riley, 1962).

The suitability of suggested organic amendments application rates in crop production was determined using the fresh weight of the pods and the harvest index as suggested by Donald and Hamblin (1976). Harvest index was calculated using the following equation.

$$\text{Harvest Index} = \frac{\text{Dry weight of the pods}}{\text{Dry weight of the total plant biomass}}$$

The data were statistically analyzed with analysis of variance (ANOVA), at 0.05 probability level using Microsoft Excel 2010 data analysis tool pack, and SAS 9.1 statistical software. The standard SAS coding for a RCBD experiment was used in the analysis. Mean separation was done using Duncan grouping. Priority was given to find out the statistical significance of the measured parameters between the samples with and without intermixed 2% CE.

RESULTS AND DISCUSSION

The highest N content of all the treatments was observed at the initial stage (40-130 mg/kg) (Table 3). All GL, CM, and GM are reported as economical manures rich in nutrients trapped in organic molecules, especially N and P (Gana, 2011; Guo and Li, 2012; Okon and Udofot, 2011). With the mineralization, these manure types are found to release nutrients in available form. The C/N ratios of the GL, CM, and GM were 15.9, 28.4, and 17.9 respectively. Other research findings suggest that crop residues with C/N ratio below 30 result in net N mineralization, while residues with C/N ratio greater than 30 favor immobilization (Mohanty *et al*., 2013). Hence the low C/N ratio (<30) of the organic amendments might have caused immediate mineralization of N rich low molecular organic compounds resulting in increased available N content at the start. Azeez and Averbeke, (2010) observed a similar situation where the N mineralization showed a polynomial relationship with the time having an initial rapid available N release.

All the organic manure amended samples and the control showed the least available N contents (8-20 mg/kg) at the vegetative stage except that of the chemical fertilizer treatment. This can be credited to the highest N demand of the crop during the vegetative growth, especially for early seedling vigor and crop biomass accumulation (Sinclair and Horie, 1987). In the chemical treatment, the instant and adequate supply of N might have not caused a considerable reduction at the vegetative stage.
The N content increased from the flowering stage (13-20 mg/kg) to the harvesting stage (20-30 mg/kg) which might be due to lesser uptake of N after the vegetative growth. In organic manure amended samples the nutrients provision through the gradual OM decomposition over time may have let to accumulate N in the soil under low plant utilization at the flowering and harvesting stages (Suge *et al.*, 2011). In the control sample and the chemical fertilizer treatment, a gradual nutrients supply through the decomposition of organic substrates cannot be expected throughout the plant growth. Therefore, the increased N content at the flowering and harvesting stages of these might be due to decomposition of dead microorganisms due to low carbon availability (Luce *et al.*, 2011).

The N content of all the samples containing 5% organic manure with intermixed 2% CE was to be higher than those with only 5% organic manure where the differences were statistically significant at 0.05 probability level at flowering and harvesting stages of CM and

Table 3: Nitrogen (N), Phosphorous (P), and Potassium (K) contents of the samples measured at initial[1], vegetative, flowering and harvesting stages of the plant growth. (Means with different letters are significantly different at 0.05 probability level. Mean separation was done separately for the samples at different growth stages)

Treatment	Initial (mg/kg)			Vegetative (mg/kg)			Flowering (mg/kg)			Harvesting (mg/kg)		
	N	P	K	N	P	K	N	P	K	N	P	K
GL[2] 5%	92.9^{b}	1.9^{d}	267^{a}	9.8^{b}	3.7^{c}	121^{b}	18.1^{ab}	1.8^{b}	408^{b}	25.2^{abc}	1.25^{a}	61.6^{b}
CM[3] 5%	43.2^{c}	12.8^{c}	56^{bc}	9.5^{b}	6.0^{c}	44.8^{c}	16.5^{bc}	2.1^{b}	378^{b}	21.9^{bcd}	1.24^{a}	108^{a}
GM[4] 5%	46.9^{c}	14.9^{c}	236^{a}	9.2^{b}	7.8^{bc}	206^{a}	15.5^{c}	5.9^{a}	413^{b}	21.0^{cd}	1.25^{a}	59.9^{b}
GL 5%+CE[5] 2%	94.6^{b}	2.6^{d}	279^{a}	9.9^{b}	4.1^{c}	67.4^{c}	18.6^{a}	1.9^{b}	600^{ab}	26.1^{ab}	1.35^{a}	106^{a}
CM 5%+CE 2%	51.5^{c}	30.4^{a}	94^{bc}	15.5^{b}	53.7^{a}	59.8^{c}	18.8^{a}	3.2^{b}	476^{ab}	26.6^{a}	1.28^{a}	122^{a}
GM 5%+CE 2%	48.2^{c}	17.6^{bc}	282^{a}	17.2^{b}	14.6^{b}	56.1^{c}	19.1^{a}	2.3^{b}	551^{ab}	24.5^{abcd}	1.31^{a}	60.1^{b}
Control	41.2^{c}	1.3^{d}	4.1^{c}	9.4^{b}	3.1^{c}	41.9^{c}	13.4^{d}	1.6^{b}	376^{b}	20.4^{d}	1.21^{a}	48.2^{b}
Chemical	127^{a}	20.8^{b}	126^{b}	35.4^{a}	2.7^{c}	50.4^{c}	18.6^{a}	3.2^{b}	574^{a}	21.6^{cd}	1.29^{a}	70.1^{b}

[1] 2 weeks after manure incorporation, [2] *Gliricidia sepium,* [3] Cattle Manure, [4] Goat Manure, [5] *Casuarina equisetifolia*

GM amended samples. Increased water repellency is found to lower the water movements in soil, resulting in prolonged nutrient retention especially N (Fernelius *et al*., 2015). Some conflicting ideas were presented by Aamlid *et al*., (2009) stating that the water repellent soils can create nutrient losses especially nitrates through the leaching across the repellency induced preferential flow paths. Pieces of evidence have supplemented by Muller and Deurer, 2011 to support these findings by exploring that, the remediation strategies for water repellent conditions have resulted in reduced mobility and leaching of chemical substances from soils.

However, the negative impacts of water repellency on nutrients availability can be expected only in extremely repellent soils where the surface water infiltration is completely inhibited. Findings of a previous related study have revealed that the addition of extra 2% CE with 5% organic manure does not increase the water repellency in tropical Ultisols in extreme levels (Liyanage and Leelamanie, 2016). As an organic material, hydrophobic CE contains some amount of available nutrients as indicated in the Table 2. However, the C/N ratio of CE was comparatively higher (>50) compared with the other organic amendments. Hence the increments of the nutrients in those with extra CE cannot be attributed solely to the original nutrient content of CE because, OM with a high C/N ratio is not easily degradable (Lynch *et al*., 2016). Therefore, the significant increments in N levels under extra 2% CE addition in the present study might be a combination of both the slightly induced water repellency and the extra nutrients supplementation by the added CE. This explains that the addition of 2% CE along with 5% organic manure does not cause harmful impacts on N availability in soils.

In all the samples, the P content was high in initial (1-35 mg/kg) and vegetative stages (3-55 mg/kg) compared with flowering (1-6 mg/kg), and harvesting (<2 mg/kg) stages. The highest P content varied differently between the initial and vegetative stages in different organic manure amended samples. In all the treatments, the least P content was at the harvesting stage. In all the samples with GL and 5% CM with extra 2% CE, the P content increased to the vegetative stage. At the flowering stage and the harvesting stage, the P content decreased gradually. In all the samples with GM and 5% CM, the initial P content decreased sequentially to the harvesting stage. According to these findings, occurrence of considerable variations in the fate of P in the soil with respect to the addition of different organic manures was observed. It has been explored that, the P adsorption capacities of soils are dependent on the type of organic fertilizer applied and the available soil type (Bolster and Sistani, 2009). Experiments have shown that when poultry, cattle, and goat manures are applied to highly weathered tropical soil, the P-sorption efficiency of the soil and P-buffering capacity decreases under an increasing incubation period (Azeez and Averbeke, 2011). Variations in organic products from supplements have been reported to influence P sorption as well (Hue, 1991). Therefore, the variations in P dynamics might be dominated by the characteristics of the added organic substances as well as the other indirect effects of precipitation, runoff, and cultural practices (Grant *et al*., 2001). However, the samples with the highest P content at the vegetative stage might be results of the accumulation of mineralized P under low plant utilization. The increasing demand for P for energy transfer and protein metabolism processes essential for reproduction (Fageria *et al*., 2010) might be the reason for the low P content at the flowering and harvesting stages, which was observed in all the organic manure amended samples.

Compared with the samples with only 5% organic amendments, the P contents of those with intermixed 2% CE were statistically similar or higher in most of the stages except at the flowering stage of GM amended soils. Based on these results it can be concluded that the intermixing of 2% CE with GL, CM, and GM would not be disadvantageous during the plant growth. In the initial and vegetative stages, the samples with CM and extra 2% CE showed significantly higher P content than those with only 5% organic amendments. These findings reveal that the application of extra 2% CE would be beneficial to diminish the early season limitations in P availability which can result in restrictions in crop growth (Grant *et al*., 2001) and the significance of the impact depends on the type of the organic manure.

The chemical fertilizer treatment showed comparatively low P content at the later plant growth stages. Generally, inorganic fertilizers are intended to increase the availability of nutrients in the soil, especially macro elements such as N, P, and K (Lehmann *et al*., 2003). However, the instantly supplied nutrients by inorganic fertilizers might be subjected to leaching resulting low nutrients availability because, it is widely accepted that under organic manure addition, the leaching becomes low and nutrient use efficiency increases in significant levels (Lasmini *et al*., 2015).

In all the amended soils with organic materials, the initial K content was around 50-280 mg/kg. Comparatively low values of K were in vegetative and harvesting stages (40-210 mg/kg and 50-120 mg/kg respectively), and high values in the flowering stage (onset of flowering) (370-600 mg/kg). Potassium plays a major role in number of cellular and physiological activities including maintenance of intracellular osmotic balance, enzyme activation, protein synthesis and transportation, photosynthesis, cell extension, stomatal regulation, seismonastic movements, phloem transport, cation-anion balance, and stimulation of primary production (Steingrobe and Claassen, 2000). The high demand for the K for these processes might be the reason for reduced K content at the vegetative and harvesting stages.

When comparing with the samples of only 5% organic amendments, K content of the samples with intermixed 2% CE was statistically similar or higher in all the stages except at the vegetative stage of GL and GM amended soils. These results revealed that the addition of 2% CE along with 5% organic amendments would be beneficial in most of the growth stages especially at later growth stages, and there would be less disadvantageous effects of K losses.

According to the yields obtained with different treatments, the highest yield was observed in the plots amended with 5% GL intermixed with 2% CE, while the lowest was in the control (Figure 1). All the samples intermixed with 2% CE resulted in higher yields than those with only 5% organic manure. Among these, 5% GL and 5% GM intermixed with 2% CE resulted in significantly higher yields than the chemical fertilizer amended plot as well. These findings are in agreement with the findings of nutrients availability as the addition of 2% CE was found not to be unfavorable on nutrients availability. The higher original hydrophobicity of CE has not appeared to create negative effects of extreme water repellency under the current application rates. The slightly induced water repellency combined with the provision of some original nutrients contain in CE might have resulted in higher yields in the treatments blended with 2% CE. Therefore, the addition of extra 2% CE can be expected to not to have unfavorable impacts on crop yields compared with soils treated with only 5% organic manure.

When considering the harvest indices of different treatments, the control sample showed the lowest harvest index (0.28) while the 5% GM intermixed with 2% CE showed the highest (0.87) (Figure 2). All the samples with extra 2% CE showed higher harvest indices than those with only 5% organic manure where the differences were statistically significant in GM and CM amended samples. Higher harvest index is a positive indicator of improved crop production because it is positively correlated with the grain yield (Singh and Stoskopf, 1970). It was interesting to find out that the results of the harvest index of GM amended samples were fairly compatible with the findings of the yield component as explained by the Figure 1. Ultimately it is clear that the intermixing of a small amount of hydrophobic organic manure into common organic manure incorporated soils would be important to increase the economic yield of the tested crop species.

CONCLUSION

The addition of extra 2% Casuarina leaves along with 5% organic amendments would be beneficial in most of the growth stages, and there would be less detrimental effects on plant available N, P, and K. Extra 2% CE incorporation was not harmful on crop yield and in the case of GL and GM that was beneficial to have a significantly higher yield and to get a higher harvest index. As a whole, previous studies on organic amendments blended with hydrophobic organic manure [2% CE leaves] have proved that this strategy is useful to improve the aggregate stability under Sri Lankan conditions and increase soil OM accumulation without being harmful on soil hydrophysical properties. The findings on the nutrients availability of the present study prove that the amendment of 5% CM, GM, and GL intermixed with 2% CE would not cause detrimental impacts on nutrient dynamics, soil fertility, and plant growth performances as well. Further experiments are required under repeated and non-repeated manure applications to explore any other unfavorable impacts such as heavy metal accumulations and leaching losses of nutrients in order to confirm the suitability of the suggested organic amendments application rates.

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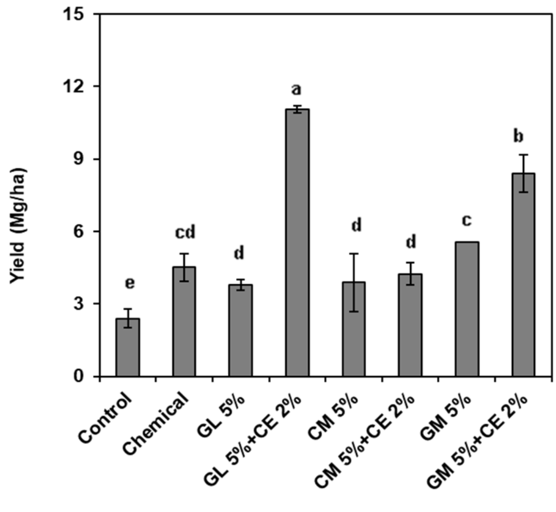

Figure 1: Yields (fresh weight) of different treatments.
GL: *Gliricidia sepium*, CE: *Casuarina equisetifolia*, CM: Cattle Manure, GM: Goat Manure.
Error bars indicate ± standard deviation.
Means with different letters are significantly different at 0.05 probability level.

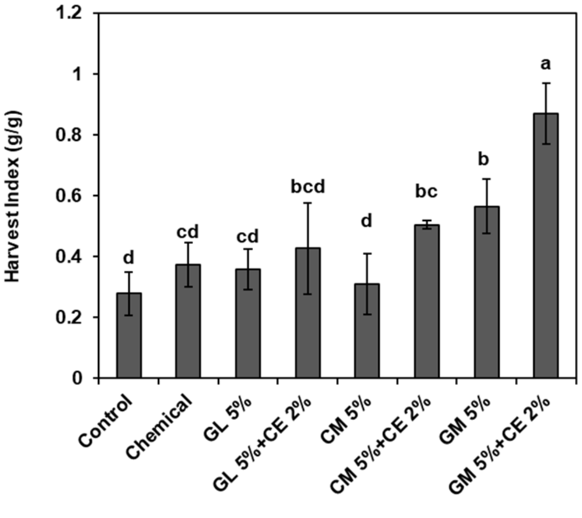

Figure 2: Harvest indices of different treatments.
GL: *Gliricidia sepium*, CE: *Casuarina equisetifolia*, CM: Cattle Manure, GM: Goat Manure.
Error bars indicate ± standard deviation.
Means with different letters are significantly different at 0.05 probability level.

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