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Research Article

Effects of Liquid Manure on Growth and Yield of Spinach (*Beta vulgaris* Var Cicla) in a Sub-tropical Environment in Swaziland

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Abstract: A study was conducted to determine the effects of liquid manure on growth, development and yield of spinach. A randomized complete block design experiment was conducted in pots, in a 50% semi shaded lath house. The four treatments were solid kraal manure applied at 40 t/ha; liquid manure applied at 40 m^3/ha; liquid manures solid remains applied at 40 t/ha and control of inorganic chemical fertilizer applied at 150 kg/ha. There were four replications per treatment. The growth parameters that were measured included the average number of leaf development per week, leaf height, leaf width, leaf area, leaf area index and the fresh biomass. For the growth parameters measured the results showed that the highest values were obtained in spinach plants fertilized with inorganic fertilizer followed in decreasing order by those fertilized with solid kraal manure, liquid manure solid remains and lastly liquid manure. The spinach fresh biomass mean yield for the treatment of inorganic chemical fertilizers was the highest at 54.4 g per plant. The lowest spinach yield of 17.9 g per plant was obtained from liquid manure treatment. The spinach yield from inorganic fertilized plants was significantly ($p \leq 0.05$) different from that of liquid manure and liquid manure solid remains. However, the yield from solid kraal manure treatment and inorganic chemical fertilizers was not significantly different, implying that kraal manure could be used to produce a high yield of the vegetable at a lower cost compared to inorganic fertilizer. Therefore it is recommended that farmers should continue to use solid kraal manure since the yields produced were similar to that of chemical fertilizers.

Keywords: Climate change, growth and development, liquid manure, Spinach (*Beta vulgaris*), yield

INTRODUCTION

The Kingdom of Swaziland is a monarchy and a land-locked country located in the south eastern part of Africa covering an area of 17,363 km^2. It is bounded by the Republic of South Africa on the north, west and south and by the Republic of Mozambique to the east. The general climatic characterization of Swaziland is subtropical with wet hot summers (about 75% of the annual rainfall in the period from October to March) and cold dry winters (April-September). Mean annual rainfall ranges from about 200 mm in the southern lowlands to 1,500 mm in the northern highlands. Mean annual temperature varies from 17°C in the highlands to 22°C in the lowlands. The population of Swaziland was estimated at 1,018,449 in 2007, with one ethnic group called Swazi. The majority of the people (75%) live in communal land and they strongly depend on subsistence agriculture and natural resources for livelihoods (FAO, 2007). Swaziland is classified as a lower middle-income country with a per capita GDP of US$ 5,300 in 2012 yet about 63% of the population fell below the national poverty line in 2010 (Central Bank of Swaziland, 2012).

Poverty in the Kingdom has been worsened by HIV/AIDS which continues to be a major obstacle to economic and social progress. In addition to its humanitarian and social consequences, HIV/AIDS costs the country severely in economic terms, as it constrains output growth, eliminates work skills and knowledge, shrinks the tax base, raises health-related costs and reduces disposable income. A study of subsistence agriculture in the country found that due to AIDS related sickness and deaths, 47% of the households had a decline in crop yield and thus contributed to the increased levels of poverty in the country (FAO, 2007) food insecurity and malnutrion. There is need for research on vegetable production using locally available material like livestock manures to alleviate food insecurity and malnutrition.

Swaziland has a population of about 620,000 head of cattle and about 275,000 goats (FAOSTAT, 2012). Excretions from livestock can be decomposed into fertile organic manure which can be used in vegetable production. The most common organic manure used is kraal manure from cattle because cattle are abundant in the Kingdom, closely followed in decreasing order by chicken manure, goats and sheep manure (Masarirambi

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et al., 2012a, b). The practice of collecting and using wastes from animal, human and vegetable sources for improving crop productivity is as old as agriculture. Manures with low nutrient content per unit quantity have longer residual effect besides improving soil physical properties compared to fertilizer with high nutrient content (Ogunlela *et al*., 2005; Anonymous, 2012a; Masarirambi *et al*., 2012a, b).

The addition of organic manures to most garden soils can improve the physical condition of the soil by incorporating bulky, organic materials and as well as feeding the organisms that live within the soil. Not only do organic manures supply plant food, but they add substances that have the power to act on the insoluble compounds already in the soil and so reduce these compounds to a form that plants can use (Anonymous, 2012b). The introduction of organic farming and the demand of organically produced crops can play an important role in reducing chemical residues in food crops (Masarirambi *et al*., 2010b, 2012a).

Deficiencies in micronutrients such as vitamin A and iron in developing countries are widespread and have negative consequences for children's growth and development (Aphane *et al*., 2002). Therefore there is a need for people to grow vegetables like spinach to supply the body with such nutrients. Vegetables form an important component of the diet of rural communities in Swaziland especially in situations of drought and famine. The most common vegetables grown and consumed in Swaziland are cabbages (*Bassica olearacia*), spinach (*Beta vulgaris* var cicla), imbuya (*Amaranthus hybridus*), ligusha (*Corchorus olitorius*), chuchuza (*Bidens pilosa*) and inkhakha (*Mormodica balsamina*) (Masarirumbi *et al*., 2010a).

Spinach (*Beta vulgaris* var cicla) has a high nutritional value and is extremely rich in antioxidants, especially when fresh or steamed (Anonymous, 2003; Olivier, 2007; Anonymous, 2010b) It is a rich source of vitamin A (and especially high in lutein), vitamin C, vitamin E, vitamin K, magnesium, manganese, folate, betaine, iron, vitamin B2, calcium, potassium, vitamin B6, folic acid, copper, protein, phosphorus, zinc, niacin, selenium and omega-3 fatty acids (Mateljan, 2012; Anonymous, 2012c). Rubiscolins (opioid peptides) can also be found in spinach (Mateljan, 2012; Anonymous, 2012c). The greatest challenge facing Swaziland today is lack of nutrients in the soil for optimum vegetable production. The use of inorganic fertilizers has proved to be the fastest and most effective way of adding nutrients and boosting crop production (Panda and Hota, 2009). However the inorganic fertilizers are too expensive and not readily available for the subsistence farmers in the country (Masarirambi *et al*., 2011a; Brown, 2011). This negatively affects crop production as optimum yields are not attained and thus unable to produce enough food for the nation. Cattle manure has long been used as a fertilizer for field crops particularly by smallholder farmers in Swaziland. Manure adds nutrients and beneficial organic material to the soil which builds soil structure and helps prevent compaction. However, despite these benefits, kraal manure decomposes slowly resulting in a slow nutrient release thus nutrients not being available as fast as they are required by the current crop (Van-Averbeke and Yoganathan, 2003; Boyanapalle, 2004; Anonymous, 2012b). Since nutrients are absorbed in solution form, dissolving nutrients from kraal manure prior to application may help accelerate the availability of nutrients to the plant, therefore the inspiration of trying to formulate liquid manure than can be used under small scale conditions.

In view of un-affordability of fertilizer due to escalating prices, more smallholder farmers in Swaziland are anticipated to turn to the use of organic sources, rather than inorganic fertilizers for enhancing crop productivity without incurring off farm cost. The question is would liquid kraal manure have a significant effect on growth rate and yield of spinach. The study sought to evaluate the effect of producing spinach with liquid cattle manure compared to solid cattle manure and other fertilizer forms.

MATERIALS AND METHODS

Experimental site and design: The experiment was conducted in pots in a 50% semi-shaded area at the Horticulture department farm of the University of Swaziland, Luyengo Campus. The area is found in the Middleveld of Swaziland, 21°34'S and 31°12'E at an altitude of 730 m. The average summer maximum temperature is 27°C and 15°C in winter. The experiment was conducted in a Randomized Complete Block Design (RCBD) with four treatments and four replications per treatment. Dairy cattle solid manure (A), was applied at 40 t/ha with four replications; liquid manure treatment (B) (Richards, 2007; Anonymous, 2010a; Watkins, 2011) was applied at 40 m^3/ha, liquid manure solid remains applied at 40 tones/ha (C) and inorganic chemical fertilizers (D) [(N:P:K-2:3:2 (37)] and lime ammonium nitrate (LAN) [28% Nitrogen] were each applied at 150 kg/ha basally and top dressed respectively. Each replication consisted of four pots which had one spinach plant each and the total number of pots was 64.

Table 1: Physico-chemical properties of the soil used in the experiment

Parameter	Amount
Nitrogen (N)	1.69%
Phosphorus (P)	64.5 mg/kg
Potassium (K)	5.08×10^{-5}cmolc kg/kg
Calcium (Ca)	0.04cmolc kg/kg
Magnesium (Mg)	1.97cmolc kg/kg
Exchangeable Aluminium	0.03cmolc kg/kg
pH ($CaCl_2$)	4.55

Table 2: Nutrient values of manures used in the experiment

Manure type	Nitrogen (%)	Phosphorus (µg/me)	Potassium ($\times 10^{-4}$ cmolckg/kg)
Solid manure	1.58	1.65	8.98
Cow dung (Kraal manure)	0.73	1.76	6.92
Liquid manure solid remains	1.6	1.61	4.33
Liquid manure	0.025	1.09	2.59
Recommended amounts for spinach (kg/ha)	100-150	70-100	150-180

Soil collection and analysis: The soil put in the experimental pots was collected from a virgin land on campus. The soils were randomly sampled and analysed for pH and nutrient content [Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg) and Aluminium (AL)] at the University of Swaziland Chemistry Laboratory. The results of the analysis were used to make fertilizer recommendations before the start of the experiment (Table 1 and 2).

Determination of Nitrogen: One gram of the soil sample was duplicated in 2 digestion tubes. A blank paper was put into 2 other digestion tubes without the soil sample. A pinch of boiling stones, one selenium tablet was added into all the digestion tubes. Twenty ml of sulphuric acid (30%) was added and the tubes were put into the fume hood for digestion until a clear liquid was produced. The solution was allowed to cool and then it was diluted with distilled cold water. The solution was then put into the distillation unit and 50 mL of sodium hydroxide solution was added. Twenty five mL of boric acid was measured into an Erlenmeyer flask and put into the distillation unit. One hundred mL of the digested solution was added into the boric acid. Five drops of phenolphthalein were added and the solution was titrated with a 0.1N hydrochloric acid (HCl). The amount of HCl used was recorded, to be used in the following Eq. (1) for nitrogen (N) content calculation:

$$N = \frac{V * Acid\ Normality * 0.014}{weight\ of\ sample} * 100\% \quad (1)$$

where,

N = Nitrogen content (%)

V = Amount of HCl used for titrating the sample minus the amount of HCl used for titrating the blank digestion tubes

Determination of soil phosphorus (P): Two duplicates of 2 g soil samples were weighed into 50 mL centrifuge tubes and 20 mL of Bray-1 solution were added. The centrifuge tubes were mounted on a mechanical shaker and shaken for 5 min. The contents of the centrifuge tube were centrifuged for 10 min on a Kubota centrifuge (model 2010) [Tokyo, Japan]. The supernatant solutions were filtered through no. 42 whatman (England) filter paper into 50 mL volumetric flasks. The amount of phosphorus in the extracts was determined calorimetrically following the ammonium molybdate blue method. Phosphorus in the extracts was measured using Biochrom spectrophotometer (model Libra S12) [UK] at a wavelength of 730 nm. The amount of phosphorus in the extracts was expressed as mg/kg (Table 1).

Determination of soil calcium (Ca), magnesium (Mg) and potassium (K): The content of Ca, Mg and K were determined as described subsequently. Two duplicates of 10 g soil samples were weighed into 250 mL Erlenmeyer flasks and 90 mL of 1N ammonium acetate solution was added. The flasks were put on a mechanical shaker and shaken for 10 min. The contents of the flask were allowed to settle and then filtered through Whatmann no. 42 filter paper into 100 mL volumetric flasks. The contents of the flasks were diluted with the extracting solution. The amount of Ca and Mg in the extracts was measured by Varian Atomic Absorption Spectrometer (Model AA 200 Spectra) [Australia] at the appropriate wavelength for each element. Potassium was measured by flame photometry using a Jenway Flame Photometer (Model PFP7) [Essex, England]. The content of the elements were expressed in cmolc/kg (Table 1).

Determination of exchangeable aluminium (Al): Five g soil samples were duplicated and weighed into 250 mL Erlenmeyer flasks and 50 mL of 1N potassium chloride (KCl) solution was added. The contents of the flask were shaken on a mechanical shaker for 30 min. After shaking, the solutions were allowed to settle and then filtered through a Whatman No. 42 filter paper into 50 mL volumetric flasks. The content of aluminium in the extracts was measured by titration against a 0.001 M NaOH solution. The content of Al in the extracts was expressed in cmolc/kg (Table 1).

Determination of soil pH: The pH was determined by duplicating 10 g soil samples and weighing them into 50 mL beakers and 20 mL of 0.01 M $CaCl_2$ solution was added. The mixtures were swirled for 5 min with glass rods and allowed to settle for 30 min before pH measurements. The pH of the soil was measured with a Hanna pH meter (model 211) [USA] by immersing the pH electrode into the supernatant solution. The pH values obtained were expressed as pH $CaCl_2$ scale (Table 1).

Determination of manure potassium (K) and phosphorus (P): The wet digestion procedure was followed in the determination of potassium (K) in the manure. Duplicate 2.5 g manure was weighed into 250 mL Erlenmeyer flasks. Ten milliliters (10 mL) of concentrated sulphuric acid (H_2SO_4) were added into each flask and placed on a hot plate at 300°C for one h. The manure was digested on a hot plate for one hour. The mixture was cooled and six drops of hydrogen peroxide (H_2O_2) were added and the flasks were placed on a hot plate for further digestion. The addition of hydrogen peroxide (H_2O_2) and heating intervals were repeated until the solution was clear. One final addition of sulphuric acid and heating intervals were made before the flasks were cooled and made up to 250 mL mark with distilled water.

The contents of K and P in the extracts were dissolved and elements were measured using a Varian Techron Absorption Spectrophotometer (model AA 200) [Perkin-Elmer, Norwalk, CT, USA]. The appropriate wavelength for each element was used in the measurement.

Determination of pH of manure used in the study: The manure was oven dried at 135°C for 24 h then subsequently prepared for pH measurement. The method used for testing soil pH was used to test for the manure pH as previously described.

Preparation and analysis of organic liquid manure: Cattle manure was obtained from the University of Swaziland farm. Flickety (2011)'s hessian bag method (anaerobic) was used to make the liquid manure. The manure was analysed for its nutrient content in the Chemistry Laboratory of the University of Swaziland, before being applied to the experimental pots. Nitrogen was analysed using the procedure outlined previously. The results of the nutrient values are shown in Table 2.

Planting and weed control: Pots were filled with the tested soil. Tyee spinach variety, an early maturing and bolt resistant variety, was used for the experiment. It was bought as seedlings and planted. Uniformity was ensured by choosing those seedlings whose leaf width and height were similar. Weeds were manually removed by hand throughout the growing period as and when needed.

Leaf width, leaf height, leaf area index and plant yield determination: The leaf growth parameters were measured on all the leaves on each plant and the average was obtained and used for data analysis. The leaf width of all the plants from the pots was measured using a 30 cm rule. This was measured from one end of the leaf to the other end (in the middle position of the leaf length).

Leaf height was measured for all the plants from the pots by using a measuring tape to determine the distance from the soil surface at the base of the leaf to the apex of the leaf. The average leaf height was determined by adding the different leaf heights from each treatment and dividing that sum by the number of plants measured for each treatment.

The Leaf Area (LA) was calculated by measuring the length and width of a leaf from each pot using a measuring tape. A leaf count was done to find the total number of leaves in all the plants. To get the average number of leaves per plant, the total number of leaves was divided by the total number of plants whose leaves were counted. To calculate the leaf area index (LAI) the following Eq. (2) was used:

$$LAI = Y \times N \times AL \times (AP)^{-1} \tag{2}$$

where,

Y = Population of plants per pot
N = Average number of leaves
AL = Average area per leaf
AP = Area of pot

Spinach yield for each pot was obtained by weighing all the leaves.

Data analysis: Analysis of Variance (ANOVA) was done using Statistical Package for Social Science (SPSS Inc., 2011) and to find the mean values and the standard deviation which were used to determine the treatments that were significantly different from each other. Upon significant F-value, the means were separated by Least Significant Difference (LSD) test at $p \leq 5\%$ (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Soil nutrient content: The soil had a relatively low nutrient content (especially nitrogen which is vital for spinach leaf growth and development) and needed to be improved to produce better yields.

Manure nutrient content: Cow dung (cattle/kraal manure), which was used for making the liquid manure, had the least nitrogen content (Table 2). After the liquid manure preparation from cow dung, the liquid manure had the least nitrogen content while the liquid manure solid remains had the highest nitrogen content. This shows that, during the decomposition process, the plant matter was converted into nitrogen and was held within the liquid manure solid remains. The findings were consistent with those of Devasenapathy (2008).

Plant growth parameters:

Number of leaves: At the start of the experiment the spinach plants had similar number of leaves. However, between weeks one and two, the highest development of new leaves was observed in Liquid Manure Solid Remains (LMSR) fertilized spinach plants and Inorganic Chemical Fertilizers (ICF) applied plants and there was no significant difference between the two Fig. 1. However, development of new leaves was slowest in Liquid Manure (LM) fertilized plants. Relatively low content of nitrogen in the liquid manure reduced the growth and development of new leaves.

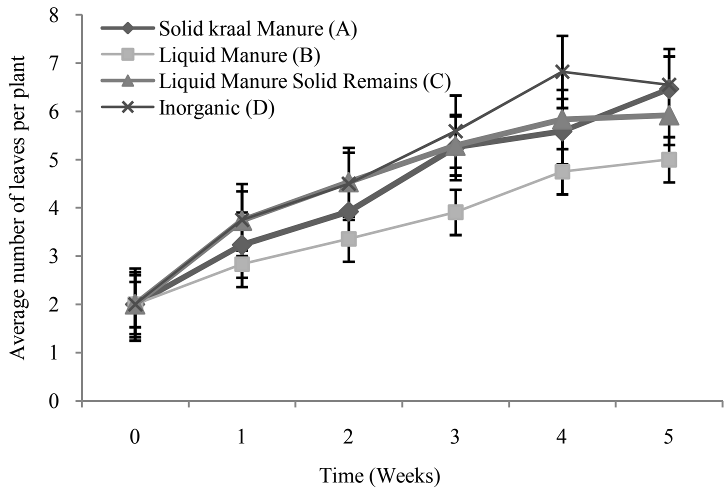

Fig. 1: Number of leaves of spinach (*Beta vulgaris* var cicla) per plant as affected by the various treatments *Bars are standard error bars above and below the mean

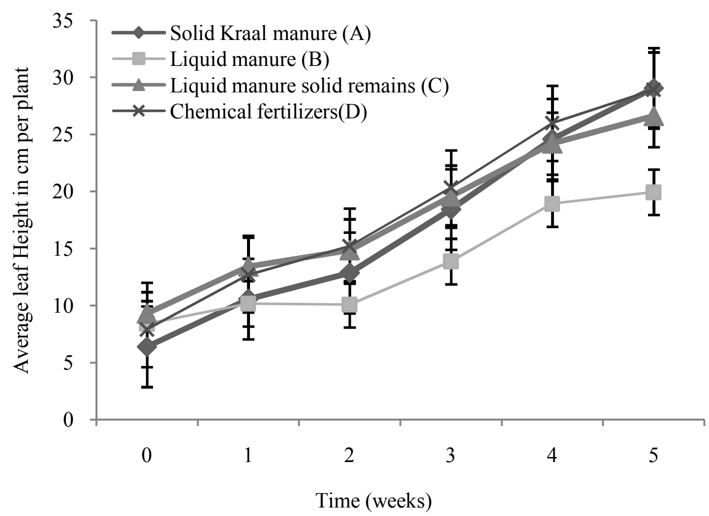

Fig. 2: Leaf height of spinach (*Beta vulgaris* var cicla) as affected by the various fertilizers, over the growth period

The high increase in the growth and development of new leaves in liquid manure solid remains fertilized plants was due to prior release of relatively copious amounts of nitrogen during the decomposition process.

Normally, inorganic chemical fertilizer nutrients are soluble, so the nitrogen was quickly released into the soil thus leading to fast leaf growth and development. However, during maturity, leaf development declined because the nutrients were probably exhausted in the soil. This resulted in the leveling of the leaf growth and development between ICF and SKM at maturity as SKM was continuously releasing nitrogen (Fig. 1). Organic manures like cattle manure have been reported to release both micro and macro nutrients slowly resulting in subsequent promotion of vegetable growth (Van-Averbeke and Yoganathan, 2003; Ogunlela *et al*., 2005; Pimentel *et al*., 2005; Kuntashula *et al*., 2006).

In weeks three and four there was no significant different between the numbers of leaves of spinach fertilized with liquid manure solid remains or solid kraal manure. This meant that the LMSR nitrogen release was the same as the SKM. Liquid Manure (LM) fertilization of spinach resulted in relatively low leaf growth and development due to relatively low nitrogen content.

Leaf height: Leaf height or plant height of spinach increased with time from week one up to week five (Fig. 2). Plants fertilized with chemical fertilizers where the tallest followed in decreasing order by those fertilized with liquid manure solid remains, solid kraal manure and lastly liquid manure which had significantly the lowest height. Presumably in that order was the availability of nitrogen and other plant nutrients required for growth and development.

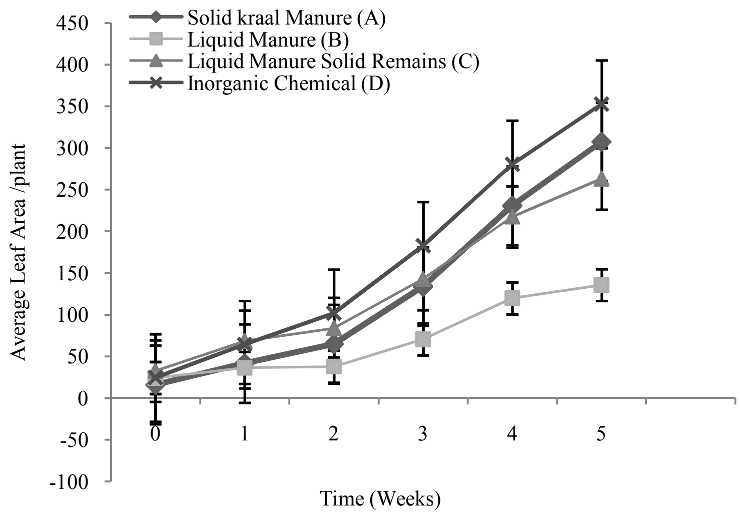

Fig. 3: Leaf area of spinach (*Beta vulgaris* var cicla) as affected by the various fertilizers

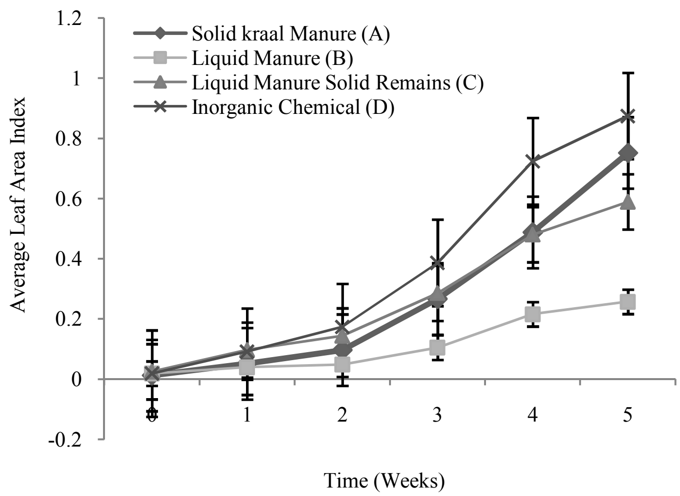

Fig. 4: Leaf area index over the growing period of spinach (*Beta vulgaris* var cicla)

Leaf area: There were significant differences in the leaf areas of spinach among the treatments (Fig. 3). The inorganic chemical fertilizers treatment produced plants that had the highest leaf area as compared to the other three treatments. The liquid manure resulted in the least leaf area of spinach plants. The inorganic chemical fertilizers were significantly different from the solid kraal manure, liquid manure and liquid manure solid remains in terms of subsequent spinach leaf area produced. The higher leaf area of spinach that was exhibited by the inorganic chemical fertilized plants was due to the fact that the nutrients were readily available for the plants while in the solid kraal manure and the liquid manure solid remains the nutrients had to undergo a i.e., after decomposition process in order to be available to the plants (Ogunlela *et al*., 2005; Pimentel *et al*., 2005). However, the rate of growth of spinach leaf area in SKM and ICF fertilized plants were comparable as the gradient for their graphs are the same from week 2 till maturity (Fig. 3).

Leaf area index: Leaf Area Index (LAI) is a measure of the photosynthetic area of the plant per unit area of land. For broad leaf plants, the higher the LAI, the more the ability for the plant to make and potentially store food. Figure 4 shows the different leaf area indices for the treatments throughout the growing period. The chemical fertilizers resulted in the highest leaf area index while liquid manure had the lowest. The photosynthetic area of the leaves was greater in the chemical fertilized plants resulting in yield which was higher than in the liquid manure. The higher leaf area

Table 3: Fresh biomass of spinach (*Beta vulgaris* var cicla) as affected by the various fertilizers

Treatment	N (Total population of plants)	Mean yield (g)
Kraal manure (A)	13	48.9a*
Liquid manure (B)	13	17.9c
Liquid manure solid remains (C)	13	35.26b
Chemical Fertilizers (D)	12	54.4a

*Mean values for different treatments with the same alphabet signify that they are significantly different from each other ($p<0.05$)

index in inorganic chemical fertilizers was caused by the relatively higher nutrient availability which increased the leaf height and width per unit area of the pot.

Plant fresh biomass: The fresh biomass mean yield of spinach from the treatment of inorganic chemical fertilizers was the highest at 54.4 g per plant (Table 3). The lowest yield was obtained from liquid manure treatment (17.9 g). According to Ewert (2004), biomass production is closely related to light interception, which is mainly determined by Leaf Area Index (LAI). Leaves with a higher leaf area index, have a larger photosynthetic area and they accumulate more biomass if all other factors are not limiting. The lowest yields in the liquid manure were caused by the small LAI compared to the other treatments.

The yield from inorganic chemical fertilizers was significantly ($p\leq0.05$) different from that of liquid manure and liquid manure solid remains. On the other hand the yield from solid kraal manure treatment and inorganic chemical fertilizers was not significantly different, implying that kraal manure could be used to produce a high yield of the vegetable at a lower cost compared to inorganic chemical fertilizer.

Similar results of plant growth promotion when fertilized by animal manure have previously been reported (Ogunlela *et al*., 2005; Ossom, 2010; Masarirambi *et al*., 2012a, b), however in the findings of Masarirambi *et al*. (2010, 2012a, b), inorganic fertilizer’s promotion of plant groth was less than that of animal manures when applied at the optimum levels.

Although the application of inorganic fertilizers resulted in more spinach yield, it needs to be borne in mind that the production of inorganic fertilizers is environmentally unfriendly and has been implicated in greenhouse gas emissions and impending irreversible climate change.

It may be interesting for future researchers to use a more porous bag in the manure preparation as most of the nutrients were held in the solid remains. One can also consider the mortality of the leaves as another factor that can be added (plant tea) as some of the leaves dried and fell off before the plants reached maturity. Farmers or other reseachers who may be interested in doing the study may also consider soaking the solid kraal manure long enough, to catalyze the decomposition process so that the release of nutrients can be relatively faster.

CONCLUSION AND RECOMMANDATION

The results from the study showed that the liquid manure had an effect on the growth development and yield of spinach albeit the least compared to other treatments. The chemical fertilizers on average effected the highest growth, development and yield of spinach followed in decreasing order by, solid kraal manure, liquid solid manure remains and lastly the liquid manure. From the results of this study it may be recommended that the farmers may continue to use solid kraal manure for spinach fertilization since it resulted in yield similar to that of chemically fertilized spinach. In addition to that, kraal manure is affordable and readily available to farmers. Mean while quest for on farm liquid manure formulation development using locally available and environmentally friendly material will be on going.

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